Alnylam® PHARMACEUTICALS

RNAi Therapeutics Using Conjugate Delivery Platform

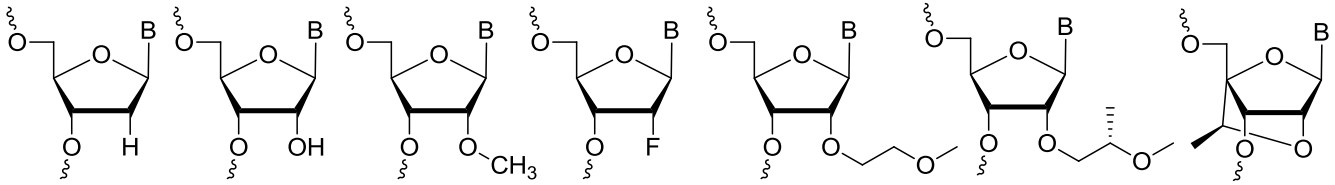
October 8, 2013

Topics to Cover

- Alnylam GalNAc-siRNA Conjugate Platform
  - » GalNAc-siRNA conjugates for subcutaneous administration
  - » Valency, Structure and Receptor Binding
- Pharmacology of GalNAc-siRNA Conjugates
- PK/PD of GalNAc-siRNA Conjugates
- Summary

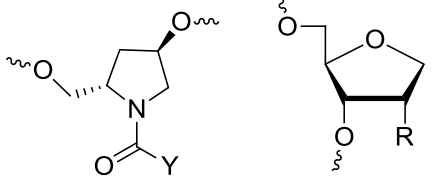
Chemical Modifications of siRNAs

Sugar Modifications



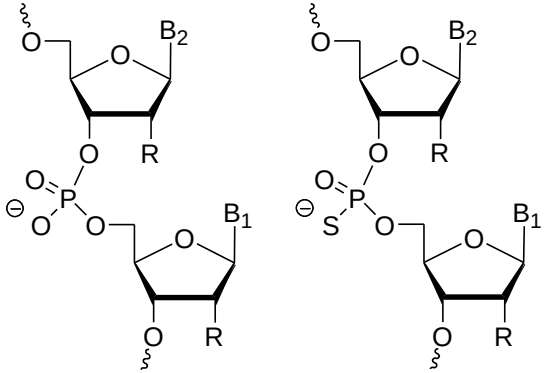
2’-Deoxy | 2’- Ribo | 2’-*O*-Me | 2’-F | 2’-*O*-MOE | SuMOE | BNA

Internal/ End Caps



Abasic

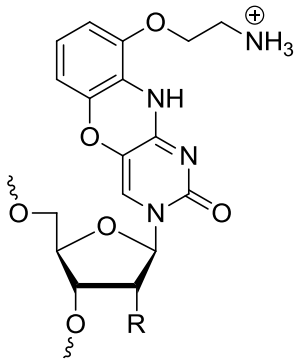
Backbone Modifications



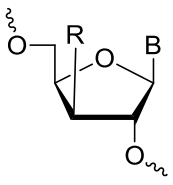
Phosphate (P=O)

Phosphorothioate (P=S)

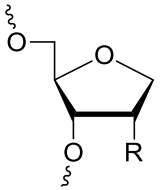
Modifications to modulate thermodynamics and improve biostability



G-Clamp (nucleobase)



Xylo



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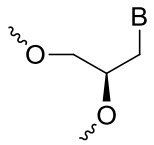
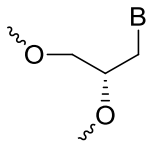
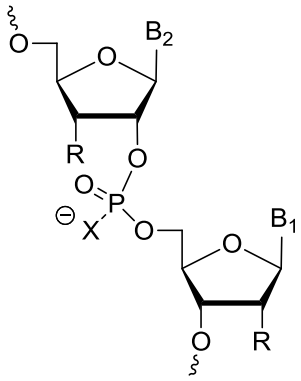




GNA



2’-5’ P-linkage

R = OH, OMe, F, H
X = O or S

Alnylam PHARMACEUTICALS

GalNAc-siRNA Conjugates

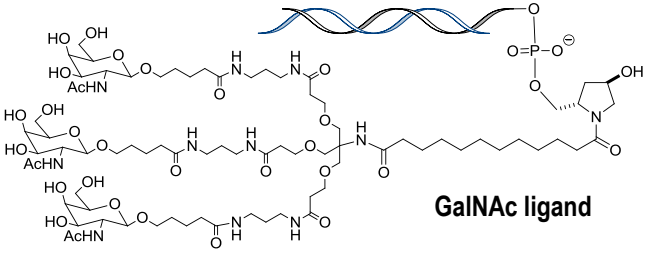
Targeted Delivery to Hepatocytes and SC Dosing

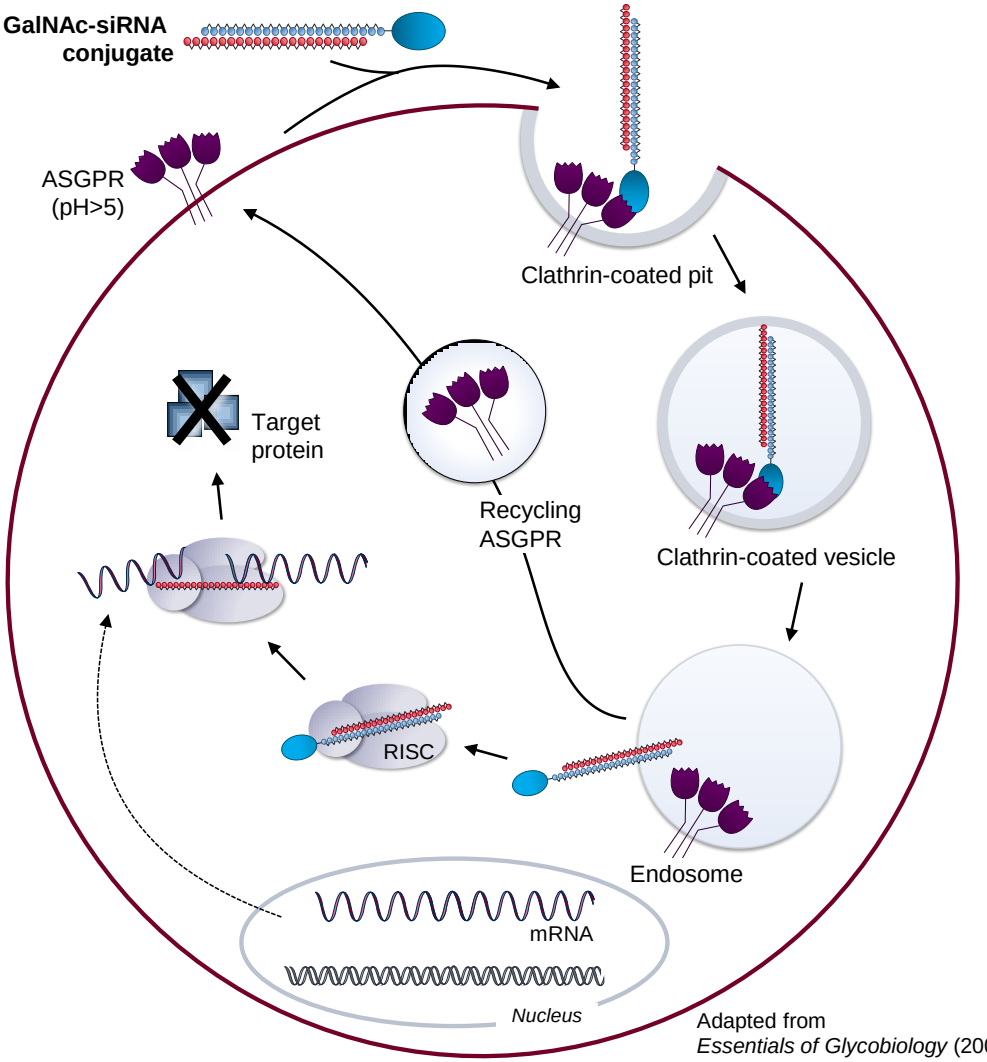
ASGPR

- Clears serum glycoproteins via clathrin-mediated endocytosis
- Highly expressed in hepatocytes
  - » 0.5-1 million copies/cell
- High rate of uptake
- Recycling time ~15 minutes
- Conserved across species

GalNAc-siRNA

- GalNAc ligand conjugated to chemically modified siRNA to mediate targeted delivery
- Trivalent GalNAc carbohydrate cluster has nM affinity for ASGPR
- Administered subcutaneously (SC)

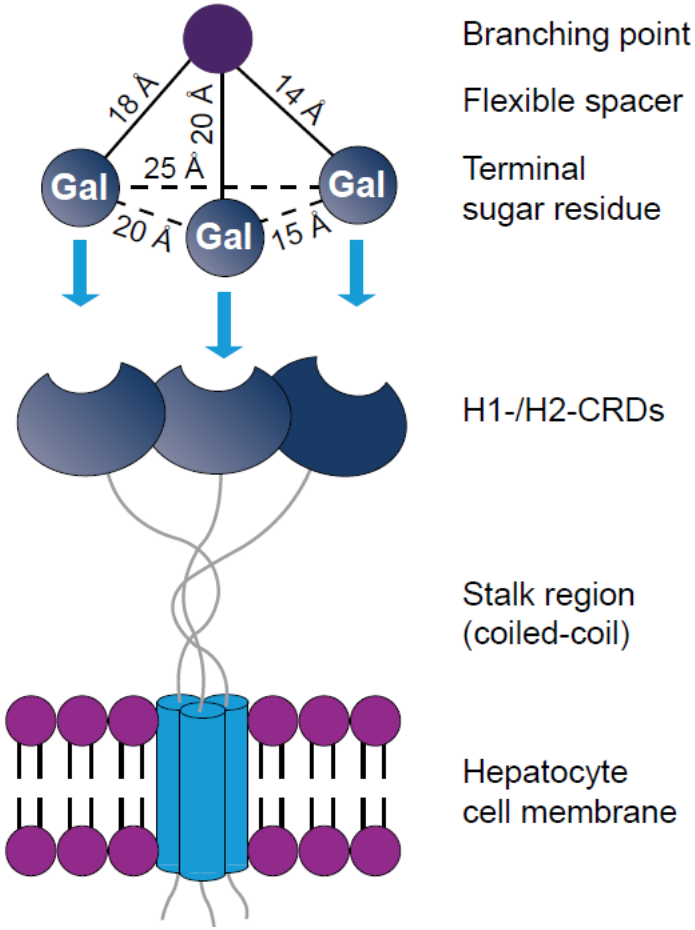


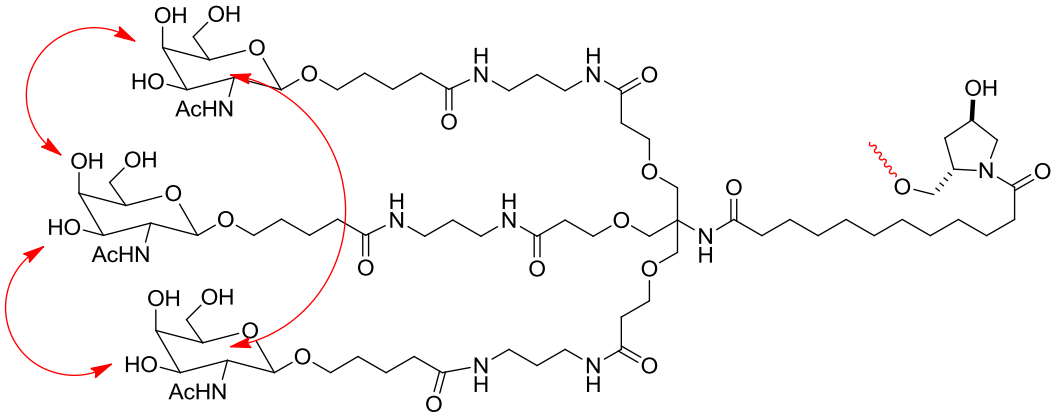


Adapted from *Essentials of Glycobiology* (2009)

Alnylam PHARMACEUTICALS

Design of Multivalent ASGPR Specific Ligand





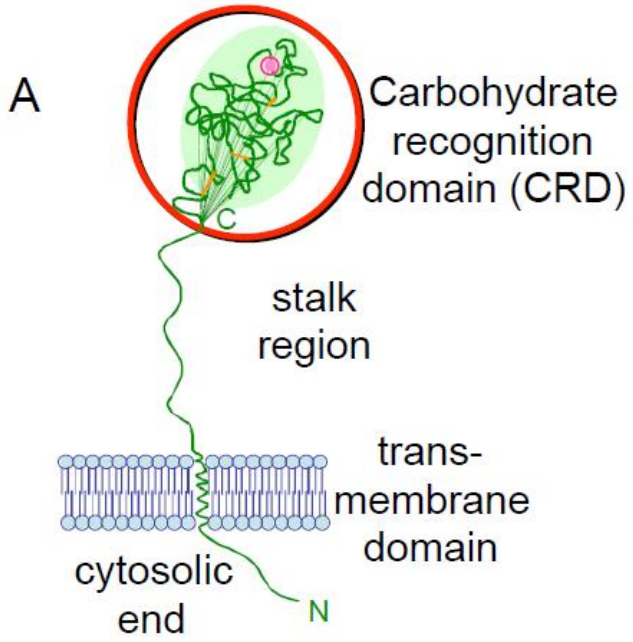
Spacing: 20 Å Triantennary GalNAc

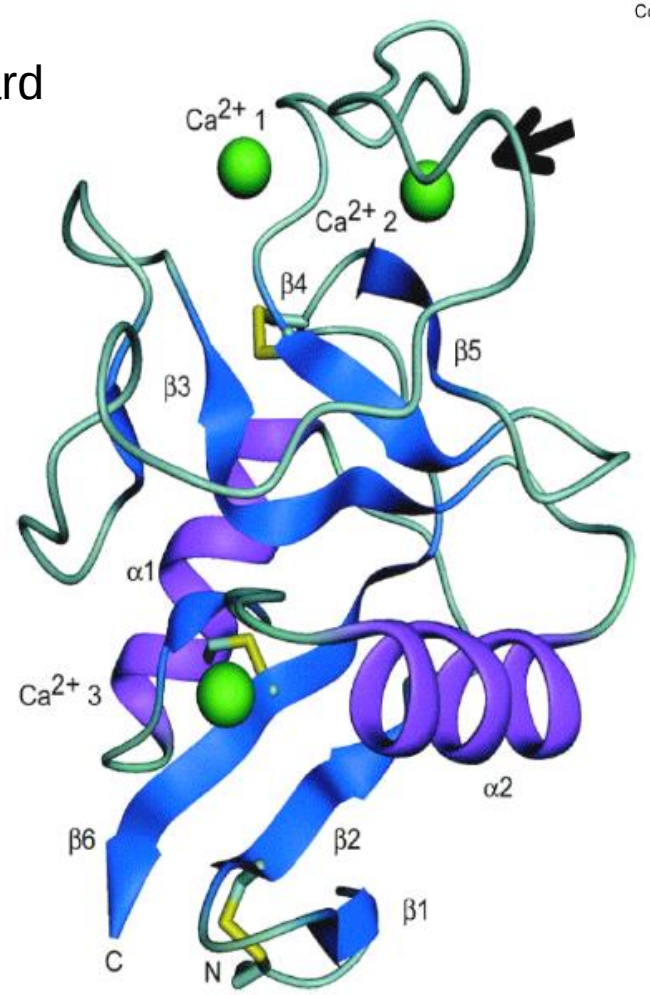
Alnylam Pharmaceuticals 2012

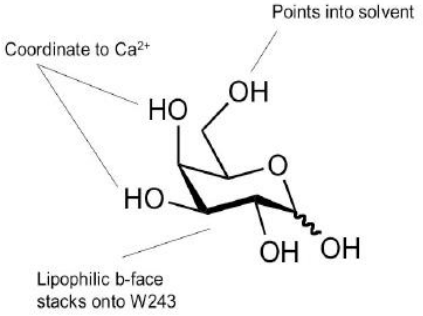
Adapted from Lee *et al., Carbohydrates in Chemistry and Biology;* 4:549 (2000)

CRD of Asialoglycoprotein Receptor

M. Meier, M.D. Bider, V.N. Malashkevich, M. Spiess, P. Burkhard
J. Mol. Biol., 300 (2000), p. 857







Meier *et al., J. Mol. Biol.;* 300:857-65 (2000)

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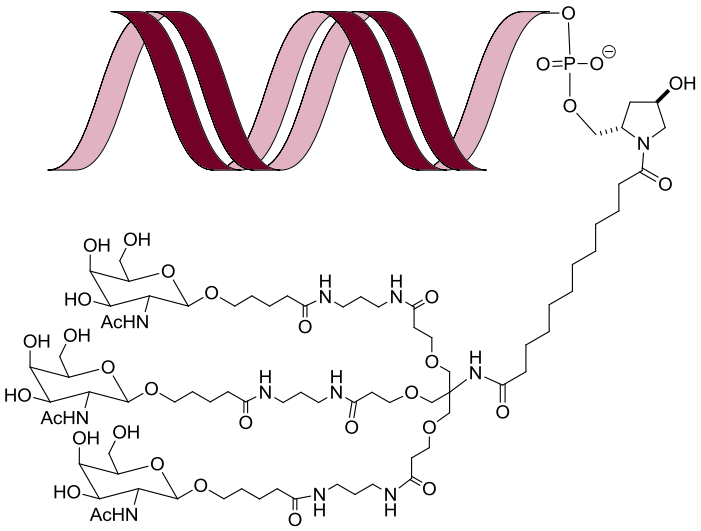
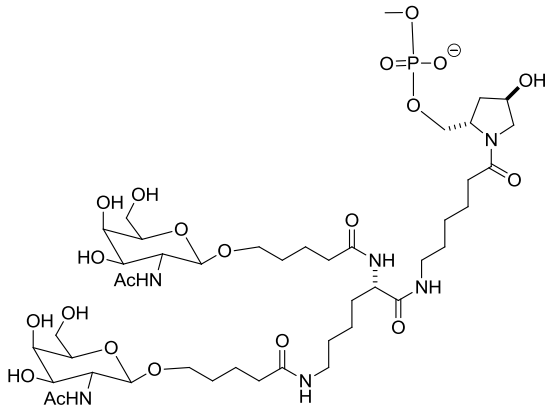
GalNAc-siRNA Conjugate Design

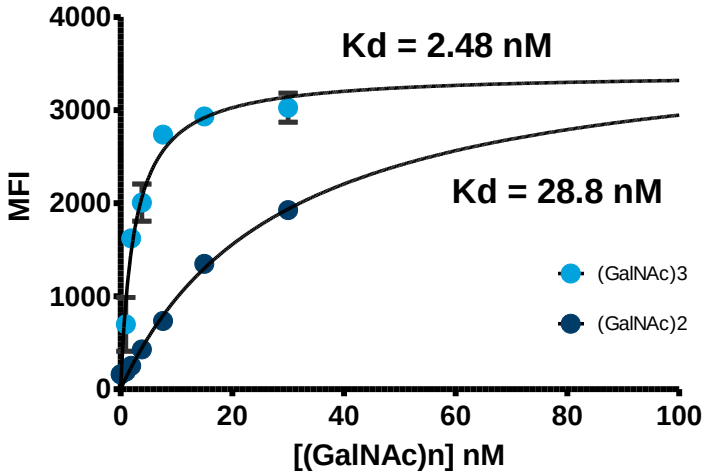
Valency Affects Binding Affinity



$GalNAc_3$ (G3)



$GalNAc_2$ (G2)

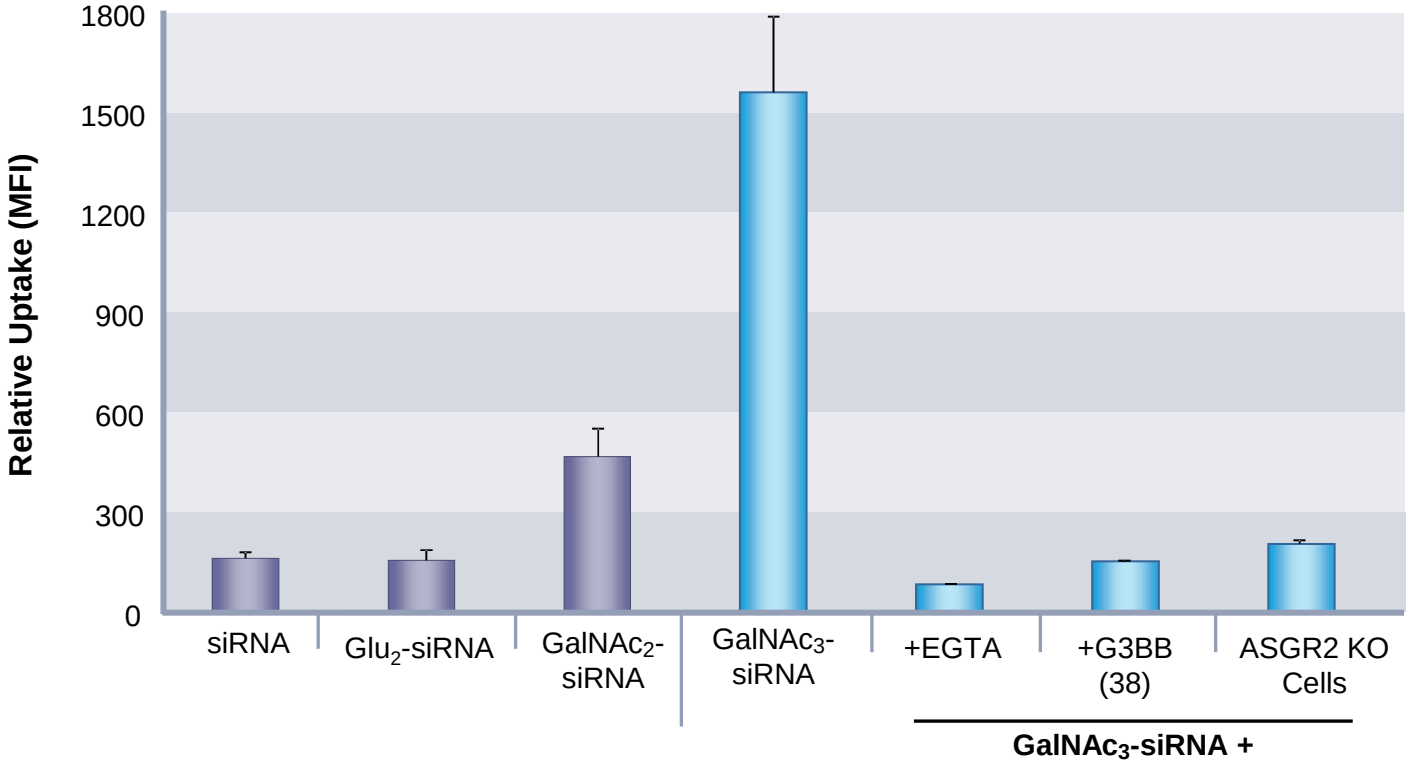


Ligand	Mouse Hepatocyte Ki
$GalNAc_2$	~24 nM
$GalNAc_3$	2.7 nM

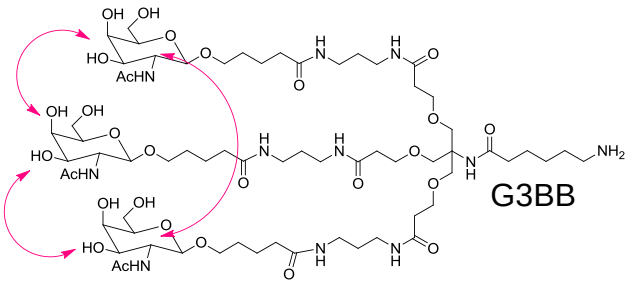
Rensen *et al., J. Biol Chem* 276:37577-84 (2001)
Biessen *et al., Bioconjugate Chem.; 13:* 295-302 (2002)

Uptake of GalNAc-siRNA Conjugates

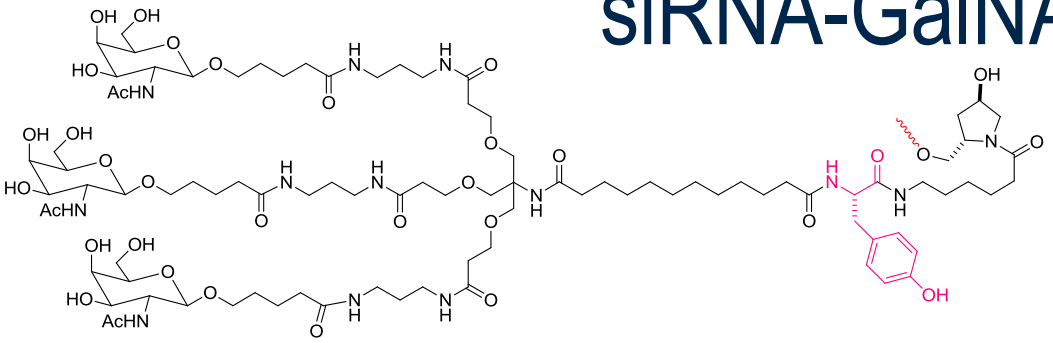
1º Mouse Hepatocytes

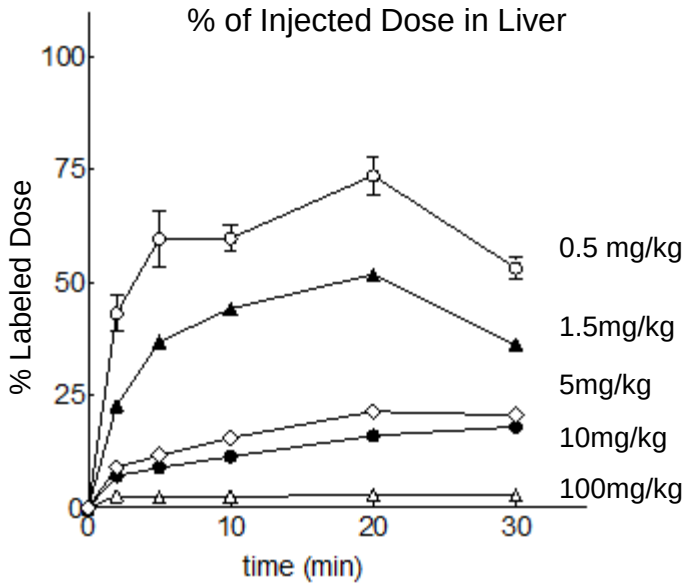


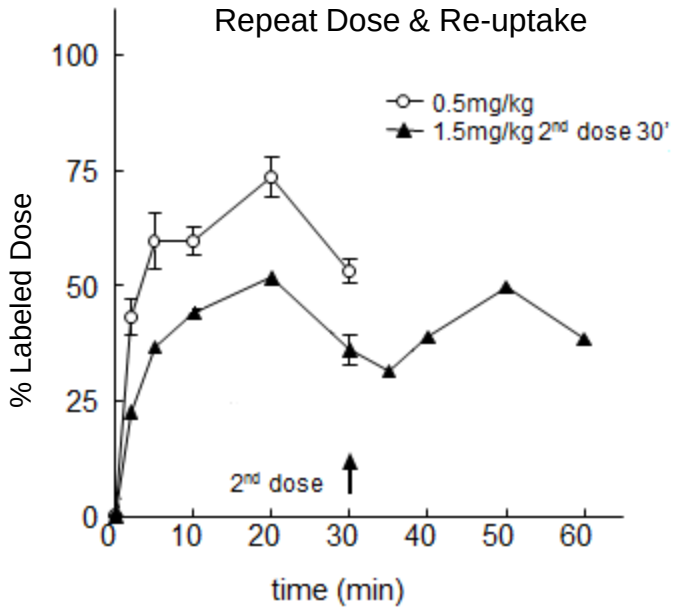
- Glucose conjugate does not mediate uptake
- $GalNAc_3$ BB & EGTA block uptake
- No uptake for ASGR2 KO Cells



IV Administration of ^{125}I labeled siRNA-GalNAc Conjugate







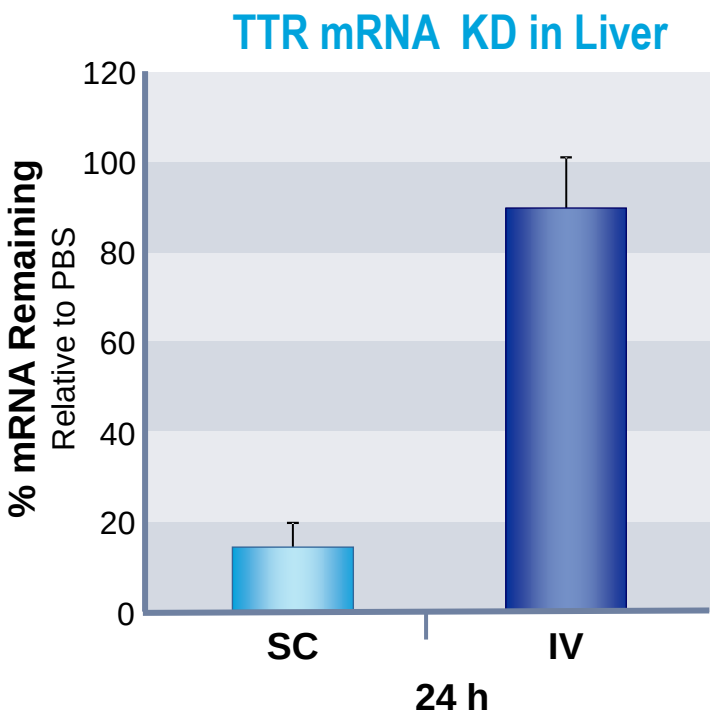
Fast uptake, saturation and recycling point towards optimizing dosing regimen

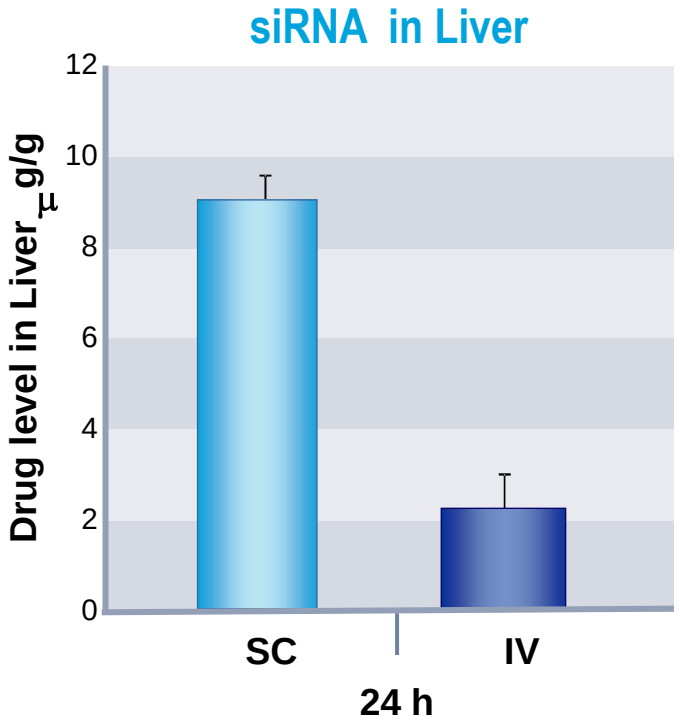
- Delayed release via SC dosing may help by engaging multiple rounds of receptor uptake
- Multi-dose SC administration as one approach to optimize receptor utilization

Collaboration with Dr Theo van Berkel

Mode of Administration Impacts Pharmacology

IV vs. SC Comparison of Efficacy and Drug Level in Mouse Liver





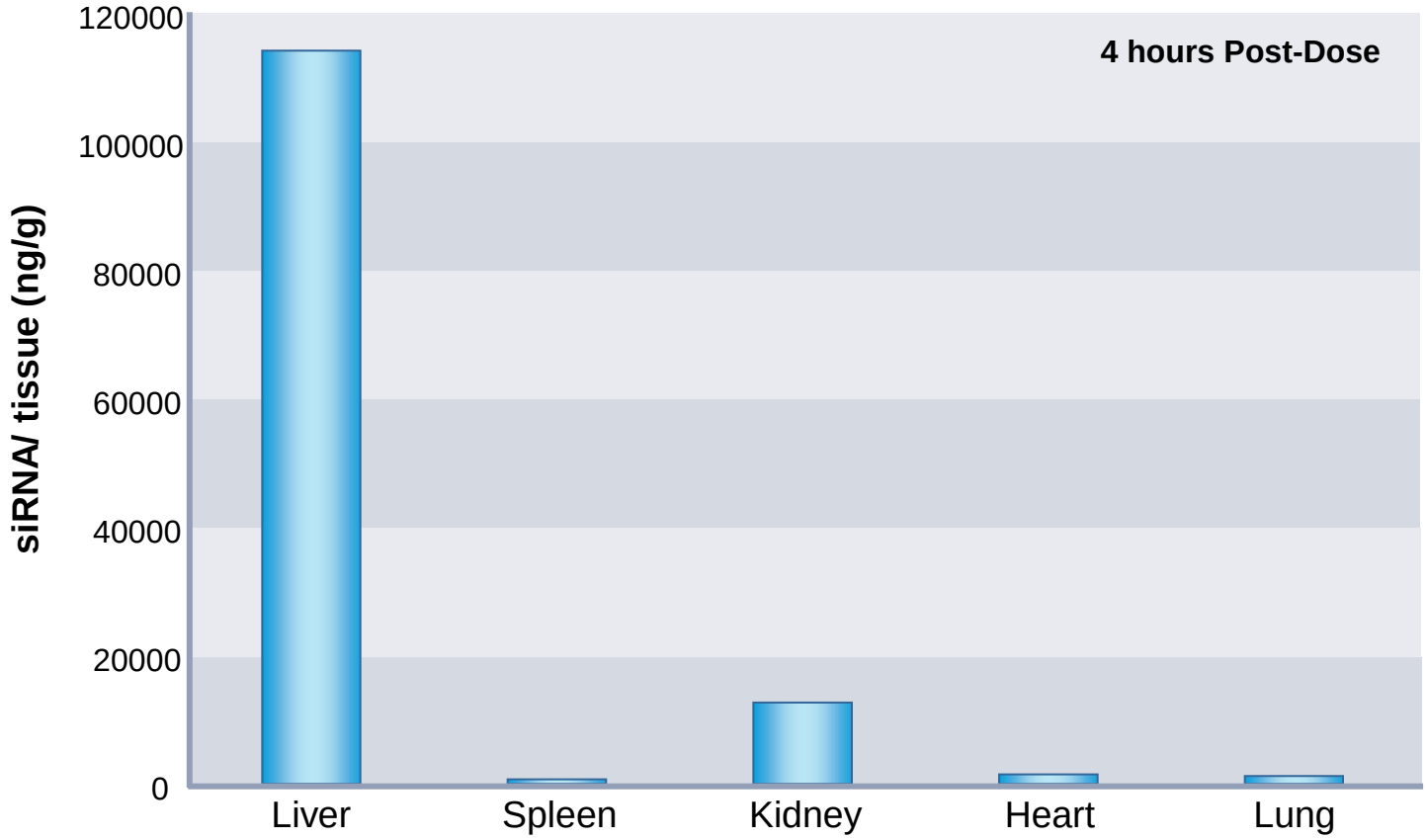
Oligo Ther Soc., Sept. 2012

Alnylam PHARMACEUTICALS

TTR-GalNAc siRNA Biodistribution
SC administration

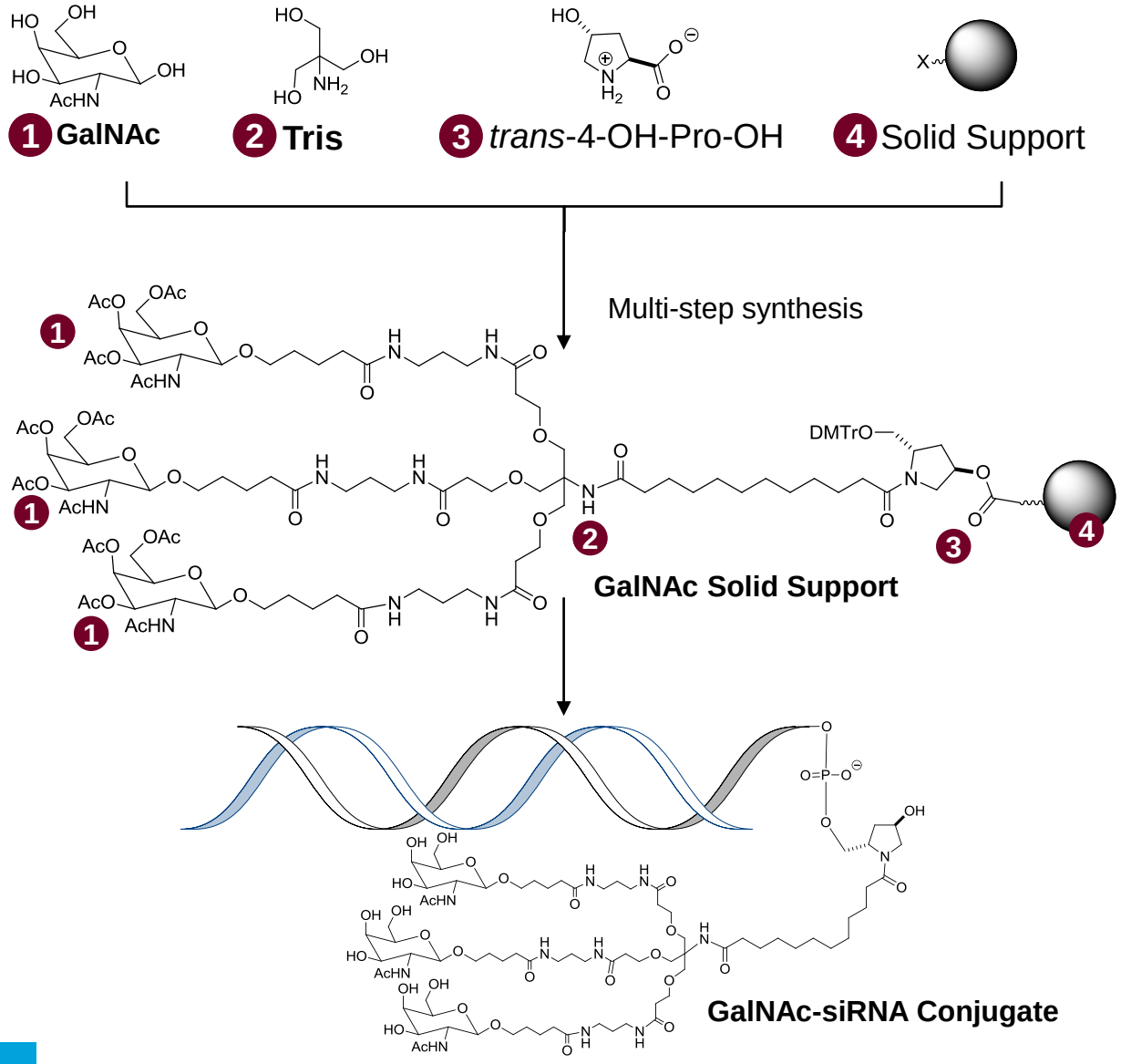
GalNAc-siRNA conjugates efficiently target liver

- Achieve liver levels of >50% delivered dose



Oligo Ther Soc., Sept. 2012

Synthesis of GalNAc Support and Conjugate



Synthesis/Process

- Developed at Alnylam
- Compatible with multiple solid supports
- Compatible with solid phase RNA synthesis and deprotection conditions

GalNAc Solid Support

- Convergent Multi-Step synthesis
- Scalable to multi-kilo

siRNA-GalNAc Synthesis

- Solid phase phosphoramidite chemistry
- Scalable to kilo scale

Alnylam PHARMACEUTICALS

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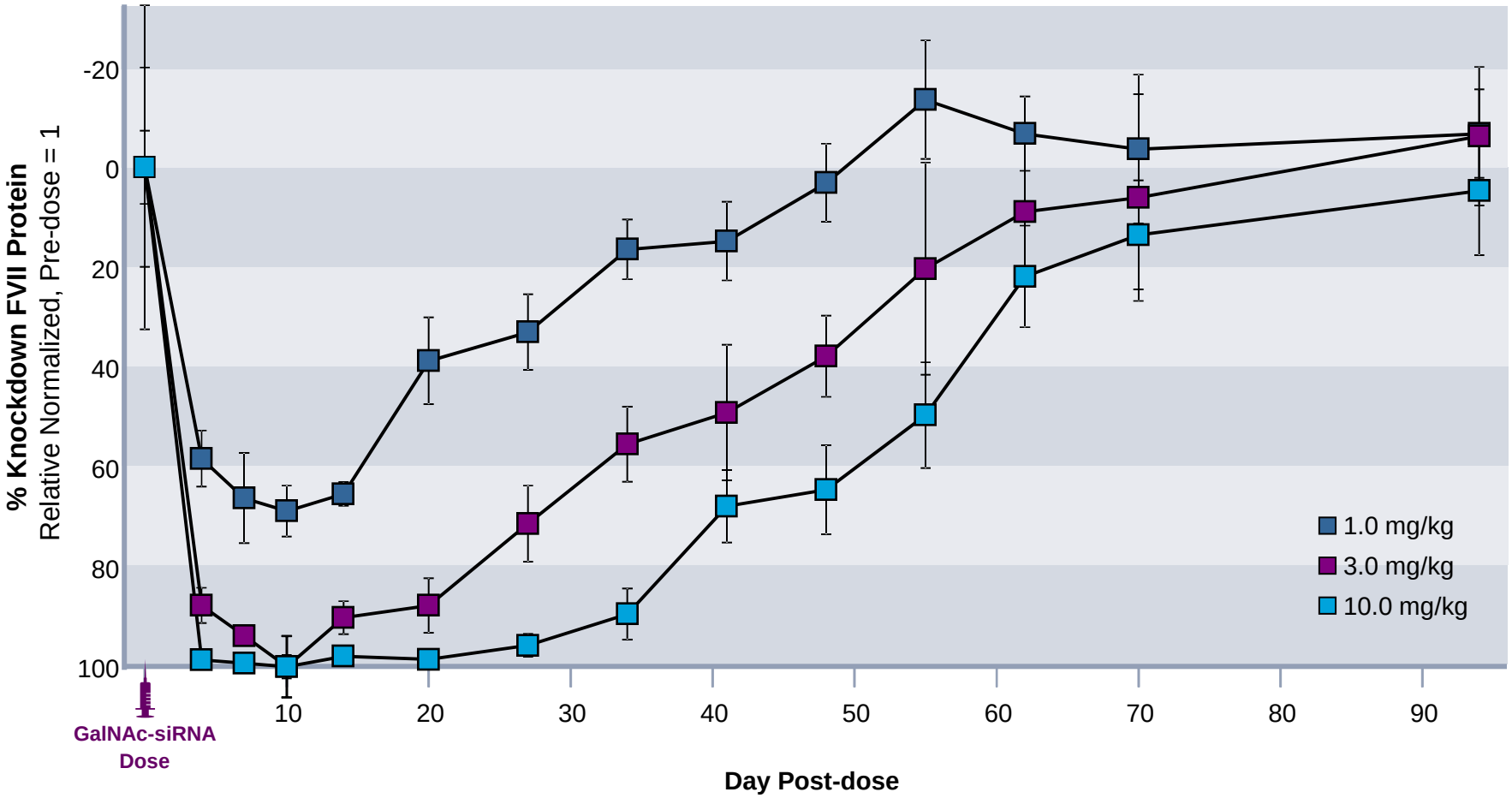
Pharmacology Translates Across Multiple Targets

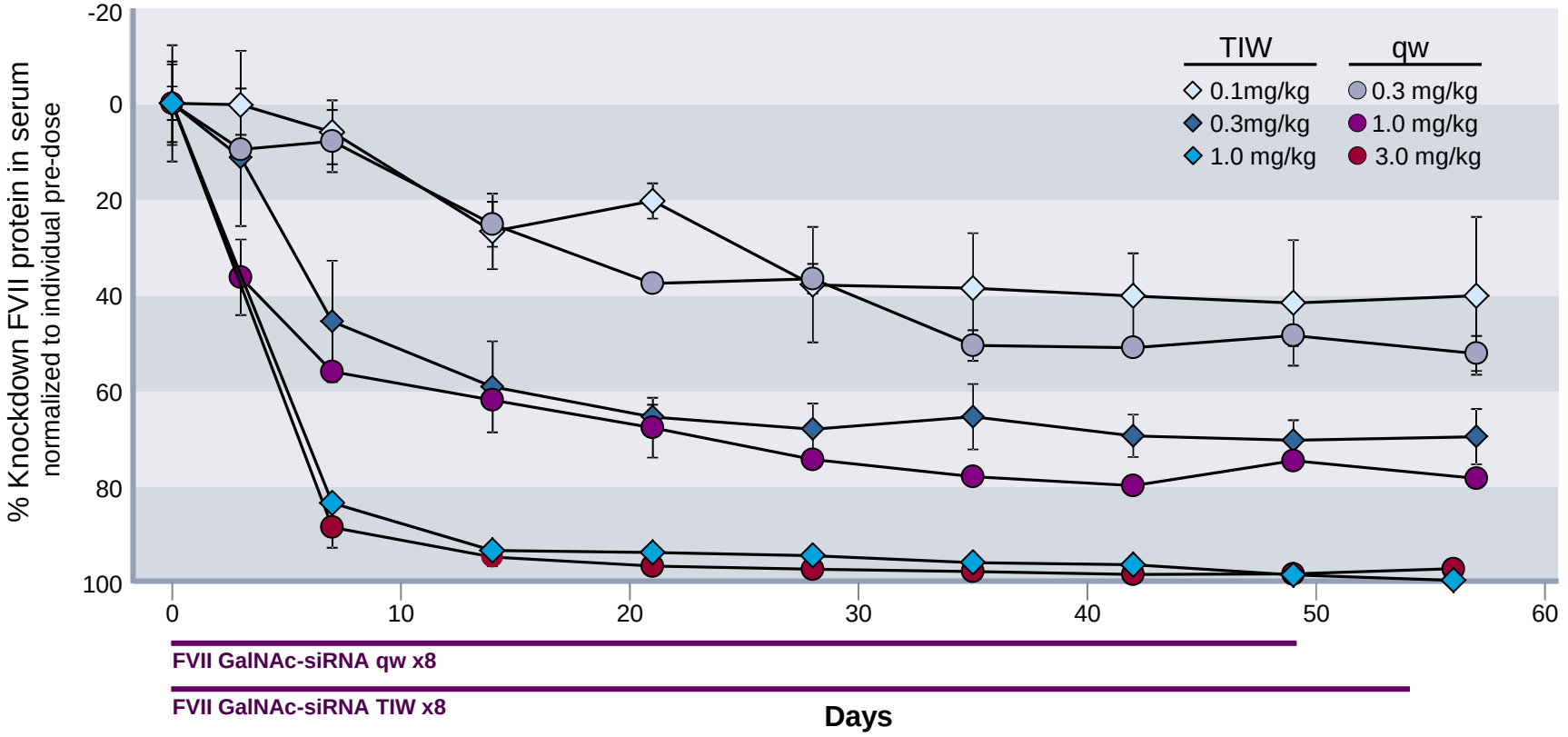
Example: Single-Dose FVII GalNAc-siRNA in Mice



Low Doses for Multi-Dose Efficacy

Example: FVII GalNAc-siRNA in Mice

- 40-50% reduction with cumulative weekly dose of 0.3 mg/kg (0.1 mg/kg TIW or 0.3 mg/kg qw)
- 70-80% knockdown with cumulative weekly dose of ~1 mg/kg
- >95% knockdown at cumulative weekly dose of 3 mg/kg

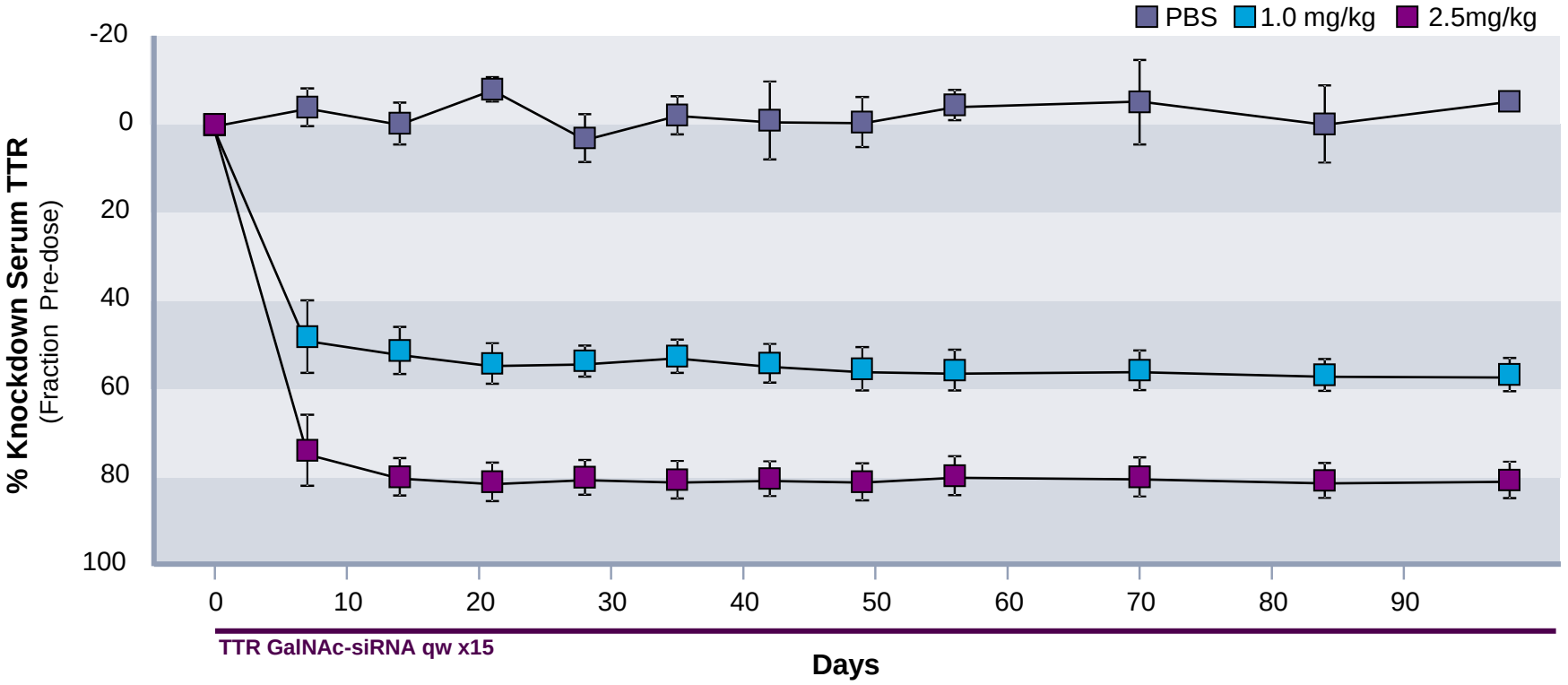


Chronic-Dose Efficacy

Example: TTR GalNAc-siRNA Ongoing Long-term Study in Mice

Steady duration of knockdown is maintained with chronic dosing

- Sustained ED50~1mg/kg and ED80 ~2.5mg/kg
  - » Rodent orthologue with improved potency compared with ALN-TTRsc
- No changes in serum TTR levels observed in PBS control group
- Demonstrate absence of tachyphylaxis or sensitization due to Changes in ASGPR level function/expression, Ab production, other factors

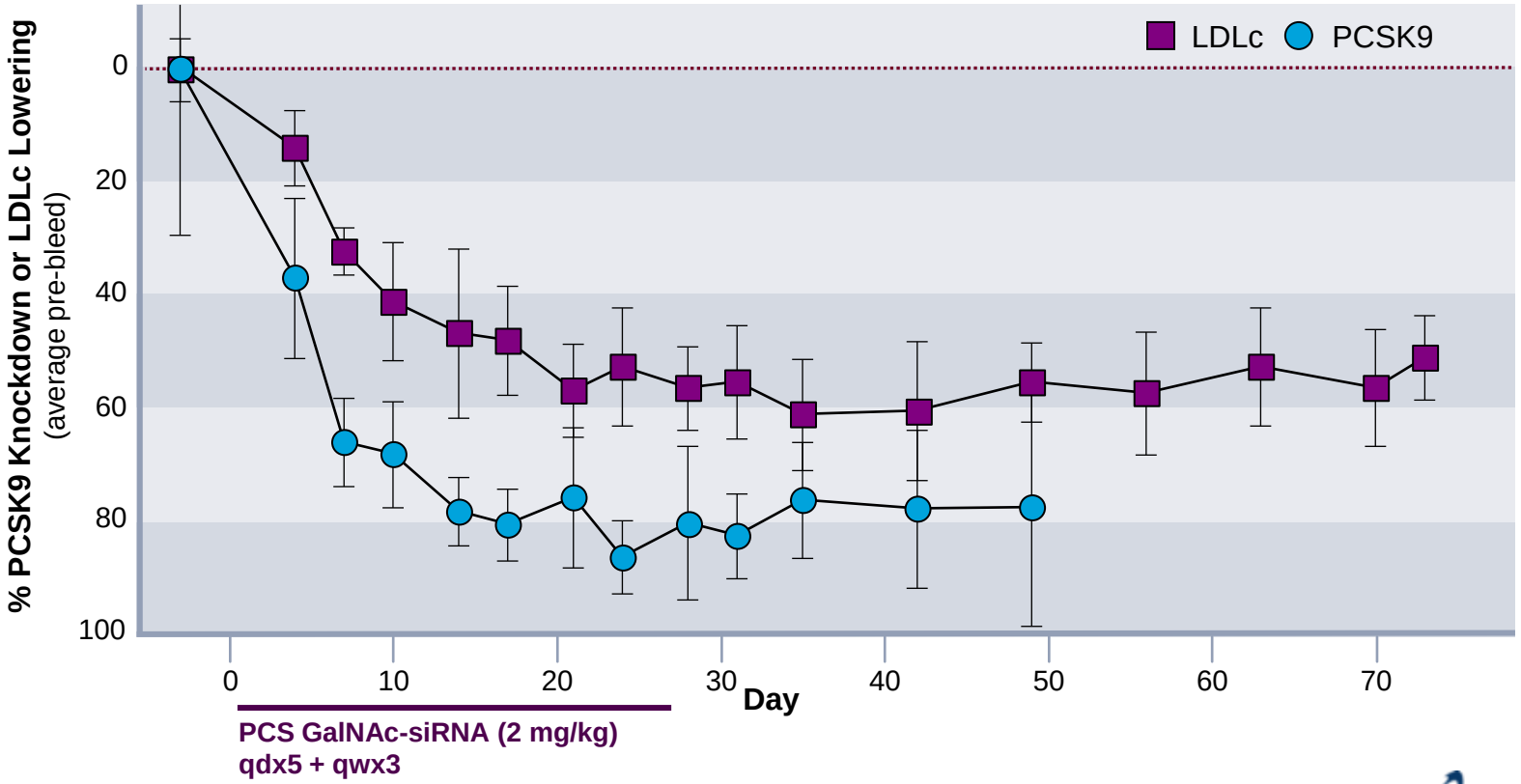


Pre-Clinical Efficacy

Example: PCSK9 GalNAc-siRNA in NHP

PCSK9 GalNAc-siRNA achieves potent PCSK9 knockdown and LDLc lowering with SC dosing

- NHP data with current lead molecule
  - » Expect Development Candidate in late 2013
- Up to 90% PCSK9 knockdown and up to 68% lowering of LDLc in absence of statins in NHP
- Goal: Competitive profile against anti-PCSK9 Mabs



Topics to Cover

- Alnylam GalNAc-siRNA Conjugate Platform
  - » GalNAc-siRNA conjugates for subcutaneous administration
  - » Valency, Structure and Receptor Binding
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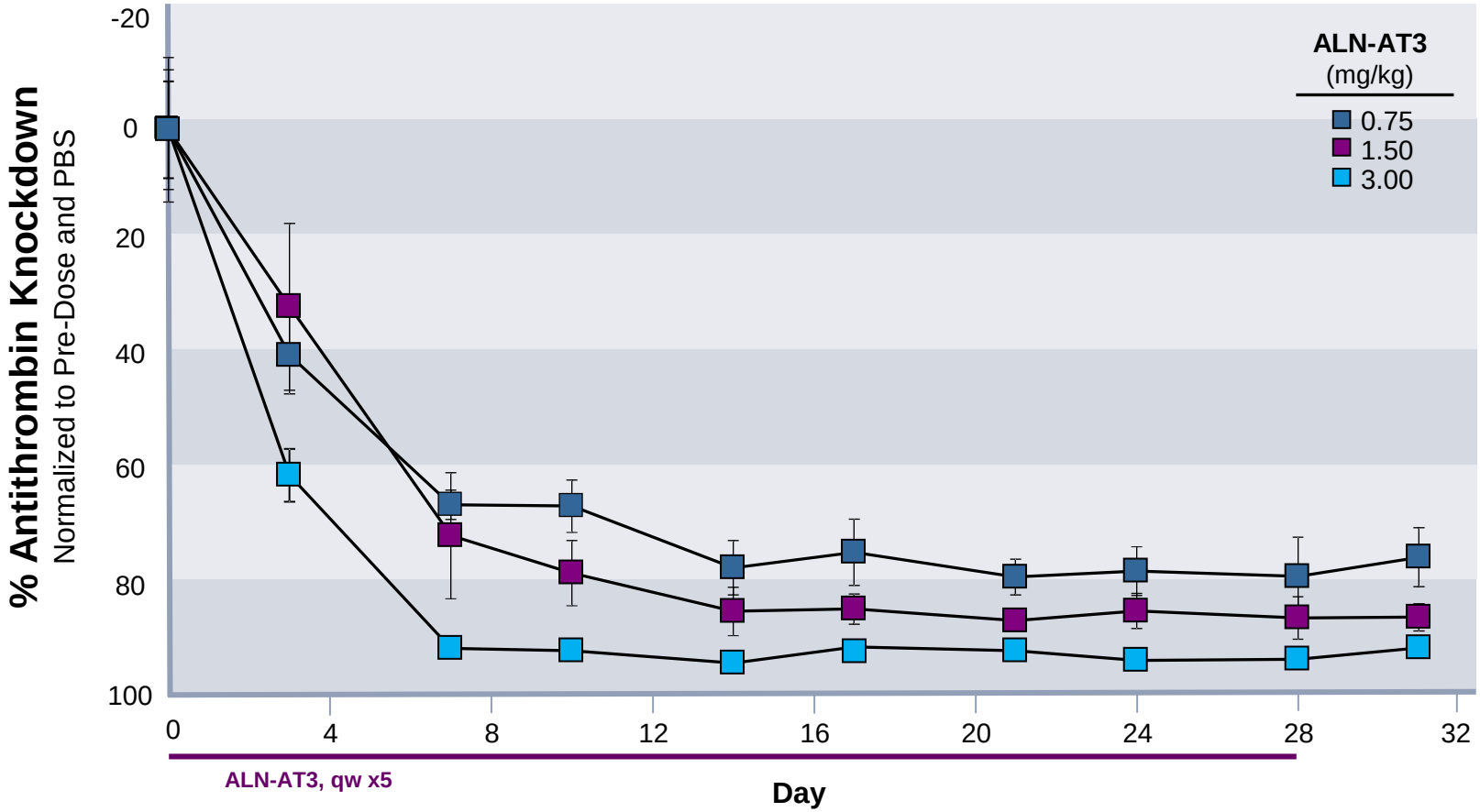
PK/PD Correlation

ALN-AT3 Pre-clinical Studies in Wild Type Mice

Weekly SC administration of ALN-AT3 results in potent and consistent AT suppression

- Repeat dose ED_{50} for AT knockdown <0.75 mg/kg
- Nadir knockdown at ~day 14



PK/PD Study Design

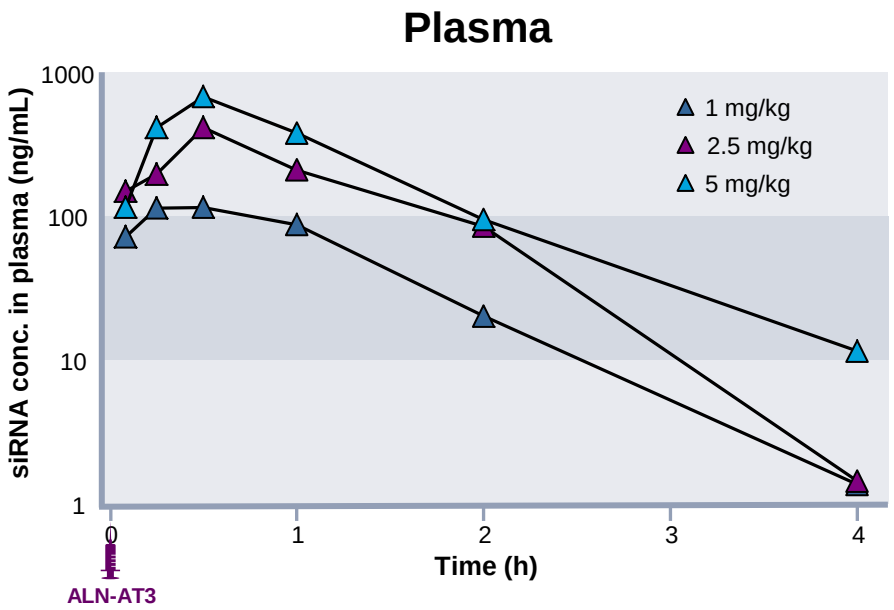
ALN-AT3 Pre-clinical Studies in Wild Type Mice

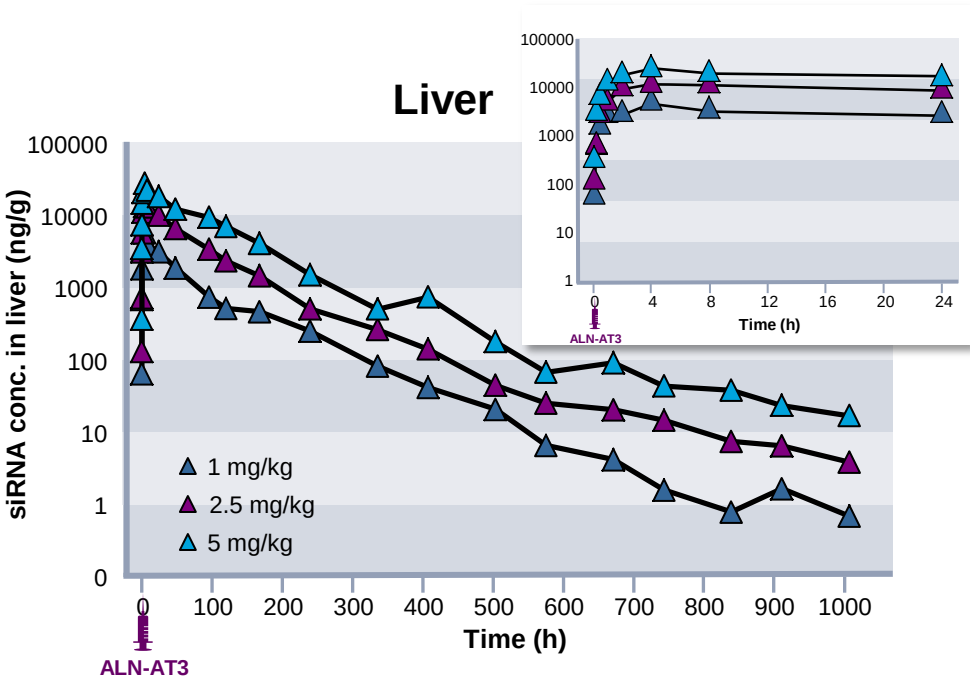
Group	siRNA	Dose (mg/kg)	Conc. (mg/mL)	Route	No. of Males	Blood and Tissues Collection Time Points
1	AT3	1	0.1	SC	44	0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 96, 120, 168, 240, 336, 408, 504, 576, 672, 744, 840, 912 and 1008 h post-dose
2	AT3	2.5	0.25	SC	44	
3	AT3	5	0.5	SC	44	

- Male C57BL/6 mice (20-30 grams)
- Organs: Liver, spleen, kidney, blood (n=2 /time point/group) at specified time points.
- qPCR assay - LLOQ Plasma = 0.004 ng/mL & Liver = 0.4 ng/g
- RISC-loaded siRNA levels were determined by Ago2 IP followed by RT-qPCR
- Plasma and liver samples at each time point were analyzed for AT protein in Plasma and AT mRNA in liver

PK Profiles in Plasma and Liver

ALN-AT3 Pre-clinical Studies





AT3 Conjugate in Plasma			
Dose (mg/kg)	1.0	2.5	5.0
Apparent t1/2β (h)	0.46	0.38	0.61
Tmax (h)	0.5	0.5	0.5
Cmax (µg/g)	0.117	0.420	0.686
AUC0-t (h·µg/g)	0.176	0.507	0.804

AT3 Conjugate in Liver			
Dose (mg/kg)	1.0	2.5	5.0
Apparent $t_{1/2\beta}$ (h)	112	126	126
Tmax (h)	4	4	4
Cmax (µg/g)	5.75	13.2	27.9
$AUC_{0\text{-}t}$ (h·µg/g)	302	1015	2274

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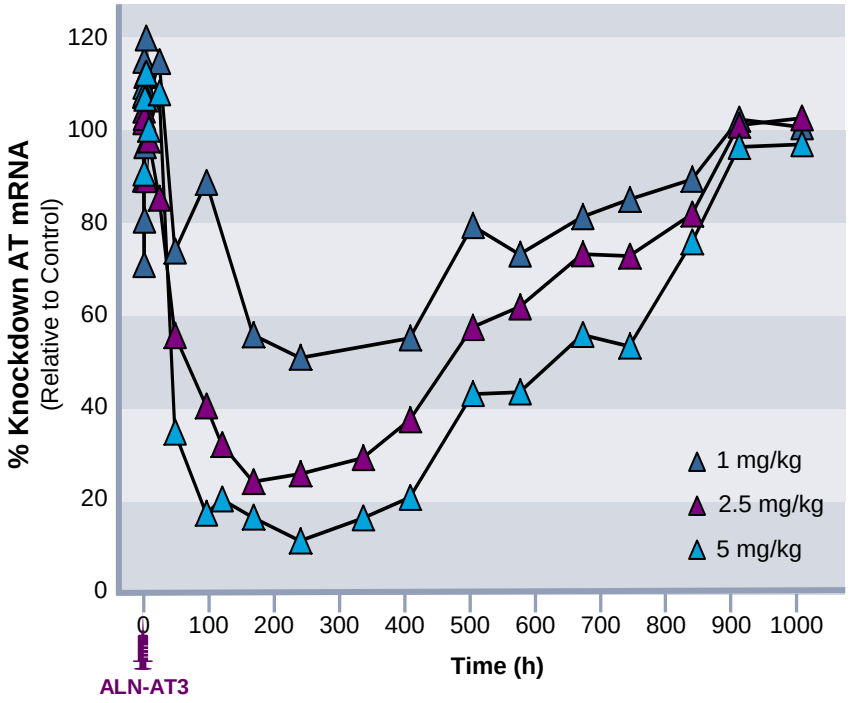
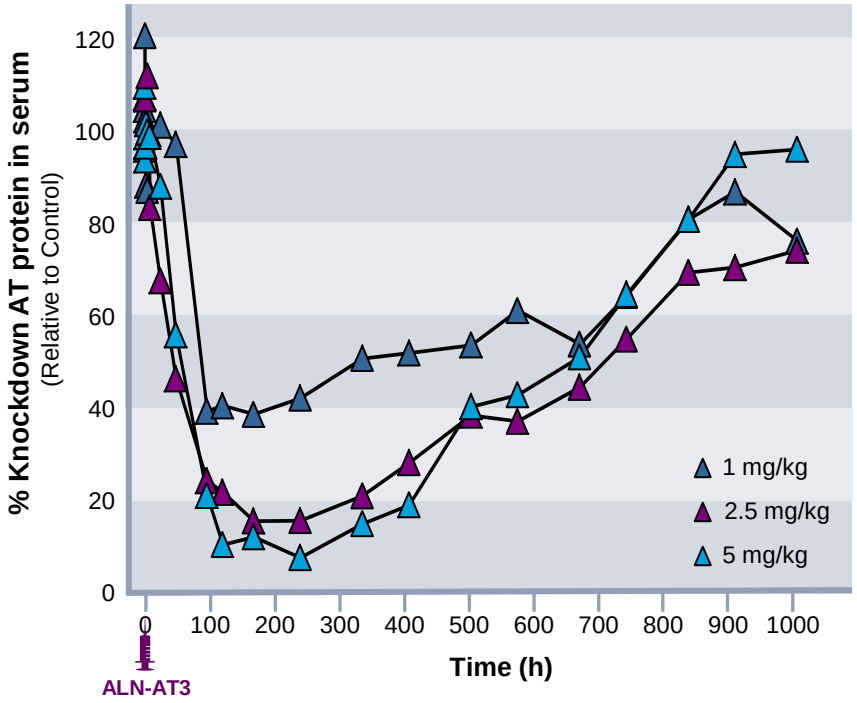
PD Profiles

ALN-AT3 Pre-clinical Studies

Silencing of AT mRNA



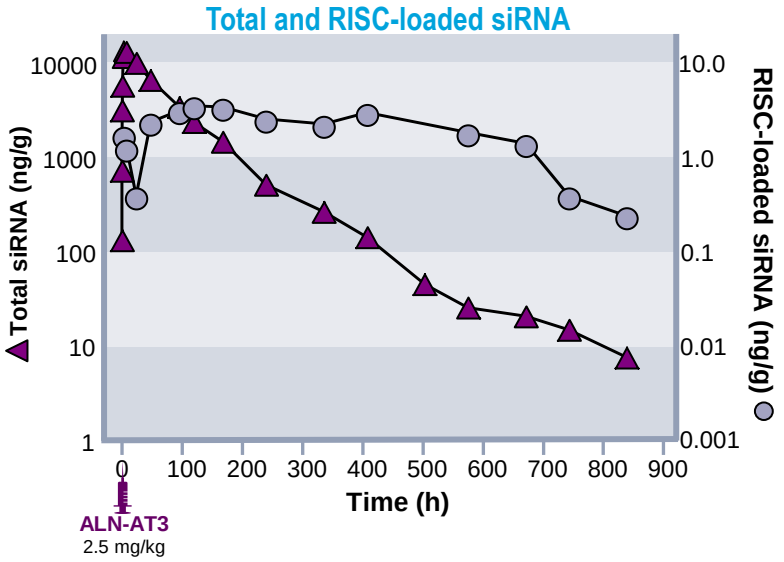
Reduction of AT Protein

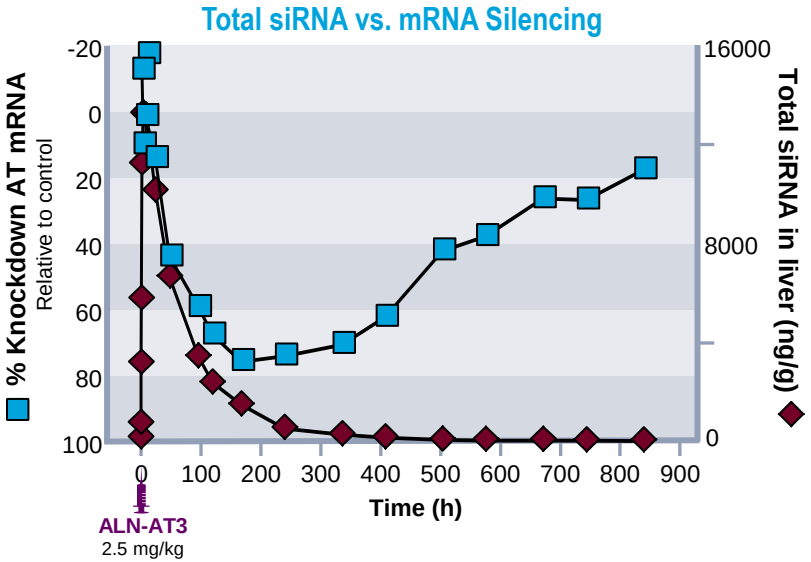


- Comparable PD profiles for AT mRNA and plasma protein after single s.c. administration of ALN-AT3

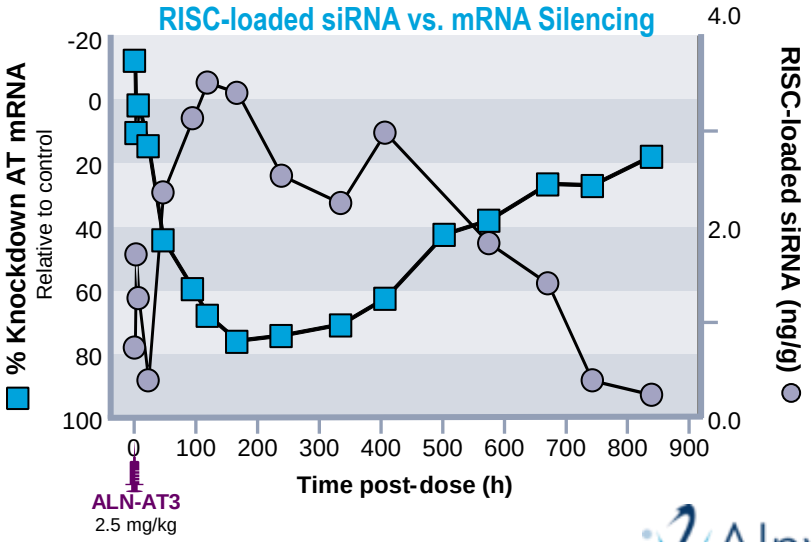
Total vs. RISC-loaded siRNA

ALN-AT3 Pre-clinical Studies





- Tmax of RISC-loaded siRNA shifted relative to total siRNA
- Rate of depletion of RISC-loaded siRNA slower than total siRNA
  - » May be due to enhanced metabolic stability of RISC-loaded compared to free siRNA and further replenishment of the drug from liver
- Amount of RISC-loaded siRNA correlates well with silencing activity

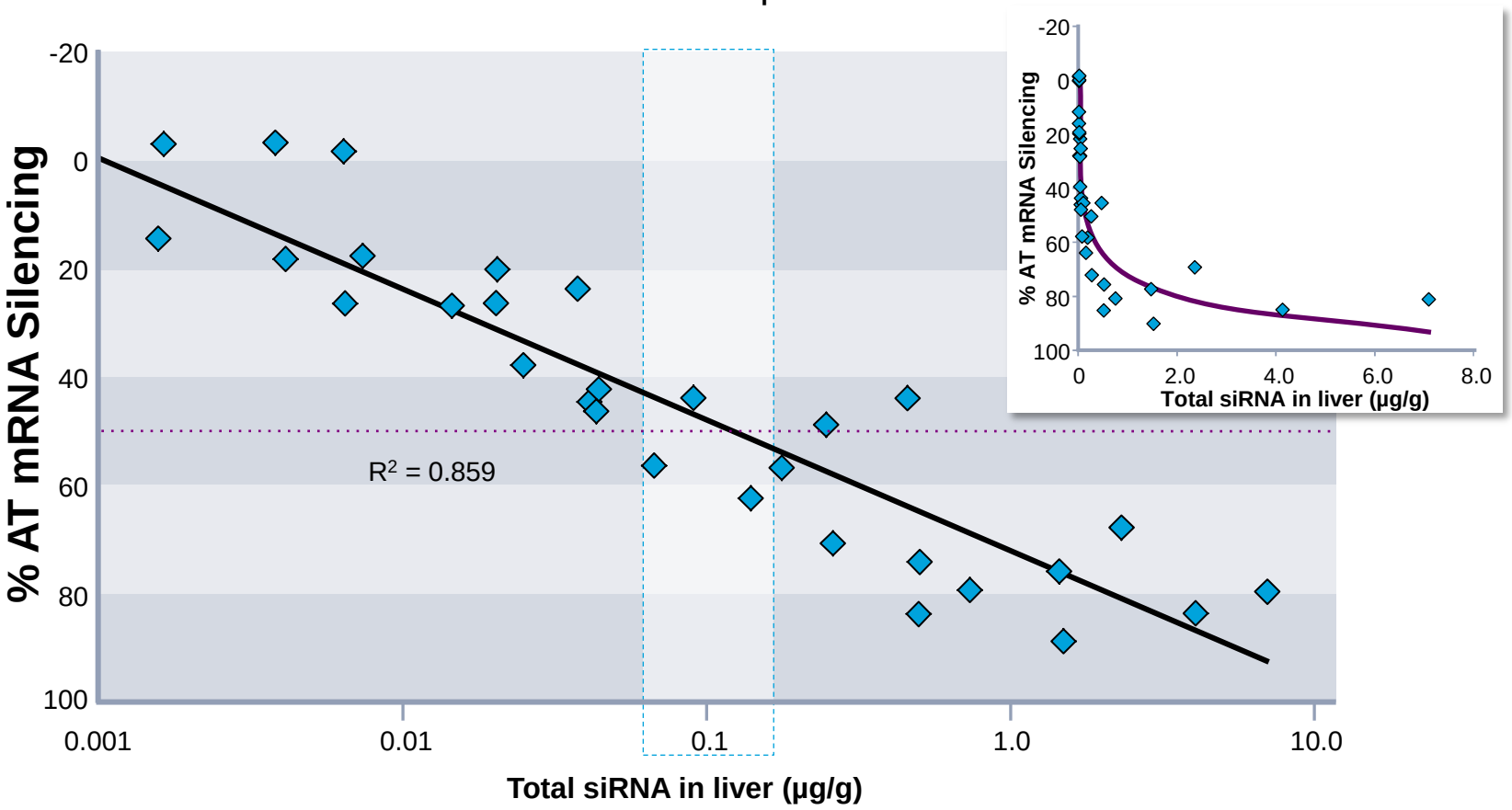


PK/PD Relationship

ALN-AT3 in Liver

Target gene silencing achieved at low liver tissue exposure

- EC50 achieved at ~0.1 μg/g tissue
  - » Compares very favorably with other oligonucleotide platforms requiring >100 μg/g tissue levels[1]
- Renal excretion and no accumulation in extra-hepatic tissues



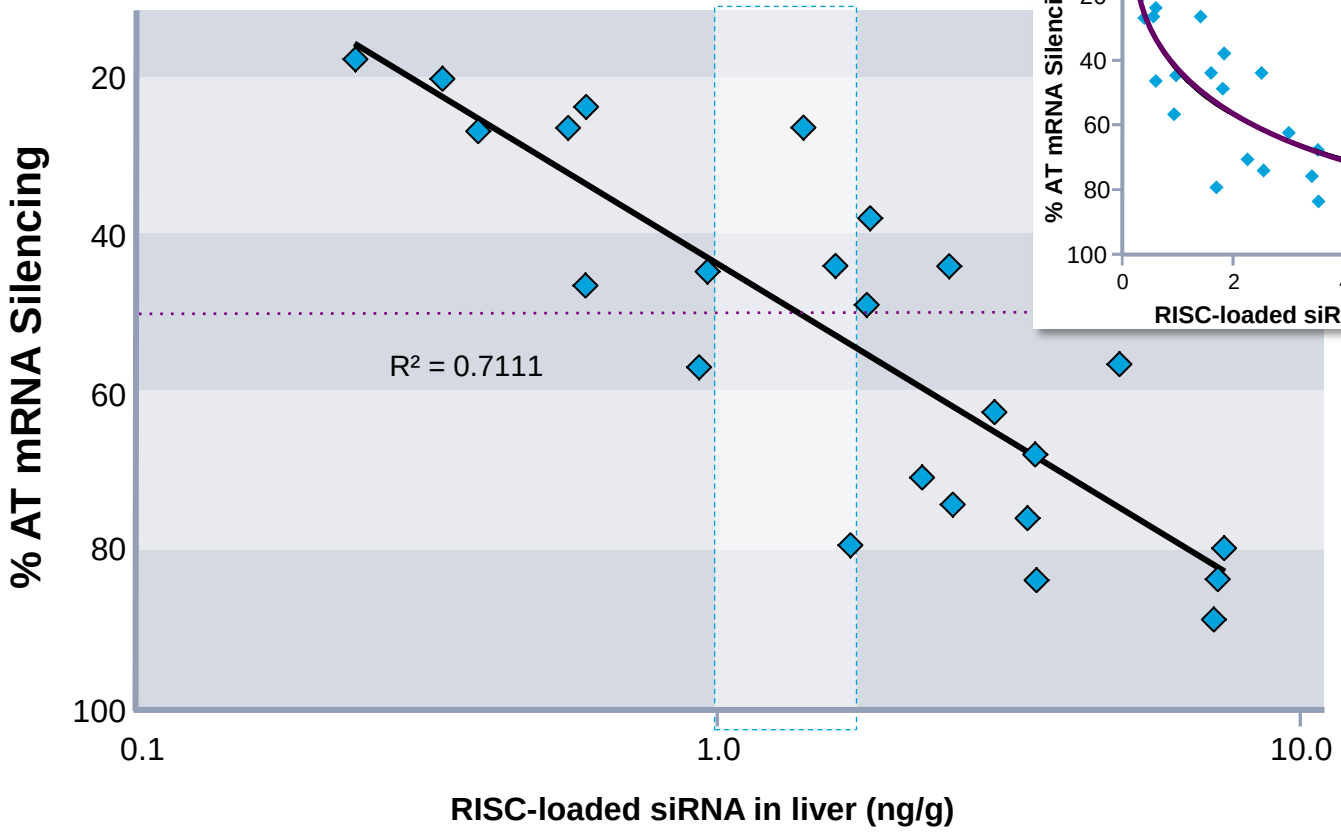
[1] *Mipomersen FDA Advisory Committee Briefing Document*, October 2012

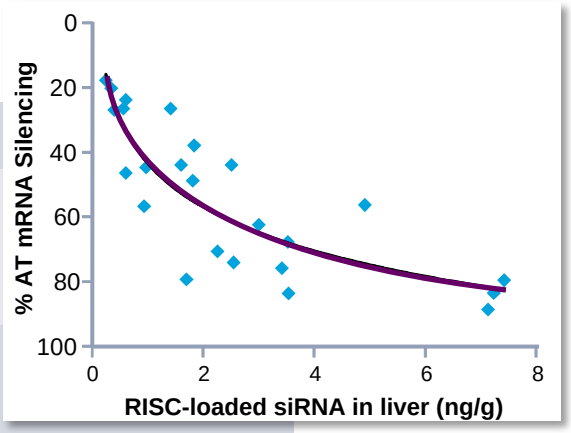
PK/PD Relationship

ALN-AT3 in RISC

Target gene silencing achieved at very low RISC-loaded siRNA concentrations

- EC50 achieved at ~1.5 ng/g tissue (~800 molecules/cell)
  - » ~100x lower than EC50 values obtained for total siRNA





GalNAc-siRNA Conjugates
Wide Therapeutic Index

Cytokine/Complement Assessment

- No evidence of inflammation (cytokine, complement) *in vitro* or *in vivo,* including NHP

GLP Toxicology Study Results

- Rat doses up to 300 mg/kg (10 doses)
  - » No in-life findings
  - » No injection site reactions
  - » No significant LFT changes
- NHP doses up to 300 mg/kg (10 doses)
  - » No in-life findings
  - » No injection site reactions
  - » No clin path or histopath findings
  - » Likely predictive species: No Adverse Event Level >300 mg/kg

Topics to Cover

- Alnylam GalNAc-siRNA Conjugate Platform
  - » GalNAc-siRNA conjugates for subcutaneous administration
  - » Valency, Structure and Receptor Binding
- Pharmacology of GalNAc-siRNA Conjugates
- PK/PD of GalNAc-siRNA Conjugates
- Summary

GalNAc-siRNA Conjugates

Broad Platform for SC Delivery of RNAi Therapeutics

- Potent, rapid, dose-dependent, consistent, and durable target knockdown with SC administration with wide therapeutic index
- Broad platform for any hepatocyte target gene utilizing efficient receptor-ligand system
- Rational design and efficient synthesis of trivalent GalNAc building block achieved
  - » Enabled successful manufacture of large scale siRNA-conjugate with simple CMC
- Combining high capacity receptor recycling with SC injection enables convenient multi-dosing paradigm
- Chronic dosing demonstrate steady-state knockdown without tachyphylaxis or sensitization due to changes in ASGPR level, Ab production, and other factors
- Dose proportionality for AUC and Cmax in plasma and liver exists
- Good correlation for mRNA and protein reduction as a function of RISC-associated as well as total siRNA for later time points (≥120 h post-dose)
- EC50 for RISC-loaded conjugate is ca. 1.6 ng/g (~800 molecules/cell) and estimated
  EC50 for total conjugate in liver is ca. 0.1 ug/g
  - » EC50 for GaNAc-siRNA is >1000-fold improvement over ASO