Nonclinical Safety Evaluation of GalNAc-siRNA Conjugates

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Alnylam® PHARMACEUTICALS

Annual Biologics Symposium
May 9th, 2018

Presentation Topics

- **Investigational RNAi Therapeutics**
  - Alnylam Pipeline
  - Summary of points to consider in toxicology assessments

- **Summary of Key Toxicology Profiles**
  - PK/PD considerations
  - Repeat-dose toxicity: target organs & pathologic effects; dose response and exposure relationships
  - Chronic toxicity
  - Exposure vs. efficacy relationship
  - Genotoxicity
  - Reproductive and developmental toxicity
  - Juvenile animal toxicity
  - Cardiovascular safety
  - Injection site reactions
  - Cytokine responses, complement effects, and immunogenicity
  - Carcinogenicity
  - Clinical translation

Alnylam Clinical Development Pipeline

Focused in 3 Strategic Therapeutic Areas (STArs):

- Genetic Medicines
- Cardio-Metabolic Diseases
- Hepatic Infectious Diseases

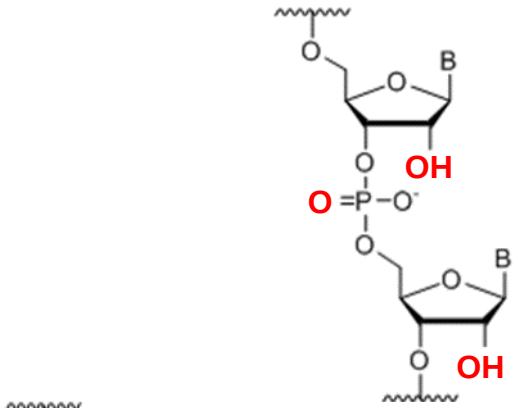
		HUMAN POC[1]	BREAKTHROUGH DESIGNATION	EARLY STAGE (IND or CTA Filed-Phase 2)	LATE STAGE (Phase 2-Phase 3)	REGISTRATION/ COMMERCIAL[2]	COMMERCIAL RIGHTS
Patisiran	*Hereditary ATTR Amyloidosis*	✓	✓			●	Global
Givosiran	*Acute Hepatic Porphyrias*	✓	✓		●		Global
Fitusiran	*Hemophilia and Rare Bleeding Disorders*	✓			●		15-30% Royalties
Inclisiran	*Hypercholesterolemia*	✓			●		Milestones & up to 20% Royalties
ALN-TTRsc02	*Hereditary ATTR Amyloidosis*	✓		●			Global
Lumasiran	*Primary Hyperoxaluria Type 1*	✓	✓	●			Global
Cemdisiran	*Complement-Mediated Diseases*	✓		●			Global

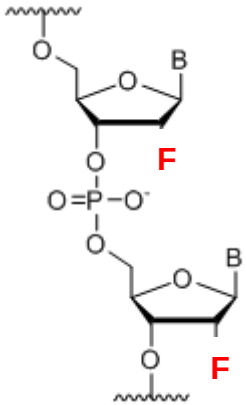
[1]POC, proof of concept – defined as having demonstrated target gene knockdown and/or additional evidence of activity in clinical studies
[2]Includes marketing application submissions

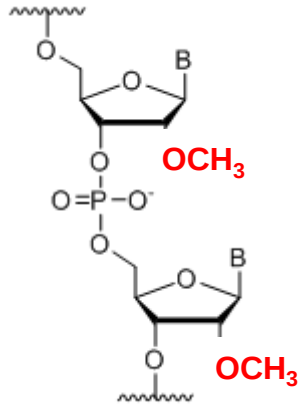
As of March 2018

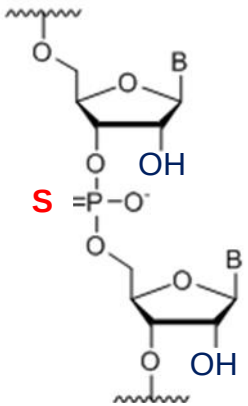
Challenges In Delivery Of siRNA Therapeutics

- **Large (15-17 kDa), net negative charge, unstable in serum**
- **Can be modified to promote active hepatocellular uptake**
  - Lipid nanoparticles
    - Require i.v. dosing
  - **Conjugation with ligand**
    - **Can be given s.c.**
- **Can be chemically-modified to increase stability in serum**
  - Block nuclease attack
    - 2’fluoro, 2’OMe, phosphorothioate linkages









Adapted from http://www.biosyn.com/oligonucleotideproduct/2-fluoro-rna-modification.aspx

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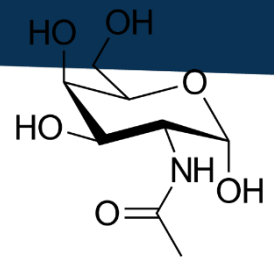
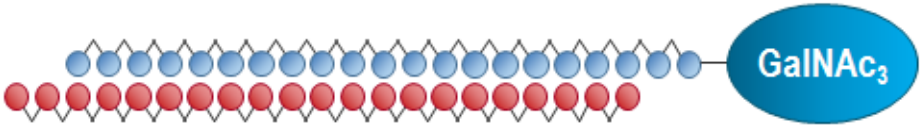
GalNAc-siRNA Conjugates

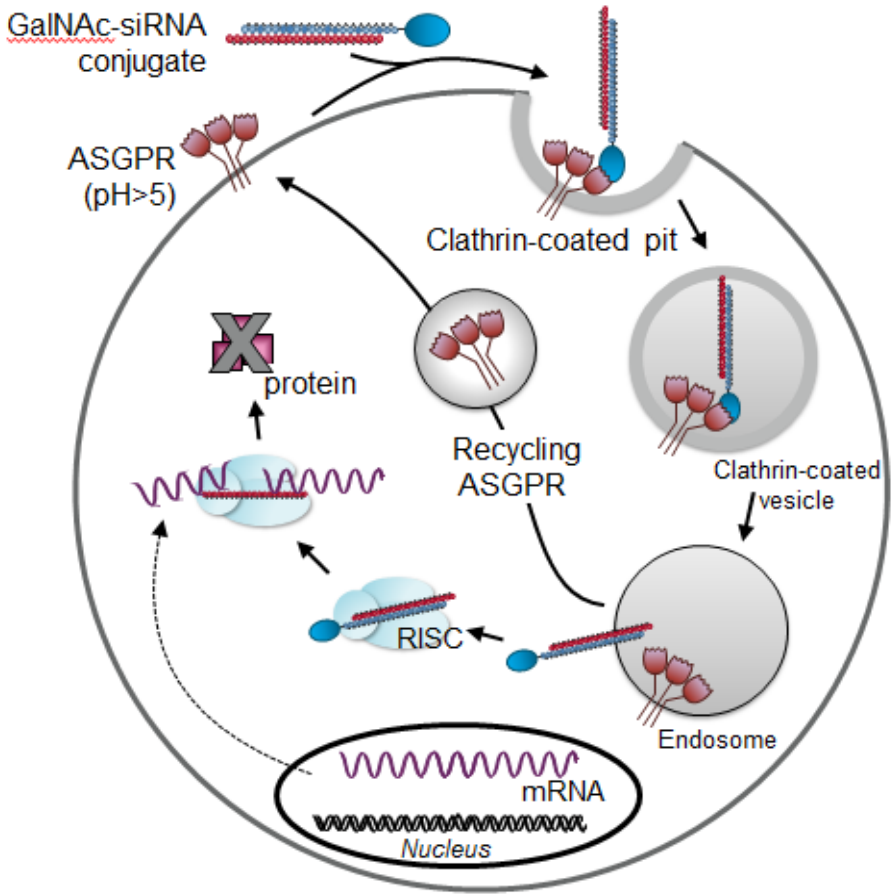
Preclinical considerations



N-acetylgalactosamine (GalNAc)

- Fate of sense and anti-sense strands
- Off-target toxicity
- Limited tissue distribution, targeted delivery to liver
- Short plasma half-life; long tissue half-life; long PD effects
- Metabolized by serum and tissue exo- and endo-nuclease digestion (stability, serum half-life)





Regulatory Challenges For RNAi Therapeutics

Preclinical considerations

Not a biologic….but not a small molecule!

Treated (more) like a small molecule by regulators

Separate regulatory guidance not in place for RNAi

Paving the way for future RNAi therapeutics to ensure timely delivery of these medications to patients!!!

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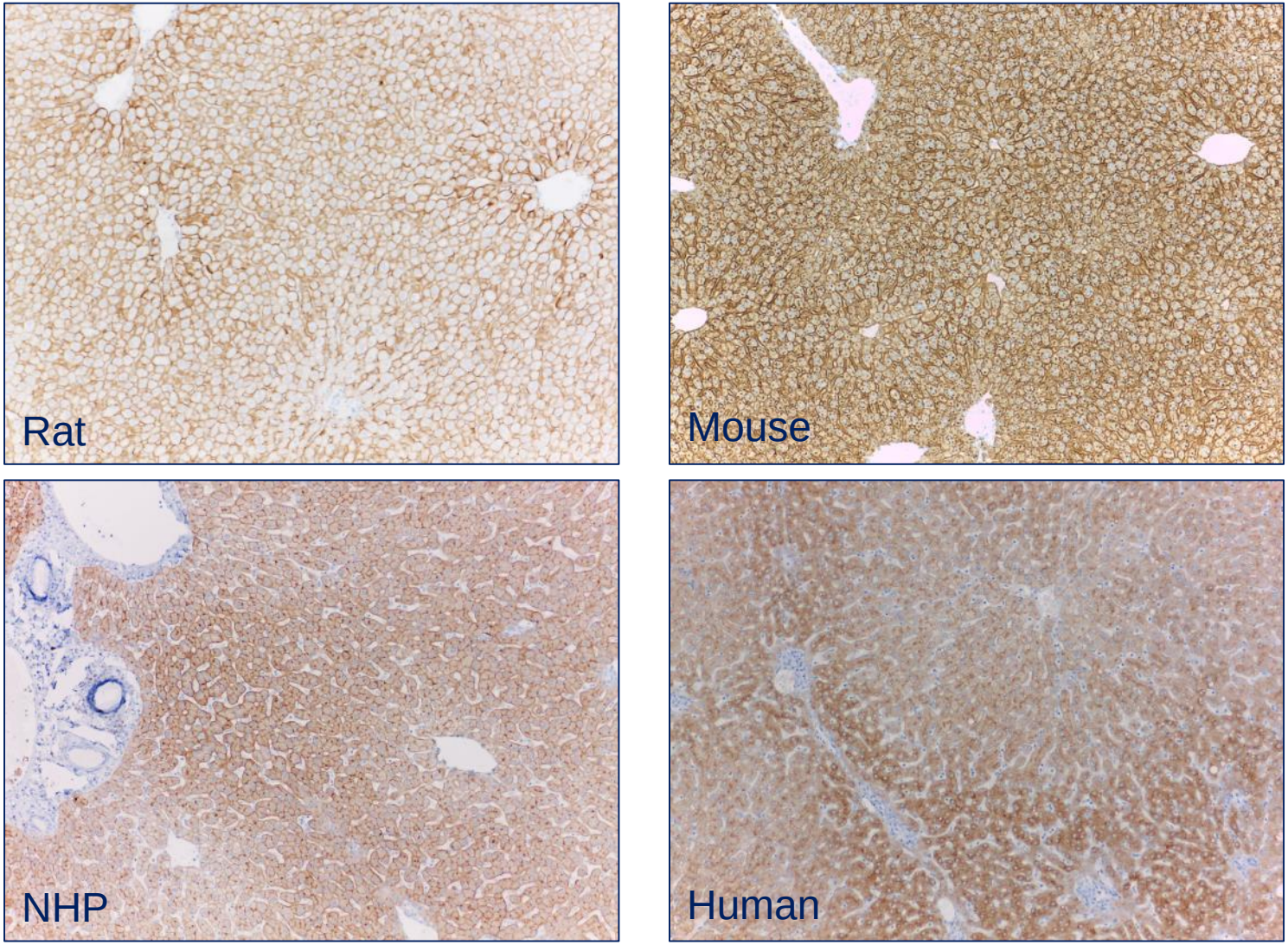
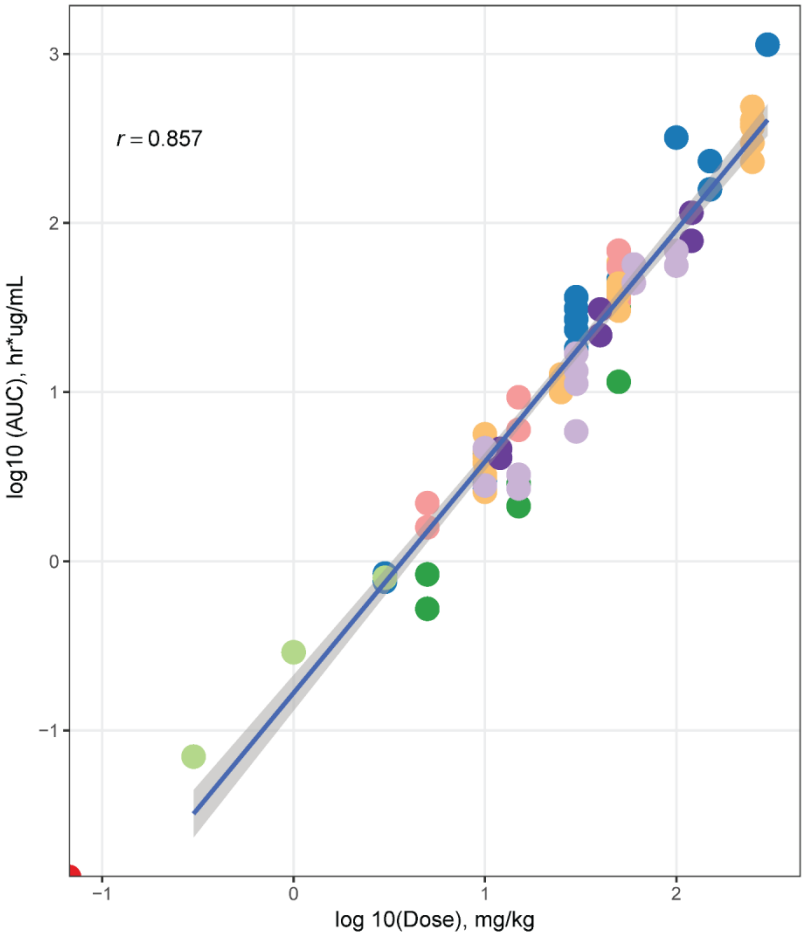
Liver: Distribution of ASGPR Expression Across Species

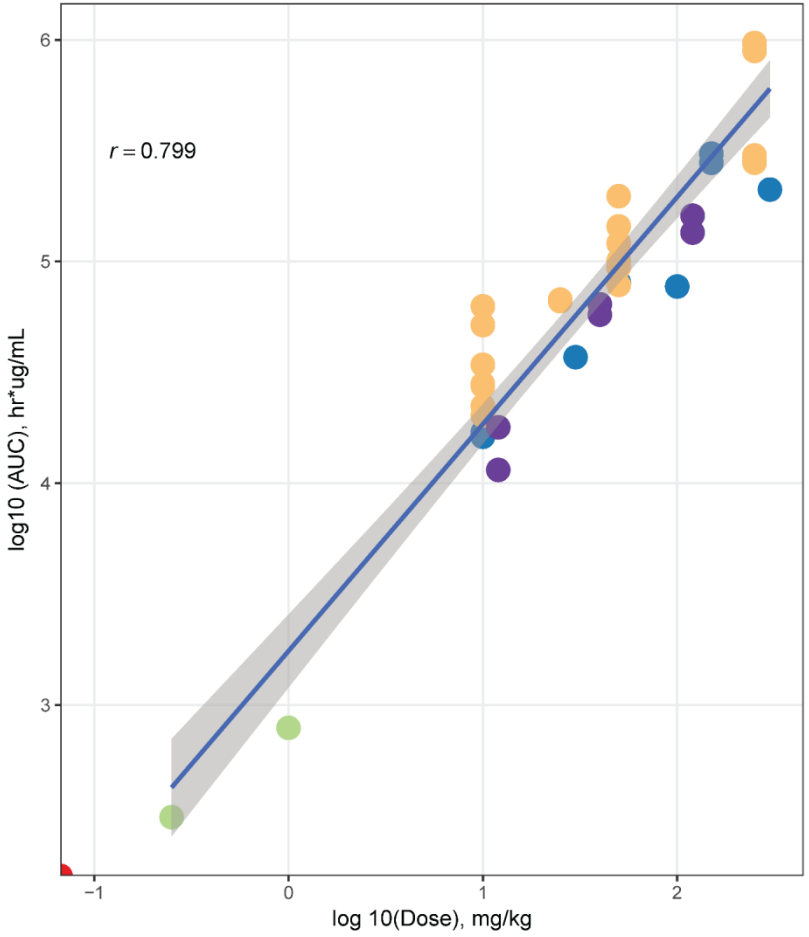


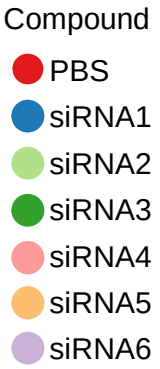
Consistent Plasma and Liver Exposure Profiles Across Programs (Rat Data)

Dose vs Plasma AUC

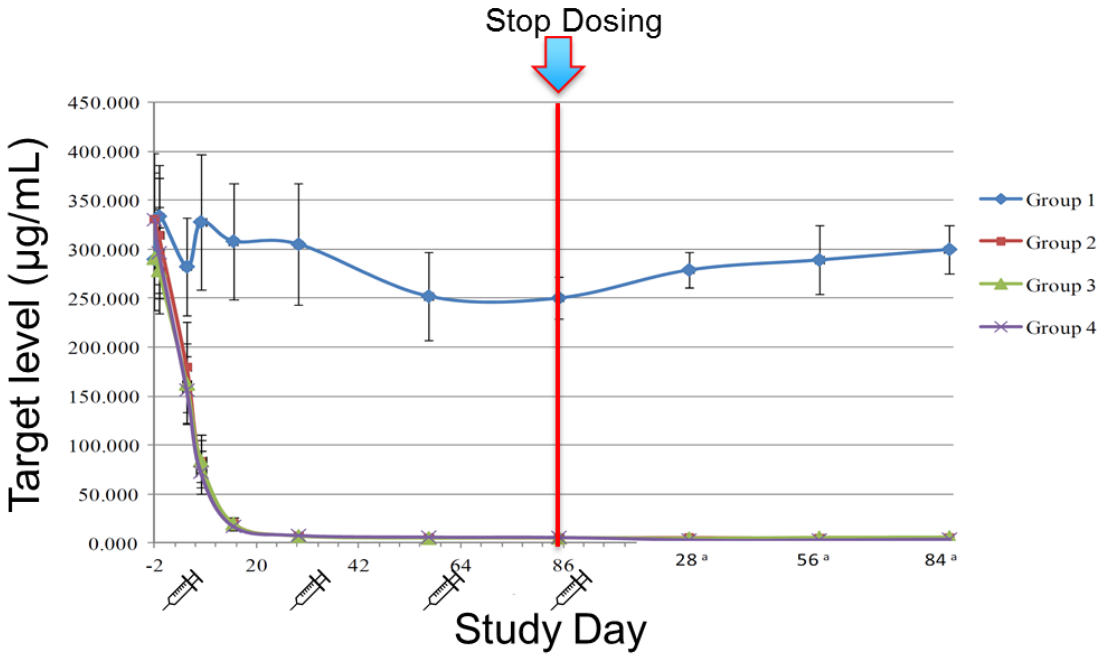


Dose vs Liver AUC





Long PD Effect and Long Tissue Half-life



- Maximum pharmacologic effect is not immediate! Typically 2-3 weeks after dosing
  - Toxicological considerations
- Test article typically still present in liver after a 13 week recovery period
  - Durable pharmacology
  - Toxicological considerations

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Lack Of Cross Reactivity Between Rodents And Humans

- **In some instances, we can get target knockdown in nonclinical species and humans**
  - We always get cross reactivity to cynomolgus monkey
- **Often, we do not have cross reactivity to rodent**
  - Can still evaluate chemical toxicity
  - Cannot investigate pharmacologically-mediated toxicity
- **Using a "surrogate" is not always appropriate or warranted**
  - Different sequence, often requiring different chemistry
  - The target in rat may have alternative function compared to human

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  - Early tox screening
  - Repeat-dose toxicity: target organs & pathologic effects; dose response and exposure relationships
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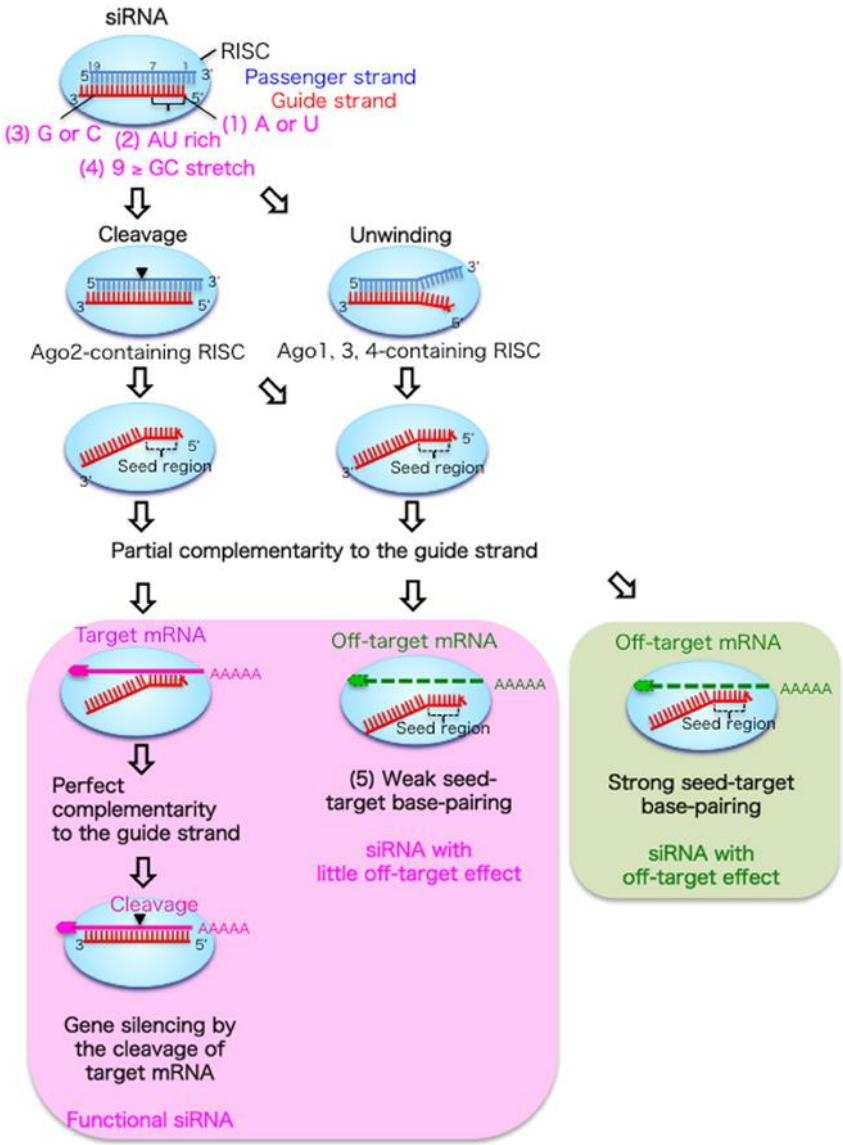
Candidate Selection: Strategies to Mitigate “Off-Target” Toxicity



- **Bioinformatics**
  - BLAST sequence against human and tox species genomes (1,000s of sequences)
    - Predict potency
    - Exclude sequences with high homology to “off-target” transcripts
- **Chemistry**
  - Modify sense and antisense strands to promote antisense loading
  - Discourage sense loading
- **Experimentation**
  - Gene expression analysis *in vitro* (~100 sequences) and potentially *in vivo* to investigate mRNA knockdown

siRNA-GalNAc Conjugates Platform-wide Toxicological Responses

Rodent (typically Rat)

- Liver
  - Hepatocellular vacuolation. Increased number and size of normal rat hepatocellular vacuoles. Contain neutral lipid.
  - Increased single cell necrosis at high doses and tissue exposures
  - Increased mitosis and regeneration at high doses and tissue exposures
- Kidney
  - Basophilic granules, proximal tubular epithelium; represents drug accumulation. Common finding with oligonucleotide therapeutics.

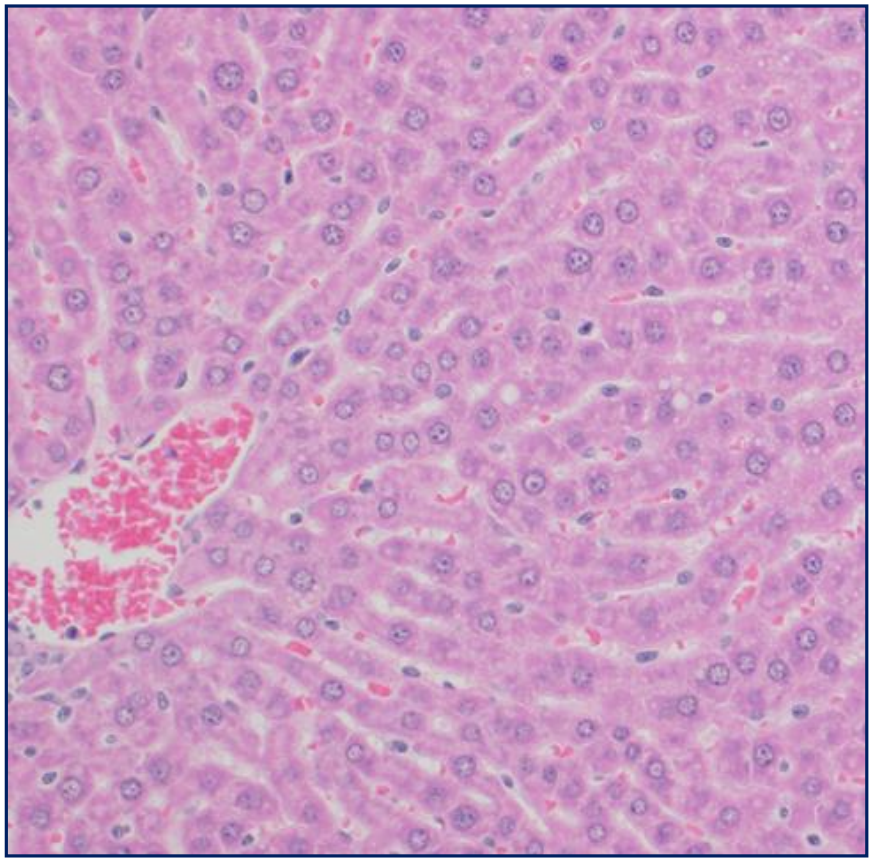
NHP

- Liver
  - Basophilic granules, Kupffer cells and hepatocytes; represents drug accumulation. Only at high doses and tissue concentrations.
- Lymph nodes
  - Vacuolated macrophages (with basophilic stippling). Phagocytosis of drug.

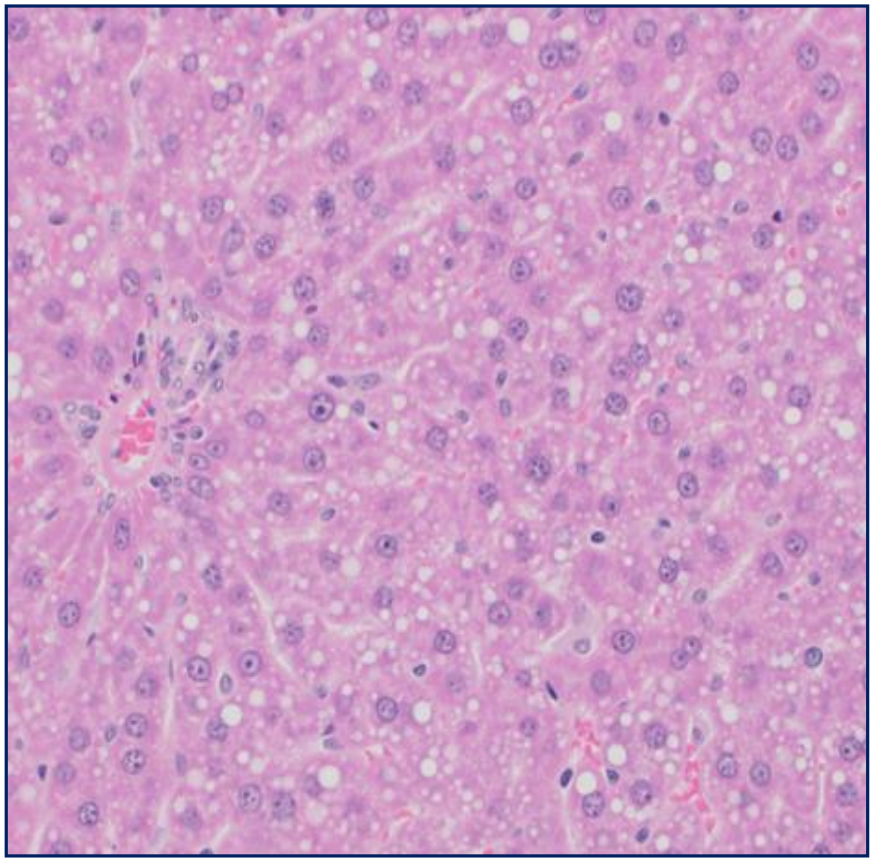
Primarily dose-dependent and the result of drug accumulation in tissue

Hepatocellular Vacuolation, Rodents Only

- Typically seen at all doses (including controls)
- Usually minimal to moderate increase above background control levels
- Dose-dependent incidence and severity
- Not associated with changes in serum liver enzyme activity
- Partially-to-fully reversible

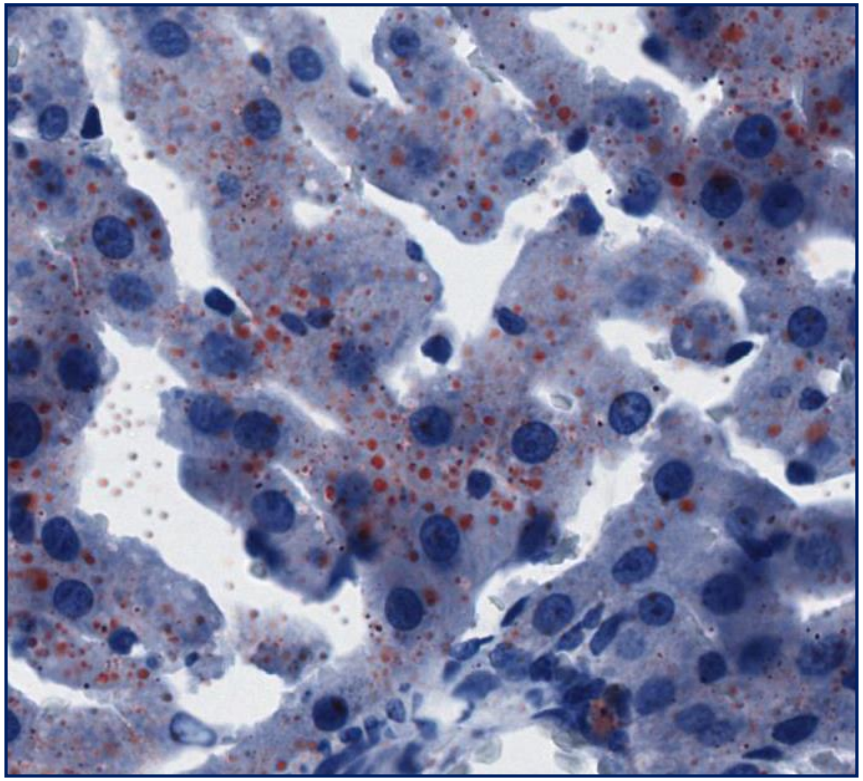


Vehicle, 20x; minimal vacuolation

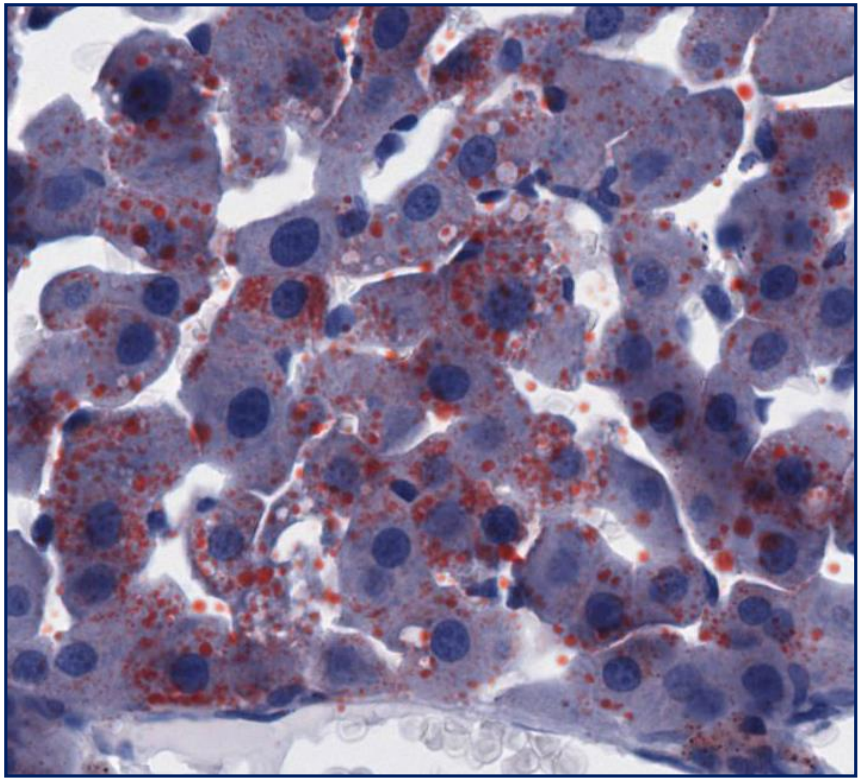


100 mg/kg, 20x; mild vacuolation

Hepatocellular Vacuolation, Rodents Only (ORO)

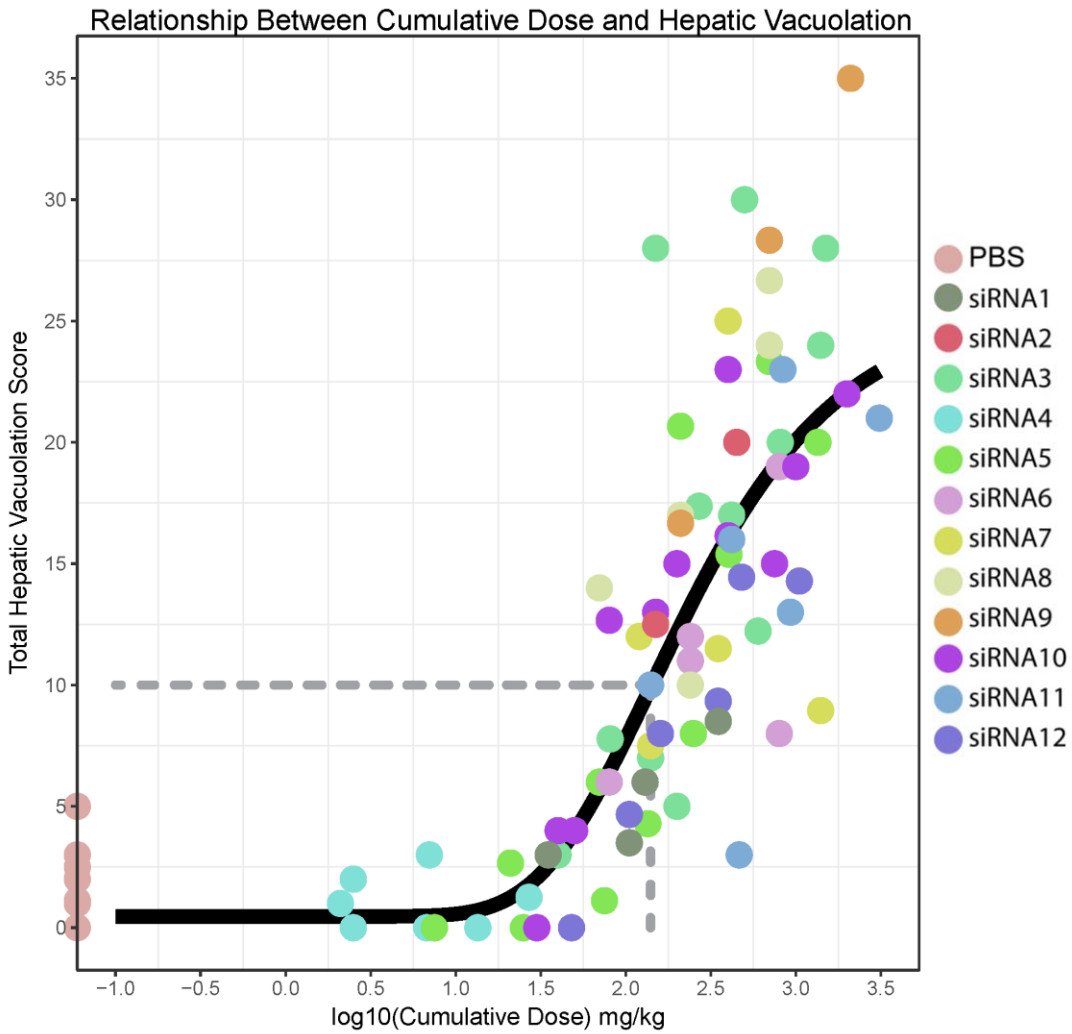


Control, Female



100mg/kg, Female

Hepatocellular Vacuolation in SD Rats



140 mg/kg cumulative dose associated with minimal vacuolation (score = 10)

Most pharmacological doses **≤ 2.5 mg/kg**

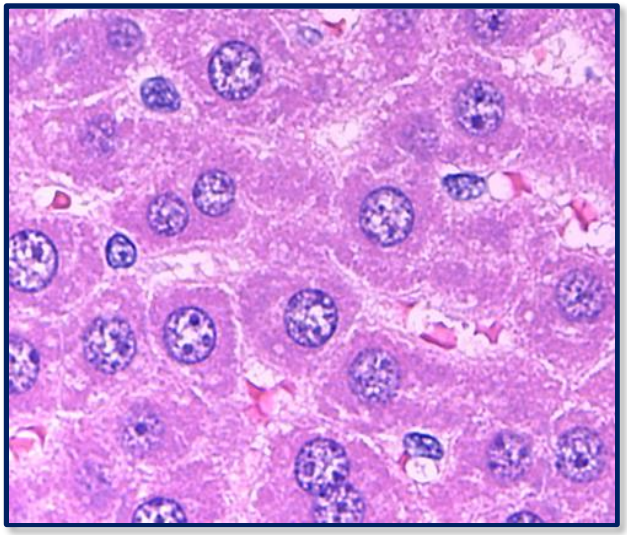
Minimal vacuolation observed at doses > 50X pharmacological dose

Black line = Model fit

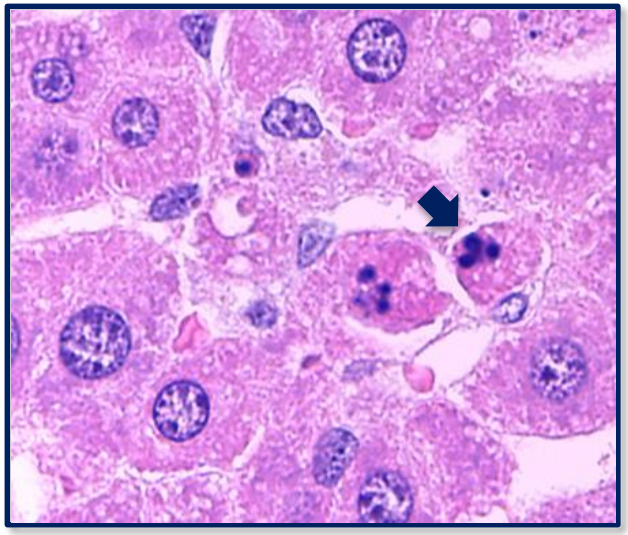
Grey line highlights concentration of drug needed to produce a vacuolation score of 10 (*e.g.* 100% of animals had minimal vacuolation)

Hepatocellular Single Cell Necrosis +/- Increased mitoses & regeneration, Rats Only

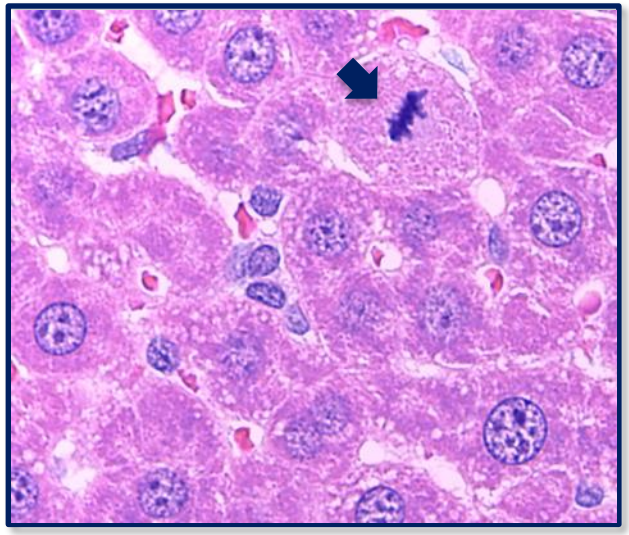
- Most severe lesion observed, occasionally with minimal increase in serum liver enzyme activity *(typically <2-fold over control)*
- Occurs at drug liver levels approaching tissue saturation generally at tissue exposure > 100x relative to effective PD tissue concentrations



Control



Single cell necrosis



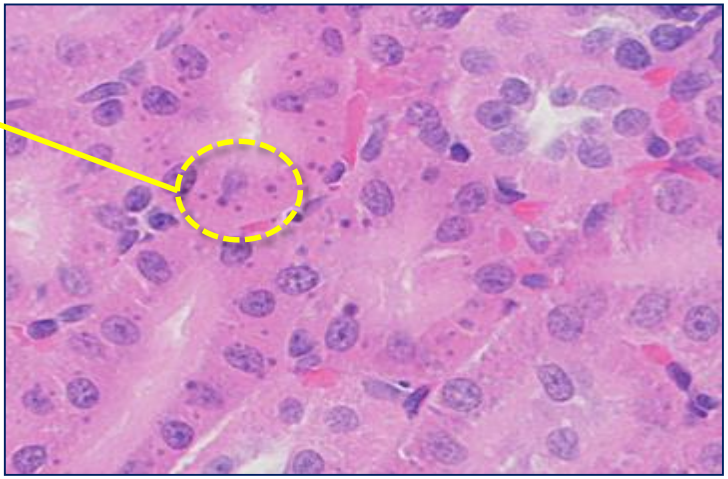
Increased mitotic figures

60x

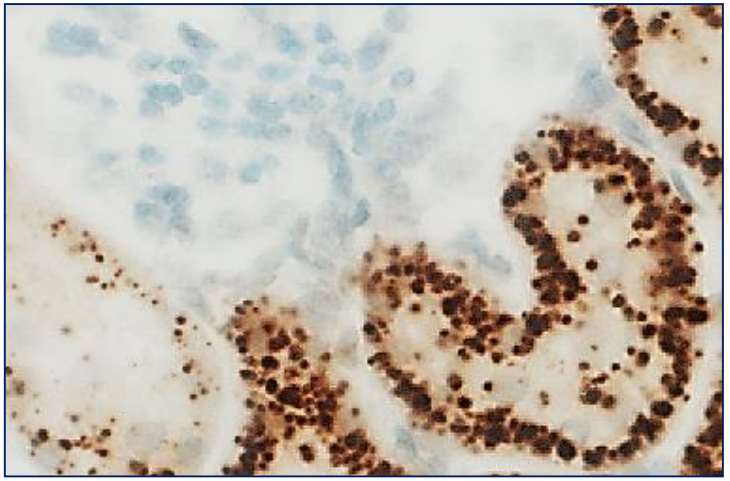
siRNA Class-Wide Toxicities: Test Article Accumulation, Rats

Basophilic granules in renal tubule epithelium

- Rat proximal renal tubules
  - Rare in NHP or other species, only at extremely high doses -- not seen in 13 week NHP studies
- Severity: Minimal to moderate, dose dependent
  - E.g. ≥ 30 mg/kg qW in rat
  - Not evident at pharmacologic doses
- Not associated with degeneration or any other renal dysfunction
- Partially reversible in recovery
- Not related to setting NOAEL



Rat siRNA 50 mg/kg qwx7



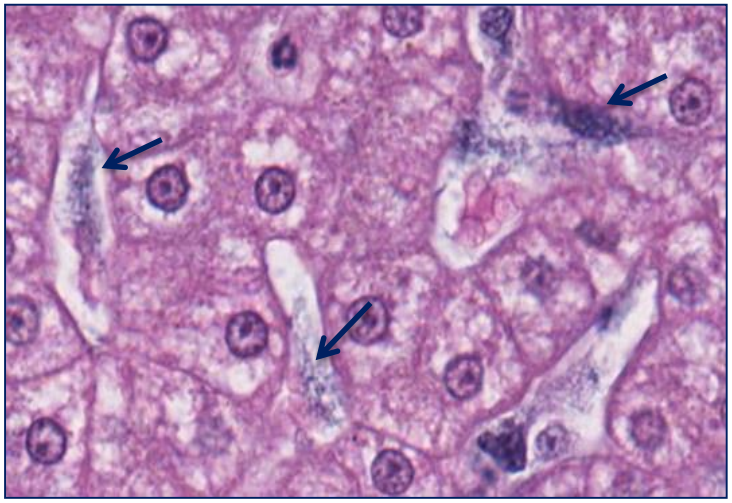
Test-article ISH

siRNA Class-Wide Toxicities: Test Article Accumulation, NHPs

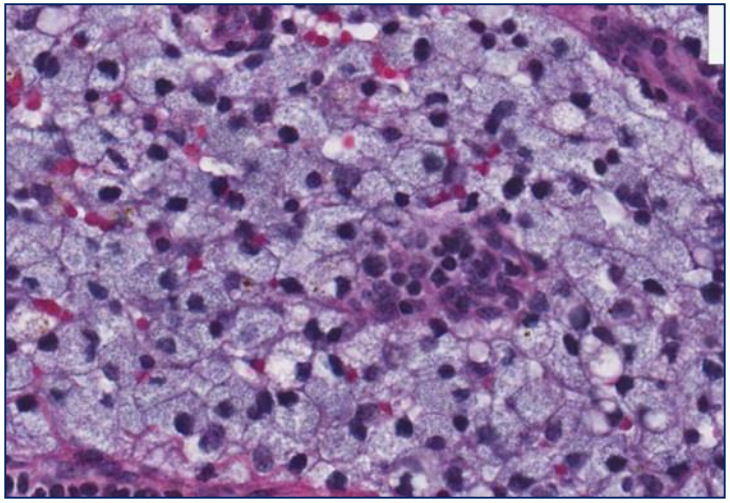
Basophilic granules in macrophages +/- vacuolation

- Kupffer cells, lymph nodes
- Severity: Minimal to moderate, dose dependent
  - e.g. ≥ 30 mg/kg qW
  - Partially reversible in recovery

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



Liver NHP (siRNA)



Lymph node NHP (siRNA)

Comparable toxicological profile following sub-chronic or chronic dosing in rats

Comparative Toxicological Profile – Typical ALNY conjugate		
Dose (mg/kg)	**qW*3**	**qM*7**
10-15	No findings	*Liver*: min.-mod. hepatocellular vacuolation
30-50	*Liver*: min.-mod. hepatocellular vacuolation	*Liver*: min.-mod. hepatocellular vacuolation Min. SCN min. karyomegaly *Kidney*: min. basophilic granules
100-150	*Liver*: mod. hepatocellular vacuolation min. mitotic figures min. SCN *Kidney*: min. basophilic granules	*Liver*: min.-mod. hepatocellular vacuolation minimal karyomegaly regeneration *Kidney*: min. basophilic granules

Compound X
Ultra-rare disease indication, no approved therapy

Chronic Tox Study in Sprague-Dawley Rats

- 0, 20, 50, 200 mg/kg (qM*7)
- No changes in target organ toxicity between 8-wk repeat dose and chronic studies
  - Liver (minimal-to-marked hepatocellular vacuolation, minimal hepatocellular hypertrophy, minimal pigment in Kupffer cells, minimal-to-moderate hepatocellular karyomegaly)
  - Kidney (minimal basophilic granules and vacuolation of tubular cells)
  - No recovery group included, but these findings were partially-to-fully reversible in the 8 wk rat tox study
- No toxicity associated with exaggerated pharmacology
  - >60X pharmacologic dose based in rats
  - 13-fold increase in circulating levels of PD biomarker
- NOAEL = 200 mg/kg (highest dose tested)
- Proposed clinical dose = 2-3 mg/kg qM

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The Standard Test Battery For Genotoxicity

ICH S2(R1)

Small Molecules Guidance*		GalNAc-siRNA Conjugate Result
Standard Battery	**Systems**	
Bacterial reverse mutation (Ames) test: *point mutations*	***Salmonella typhimurium* and *Escherichia coli***	**Negative (all programs)**
In vitro mammalian test: *chromosome damage/ point mutations*	**Metaphase chromosome aberration assay (hPBL/CHO)**	**Negative (all programs)**
In vivo mammalian test: *chromosome damage*	**Micronucleus assay (bone marrow/blood)**	**Negative (all programs)**

**Standard battery is not required for biologics per ICH S6(R1)*

Revusiran: 2-Year Carcinogenicity Assessment in Sprague-Dawley Rats

- 0, 10, 30, or 100 mg/kg (qW dosing)
- No Revusiran-related effects on survival
- Revusiran-related non-adverse decreased bodyweight gain and terminal bodyweight observed in both sexes
- No evidence of neoplasia or hyperplasia
- Target organ toxicity similar to that observed in 6-month study
  - Slight to marked hepatocellular vacuolation
  - Minimal cystic degeneration of liver
  - Minimal to moderate basophilic granules in renal tubules
  - Minimal to moderate hypertrophy of renal tubular cells

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Fitusiran: 26-Week Rat Chronic Toxicity
Juvenile and Male Reproductive Functional Outcome

Results (naive females mated with Fitusiran-treated males)

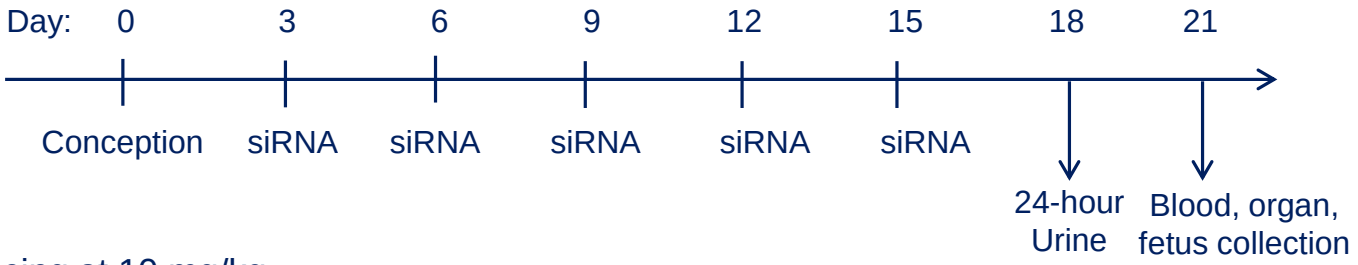
- Juvenile tox: no effects on growth (crown-rump measurement), bone measurement (tibia and femur), and sexual maturation (physical development)
- Male fertility: no effects on male reproductive performance, spermatogenic cycle assessments, sperm motility, morphology and concentration, and ovarian and uterine parameters

Summary Data (All Group Mean Values)

Fitusiran (mg/kg)	Successful Matings + Pregnancy	Corpora Lutea/ Rat	Implantation Sites/ Litter	Live Embryos/ Litter	Dead Embryos/ Litter	Early Resorptions/ Litter	Sum of Early Resorp & Dead	Pre-Implant Loss (%)/ Litter	Post-Implant Loss (%)/ Litter
0	19/20	16.5	15.0	14.2	0.0	0.8	0.8	10.20	5.47
0.25	18/20	16.3	15.4	14.6	0.0	0.8	0.8	5.48	4.92
0.5	16/20	15.9	15.1	14.3	0.0	0.8	0.8	4.46	6.14
1.0	18/20	17	15	15	0	0	0	11.8	0.0

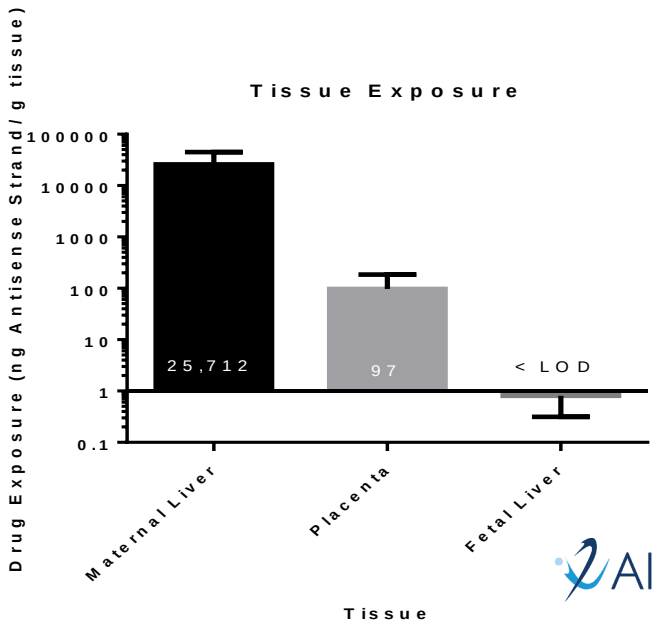
siRNA- GalNAc Placental Exposures

Inhibits Maternal Target, But no effect on Fetus



- Target Silencing at 10 mg/kg
- ~95% knockdown of mRNA in maternal liver; no significant silencing in placenta
- 80% - 95% reduction in maternal circulating protein expression product

- **siRNA Conjugate does not cross placental barrier**
  - No significant fetal liver drug exposure



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Specialty Toxicity Evaluations of GalNAc-siRNA Conjugates

- **Cardiovascular safety**
  - Single dose and multi-dose CV Safety Pharmacology studies in NHP at doses up to 300 mg/kg negative for GalNAc-siRNA conjugates. No effects on conduction or hemodynamics.
  - No expected effects on hERG or ion channel assays. Large molecule 14-17 kD.
- **Phototoxicity**
  - Oligonucleotide therapeutics are not required to undergo typical phototoxicity testing per ICH S10
- **Injection site reactions**
  - Low incidence (< 10-15%), minimal to mild, transient erythematous reactions. Histologically, minimal inflammatory cell infiltrates.
- **Cytokine responses, complement effects, and immunogenicity**
  - All GalNAc-siRNA conjugates in development evaluated and all negative for cytokine stimulation or complement activation in mice and/or NHP.
  - There has been no evidence of any anti-drug antibody formation for any GalNAC-siRNA drug candidates

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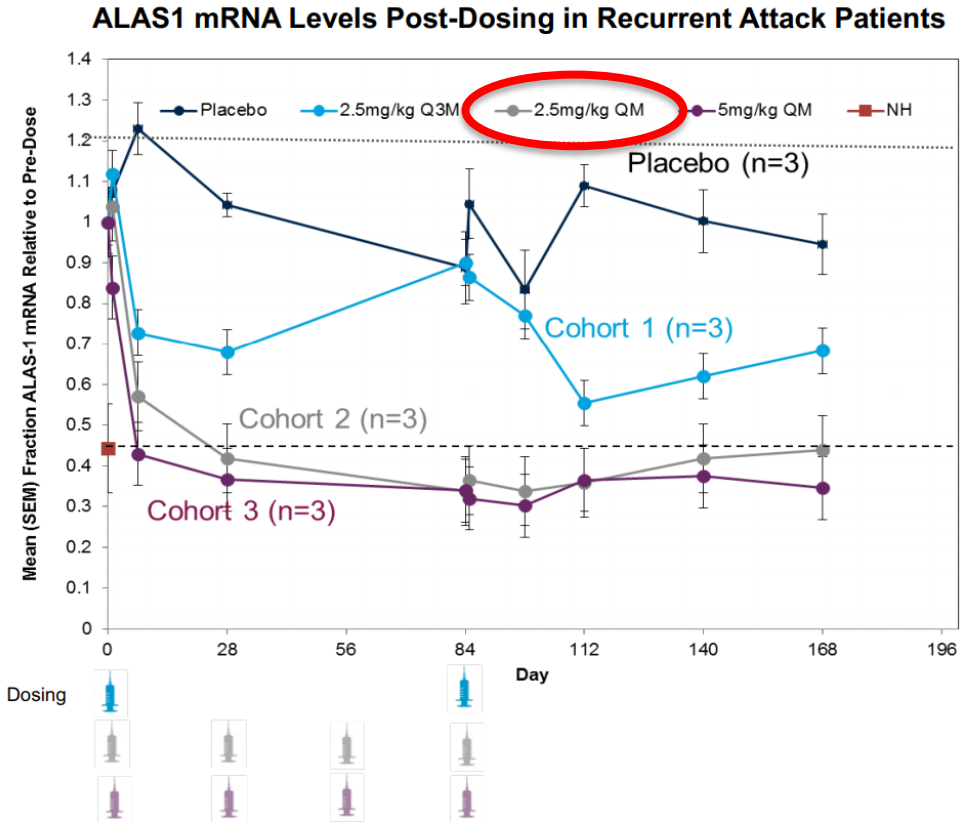
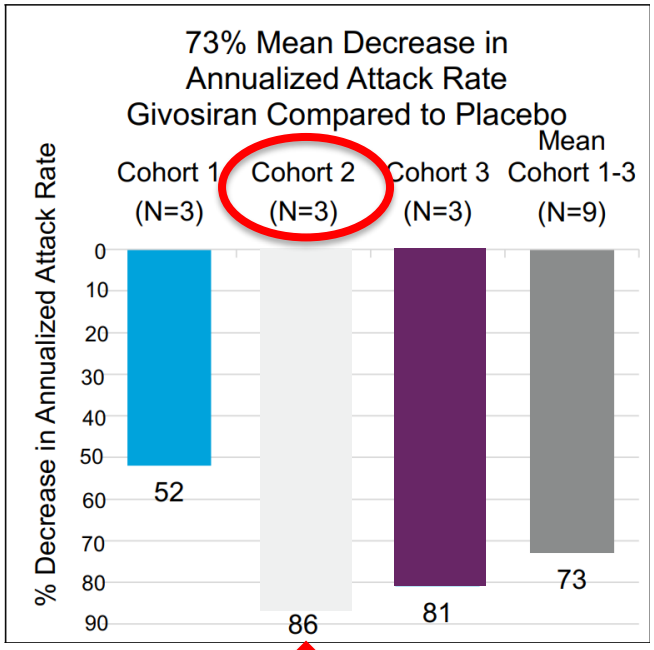
Clinical Perspective: Givosiran (ALN-AS1)
Phase 1C Study in Patients with Acute Hepatic Porphyria





2.5 mg/kg qM

13-week (qW) toxicity data			
Species	**HED NOAEL (mg/kg)**	**HED NOAEL (cumul. mg/kg)**	**Adverse Findings**
Rat	5	20	None
Monkey	50	200	None

Alnylam PHARMACEUTICALS

Summary:

Emerging safety profiles for GalNAc-siRNA Conjugates

The safety profiles of GalNAc-siRNA conjugates are generally consistent between programs

- Rat more sensitive than NHP
  - NOAEL in NHP always highest dose tested (up to 300 mg/kg)
- Target organs
  - 1º - Liver, 2º - Kidneys, Reticuloendothelial system
- Toxicity related to intracellular accumulation
  - Liver effects noted at supratherapeutic doses (> 50X pharmacological dose)
  - Evidence of reversibility following recovery phase
- Good therapeutic margins in rodents and NHP
  - Only one current program with exaggerated pharmacology limiting doses in normal subjects, not in diseased subjects

Thank You!