Advances in RNAi Therapeutics Platform

3rd International Conference on the Long and the Short of Non-Coding RNAs

Vasant Jadhav, PhD

22nd June, 2019

Alnylam® PHARMACEUTICALS

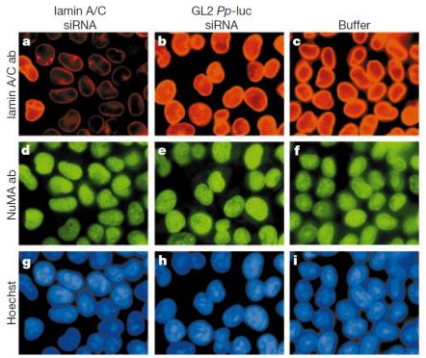
Outline

- Introduction to RNAi Platform
- New Frontiers for RNAi Therapeutics: CNS and Ocular Delivery
- Mechanistic Understanding: Durability of RNAi Therapeutics
- RNAi Therapeutics Towards Non-Parenteral Dosing

Alnylam Pharmaceuticals

Founded on the Bold Promise of Turning Nobel Prize Winning Science into a New Class of Medicine

2002: *In vitro* data by our scientific co-founders that started Alnylam



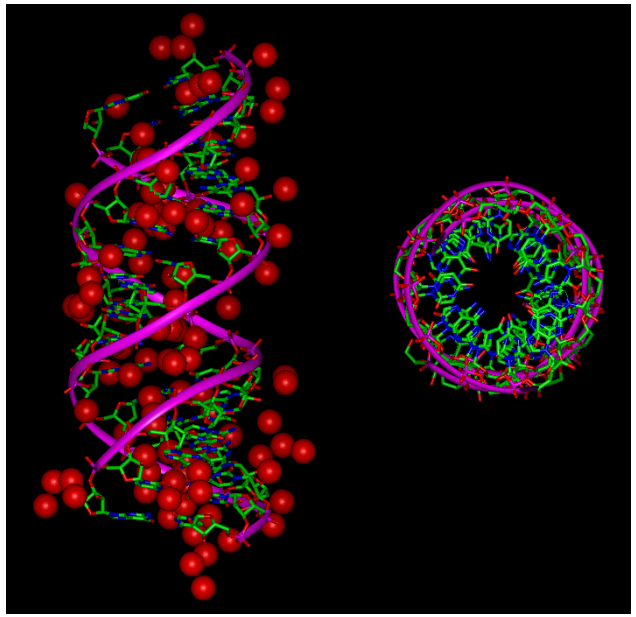
Discovery of RNAi in mammalian cells
Elbashir et al., Nature, 2001;411:494-98

16-years later

2018: Approval of first ever RNAi-based therapeutic by FDA and EMA

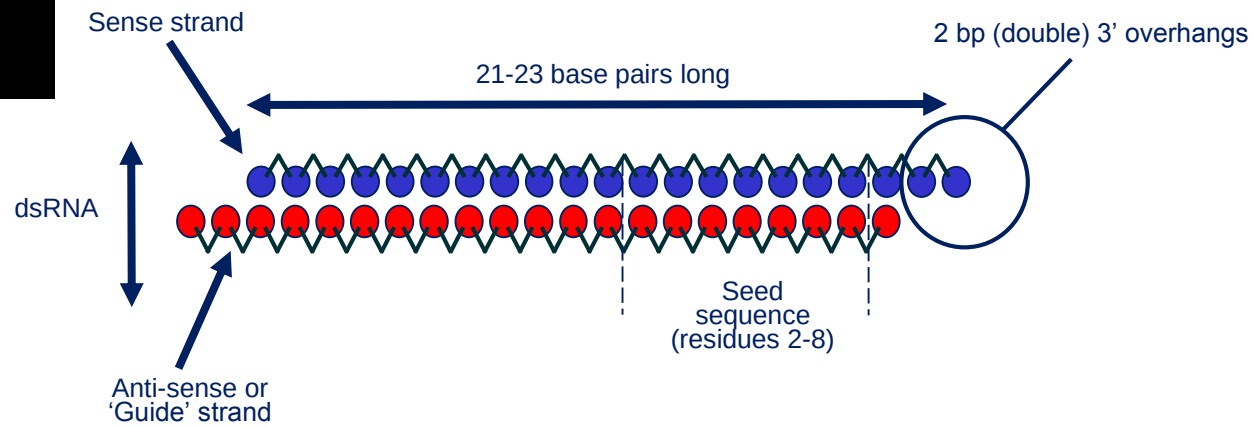
Making Drugs Out of siRNAs

The Challenge



Characteristics

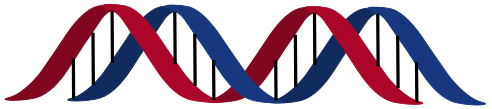
- M.W 12,000-14,000
- Size: 2 turns of helix
- 40 negative charges
- Hydrophilic
- Hydrated heavily
- ca. 5.5 nm X 2 nm
- Biostability



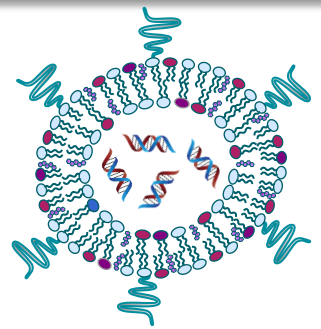
Structure adapted from *Klosterman, P. S.; Shah, S. A.; Steitz, T. A Biochemistry* (1999), 38, 14784-14792.

Addressing the Delivery Challenge

Mechanisms for siRNA Delivery to Liver



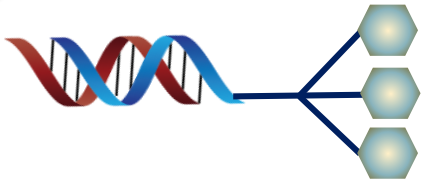
siRNA



Patisiran

Lipid Nanoparticles (LNPs)

- siRNA (limited modifications) in a multi-component lipid formulation (LNP; ~85 nm)
- Targeted delivery to liver
- Intravenous (IV) administration



Current Clinical siRNAs

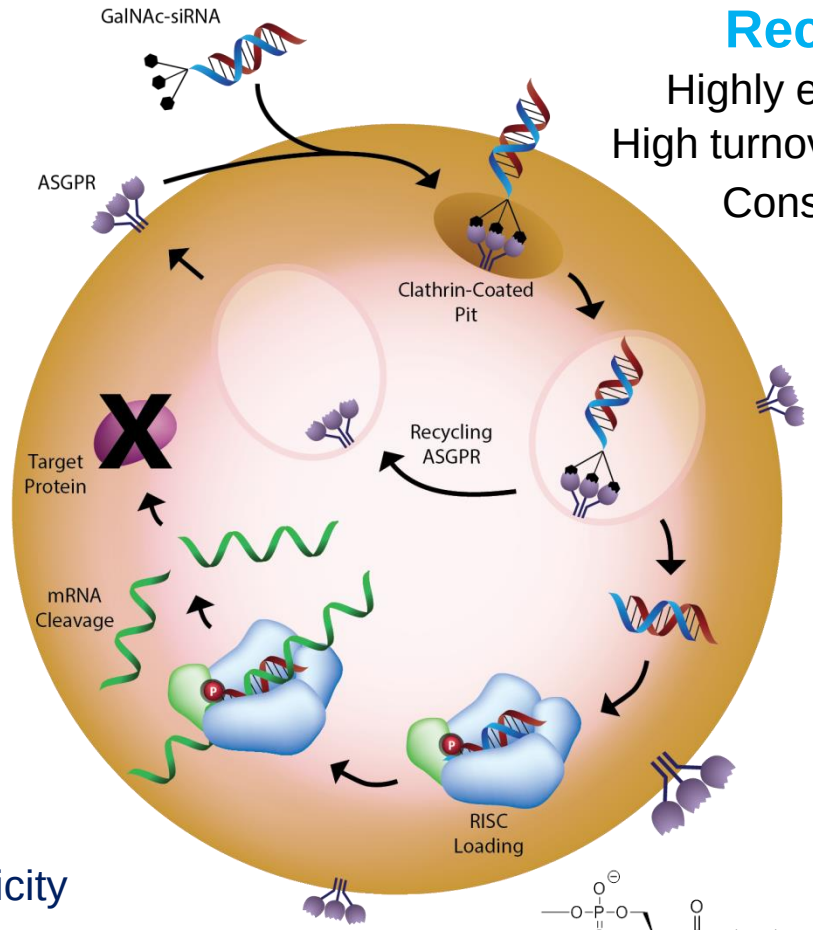
GalNAc-siRNA Conjugates

- Single chemical entity
- Tri-GalNAc ligand conjugated to sense strand of extensively modified siRNA
- Targeted delivery to liver
- Subcutaneous (SC) administration

GalNAc-siRNA Conjugates: SC-Administered Platform For Targeted Delivery To Hepatocytes

siRNA

Metabolic stability
Intrinsic potency
Duration of effect
Safety
CMC

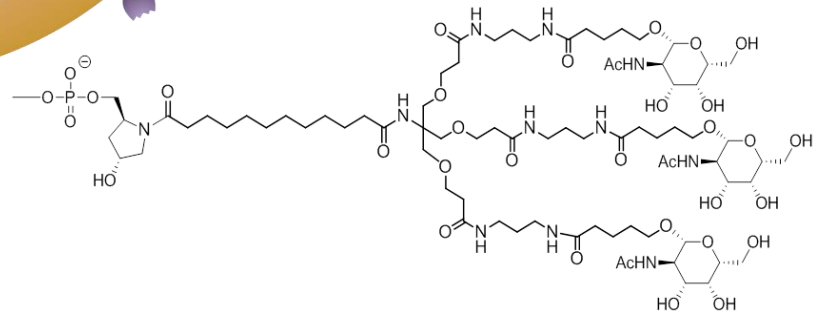


Asialoglycoprotein Receptor (ASGPR)

Highly expressed in hepatocytes
High turnover (recycling time ~15 min)
Conserved across species

Ligand

Receptor affinity specificity
Metabolic stability
Safety
CMC



Alnylam Clinical Development Pipeline

Focused in 4 Strategic Therapeutic Areas (STARs):

- Genetic Medicines
- Cardio-Metabolic Diseases
- Hepatic Infectious Diseases
- CNS/Ocular Diseases

		HUMAN POC[1]	BREAKTHROUGH DESIGNATION	EARLY STAGE *(IND or CTA Filed-Phase 2)*	LATE STAGE *(Phase 2-Phase 4)*	REGISTRATION/ COMMERCIAL[3]	COMMERCIAL RIGHTS
onpattro® (patisiran) lipid complex injection 10 mg/5 mL	*hATTR Amyloidosis*[2]					●	Global
Givosiran	*Acute Hepatic Porphyria*	✓	🏅			●	Global
Patisiran	*ATTR Amyloidosis Label Expansion*	✓	🏅		●		Global
Fitusiran	*Hemophilia and Rare Bleeding Disorders*	✓			●		15-30% royalties
Inclisiran	*Hypercholesterolemia*	✓			●		Milestones & up to 20% royalties
Lumasiran	*Primary Hyperoxaluria Type 1*	✓			●		Global
Vutrisiran	*ATTR Amyloidosis*	✓	🏅		●		Global
Cemdisiran	*Complement-Mediated Diseases*	✓		●			50-50
Cemdisiran/Pozelimab Combo[4]	*Complement-Mediated Diseases*	✓		●			Milestone/Royalty
ALN-AAT02	*Alpha-1 Liver Disease*			●			Global
ALN-HBV02 (VIR-2218)	*Hepatitis B Virus Infection*			●			50-50 option rights post-Phase 2
ALN-AGT	*Hypertension*			●			Global

[1] POC, proof of concept – defined as having demonstrated target gene knockdown and/or additional evidence of activity in clinical studies
[2] Approved in the U.S. for the polyneuropathy of hATTR amyloidosis in adults, and in the EU for the treatment of hATTR amyloidosis in adults with stage 1 or stage 2 polyneuropathy
[3] Includes marketing application submissions
[4] Cemdisiran is currently in Phase 2 development and pozelimab is currently in Phase 1 development; Alnylam and Regeneron are evaluating potential combinations of these two investigational therapeutics
As of May 2019

Alnylam® PHARMACEUTICALS

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- Introduction: RNAi Platform
- New Frontiers for RNAi Therapeutics: CNS and Ocular Delivery
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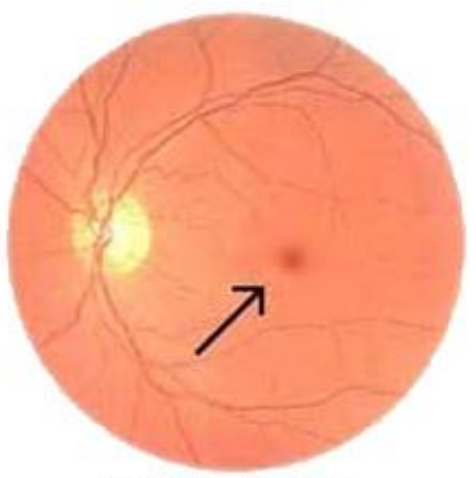
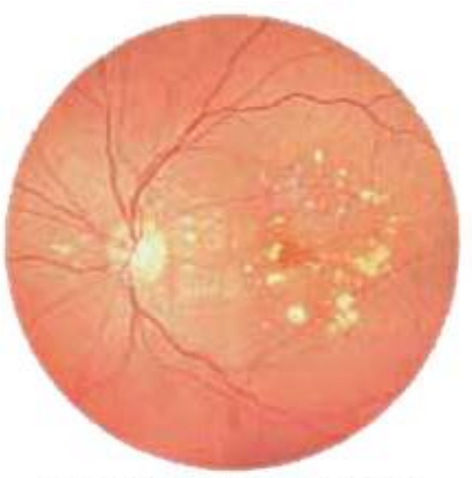
RNAi Therapeutics for CNS and Ocular Diseases


Normal Macula


Macular Degeneration

- Many dominantly inherited neurodegenerative diseases
- Many dominantly inherited eye diseases

Enormous unmet medical need across the CNS and Ocular spaces

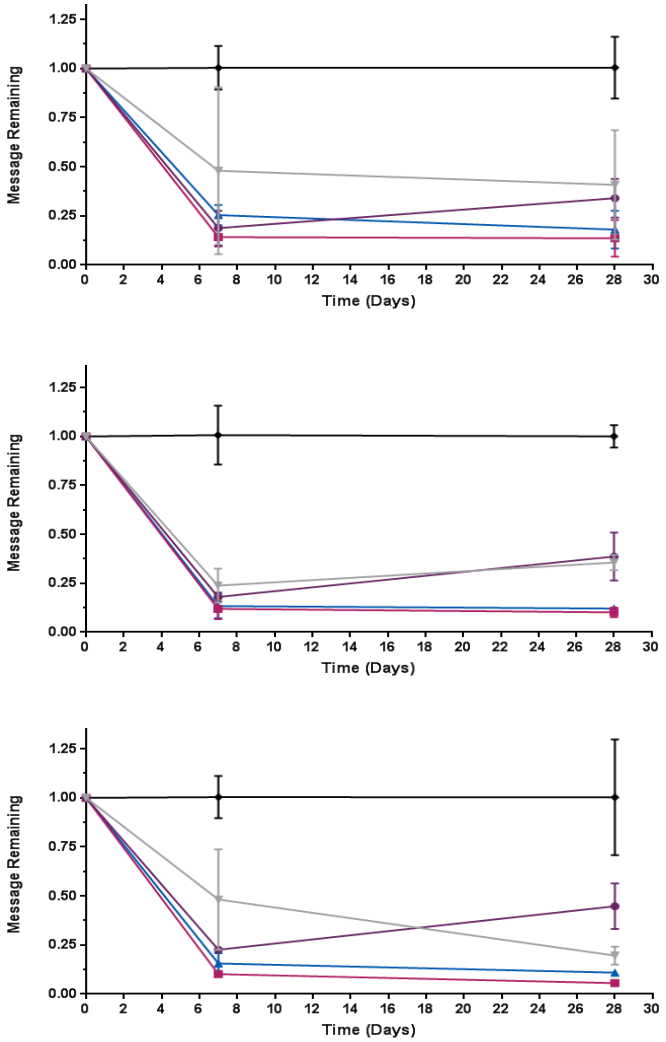
SOD1 siRNA Conjugates Demonstrate Superior Silencing in Rat CNS

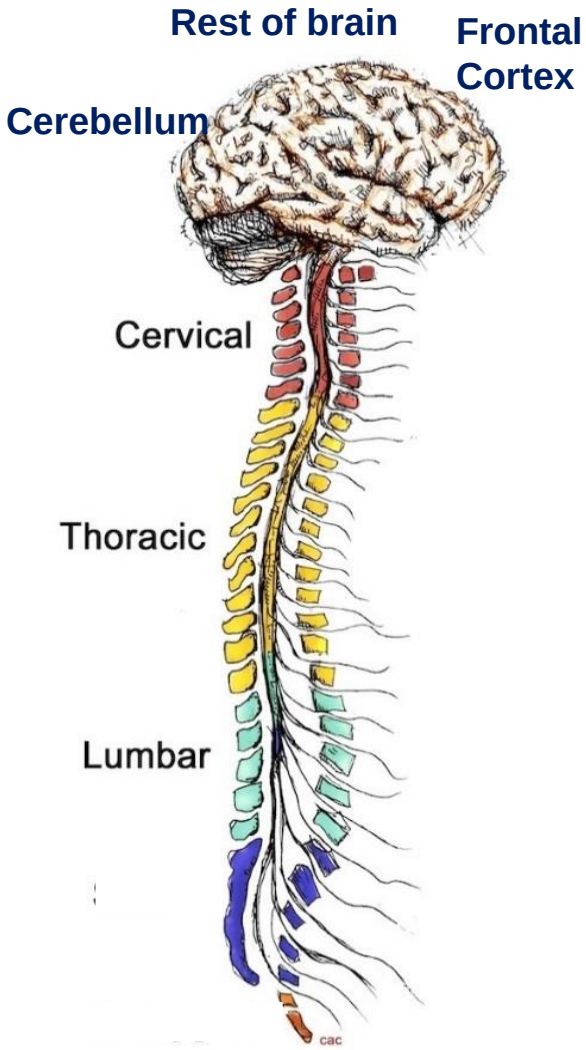
Superior silencing to parent at 10-fold lower dose

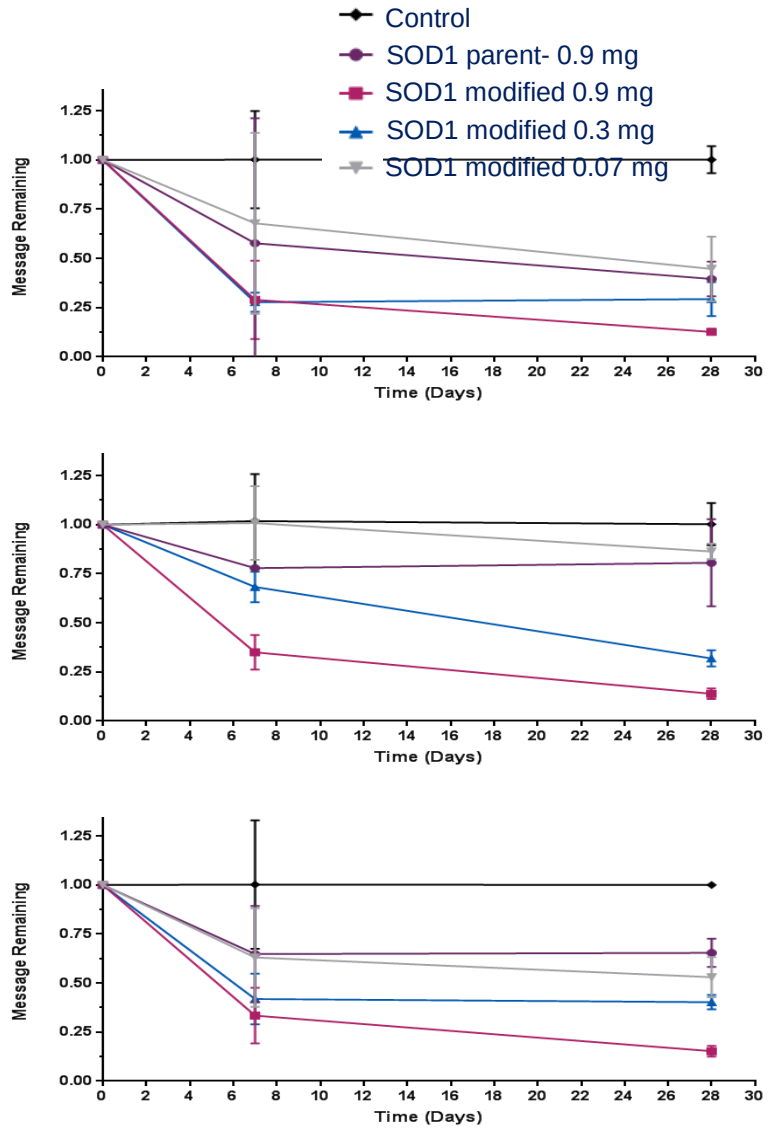
Lumbar

Thoracic

Cervical

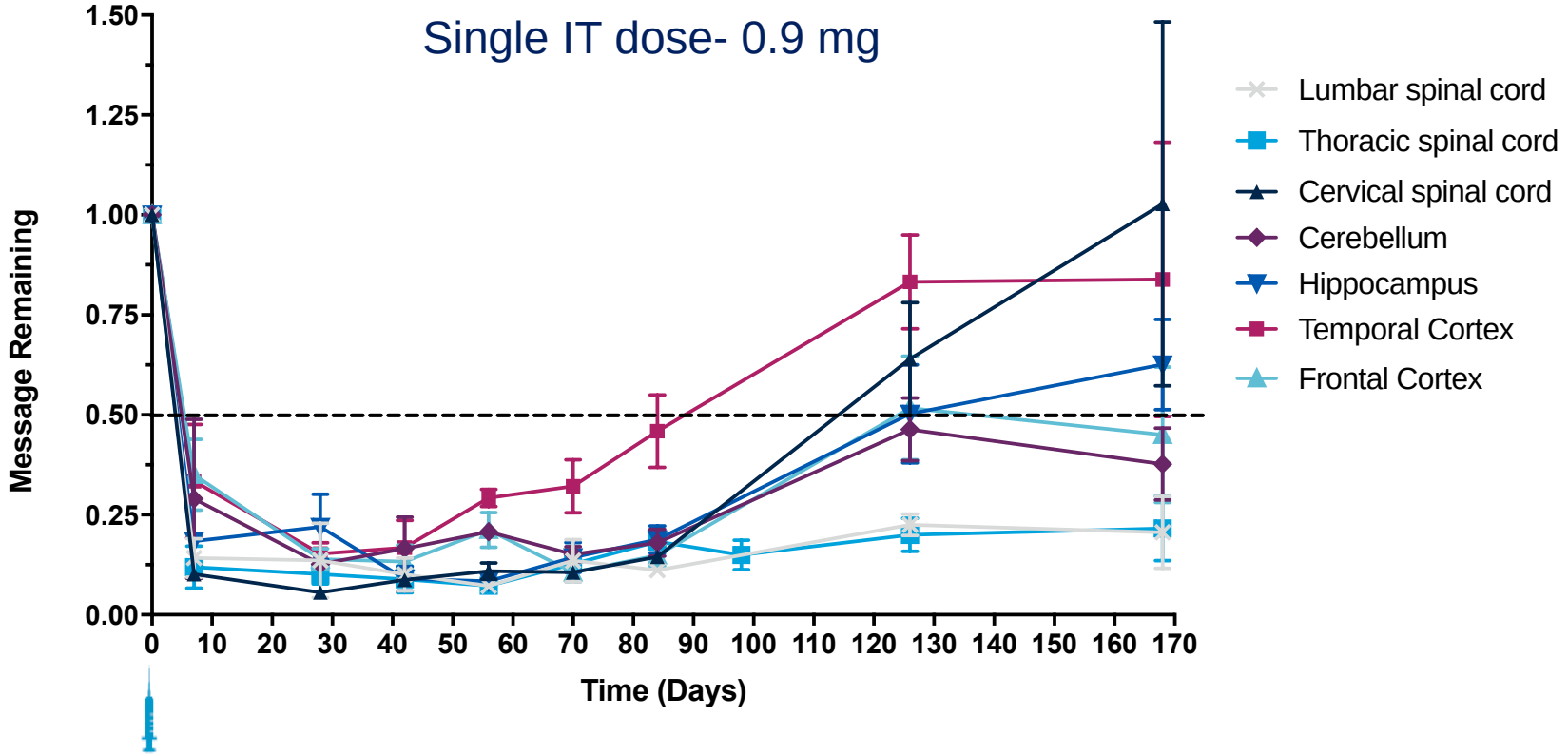






Robust Silencing Throughout the CNS

Up to 6 months of silencing in some regions of the CNS following a single dose



- Durable lowering across animals in most regions of the brain for up to 6 months
- Silencing in spine maintained close to NADIR through 6 months
- PD comparison in liver across species together with extended duration seen in rodents expected to support infrequent dosing in human

siRNA vs ASO in hSOD1 (SOD1G93A) Rats

Day 7 or 28 collection after single IT dose

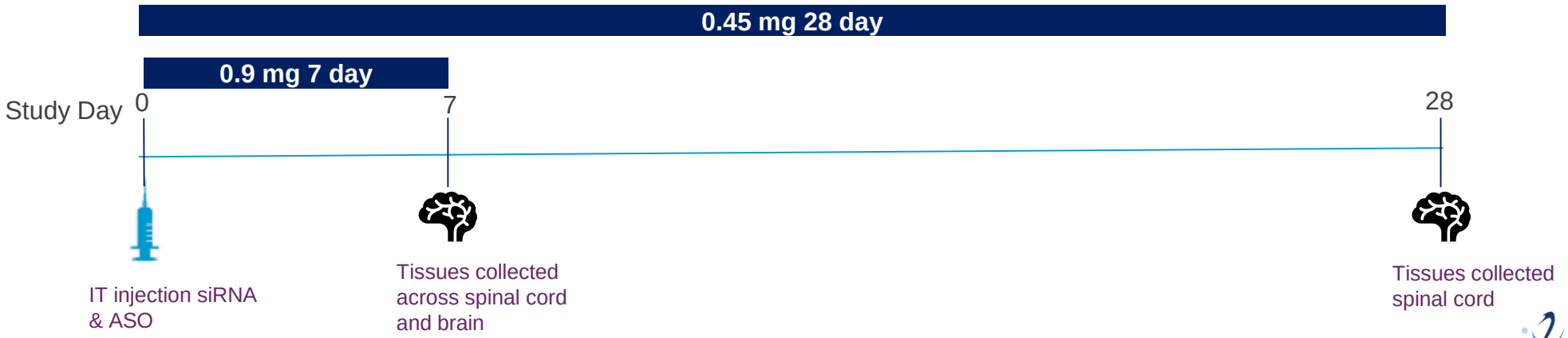
Study Purpose

- Head-to-head comparison of siRNA and ASO
  - siRNAs selected from mouse AAV-hsSOD1 screen
  - ASO 1, based on McCampbell et al. (2018)
    - Demonstrated ~75% maximum silencing at 2 weeks in the same rat model

ASO 1	CAoGGoATACATTTCTACoAGoCT

Study Design

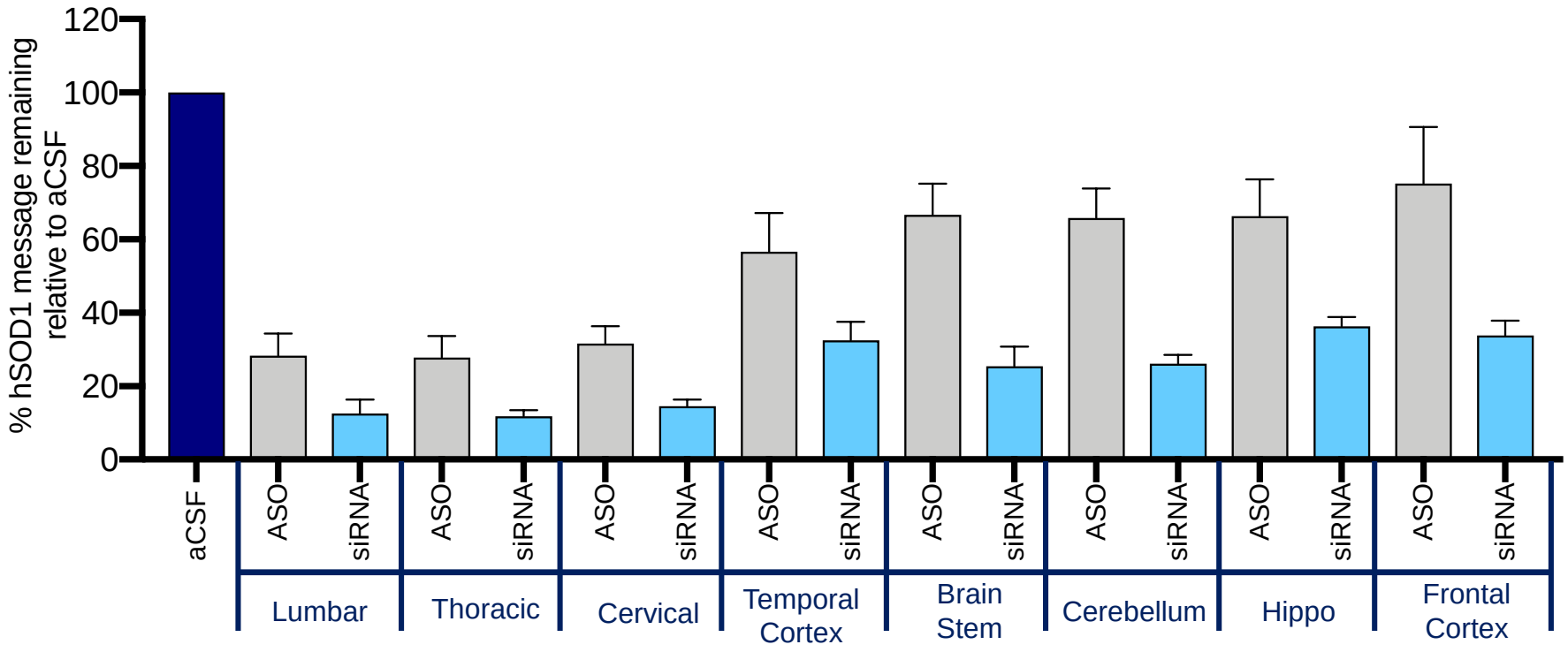
- Single IT injection of 0.9 mg assayed at day 7
  - Same dose used for siRNA and ASO
- Single IT injection of 0.45 mg assayed at day 28



[12] McCampbell et al, *J Clin Invest.* 2018;128(8):3558-3567

siRNA vs ASO in hSOD1 (SOD1G93A) Rats

Improved hSOD1 mRNA reduction with siRNA compared to ASO in CNS at day 7

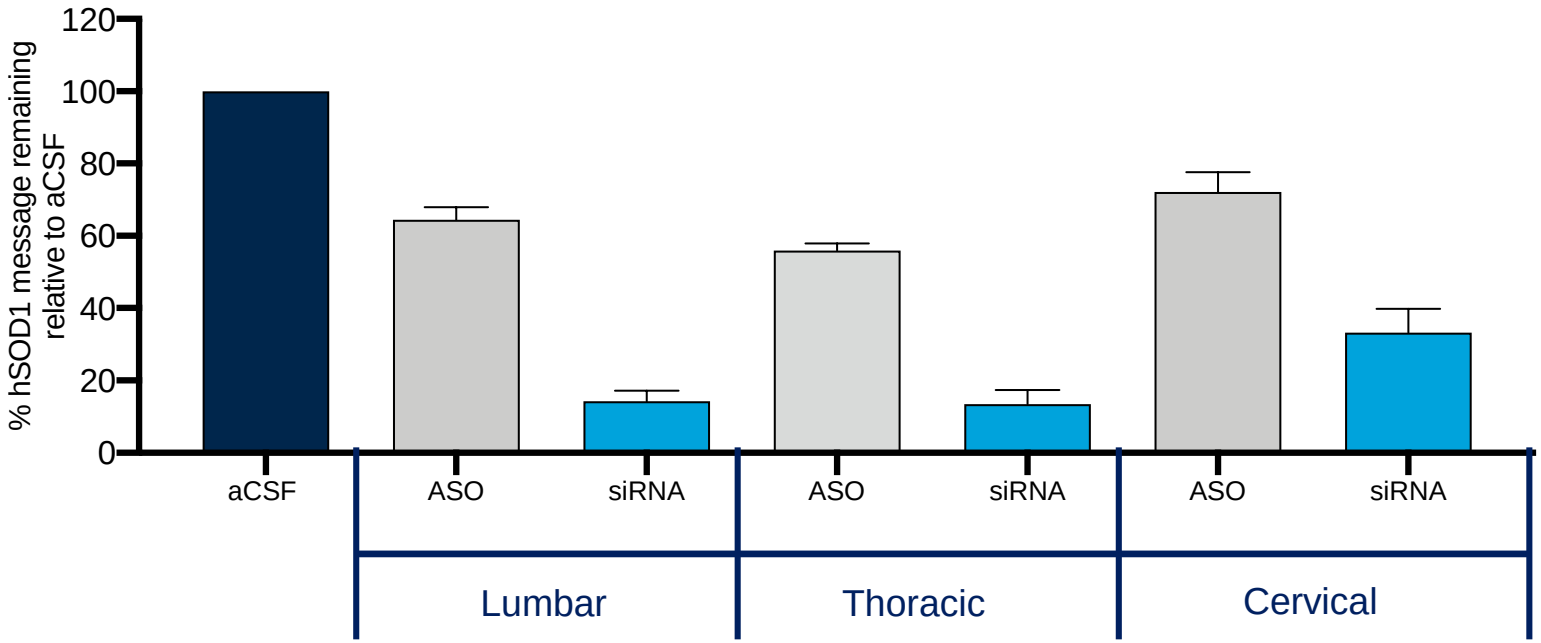


Greater silencing in all regions of the brain and spinal cord was observed using an siRNA targeting hSOD1 in this model at day 7

Single IT dose of 0.9 mg

siRNA vs ASO in hSOD1 (SOD1G93A) Rats

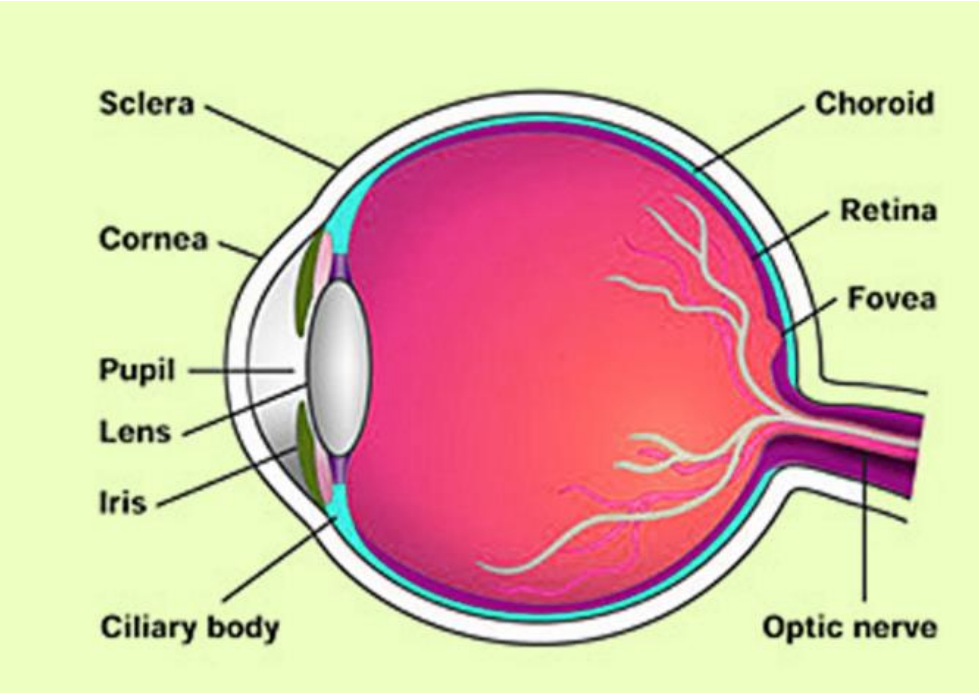
Improved hSOD1 mRNA reduction with siRNA compared to ASO in the spine at day 28

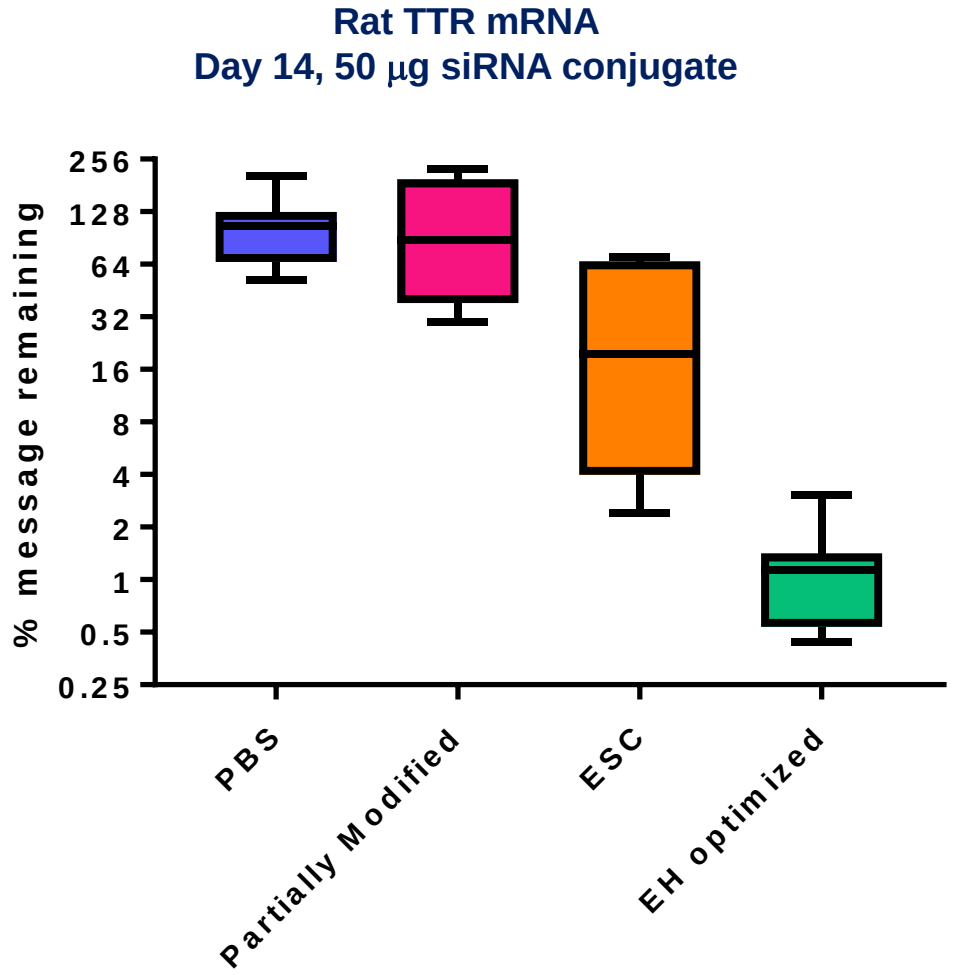


Greater silencing in all regions of the spinal cord were observed using an siRNA targeting hSOD1 in this model at day 28

Single IT dose of 0.45 mg

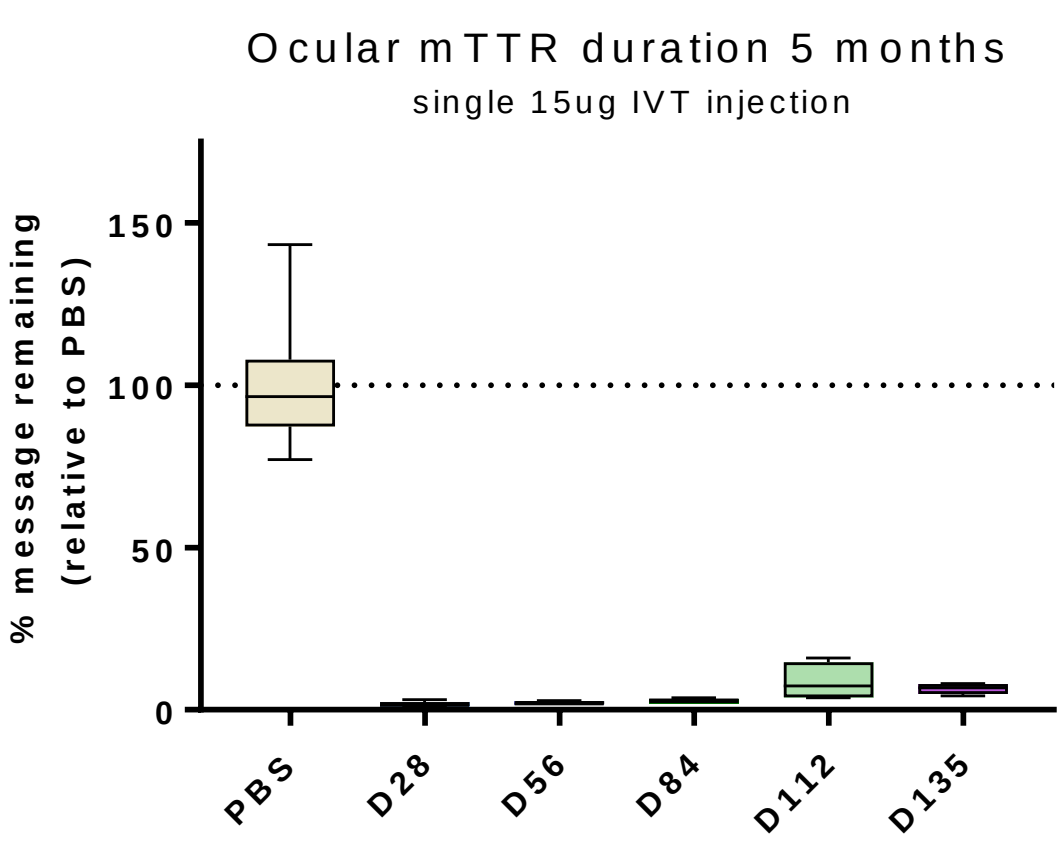
Ocular TTR Silencing by Differentially Modified siRNA Conjugates in Rat After Single Intravitreal Injection

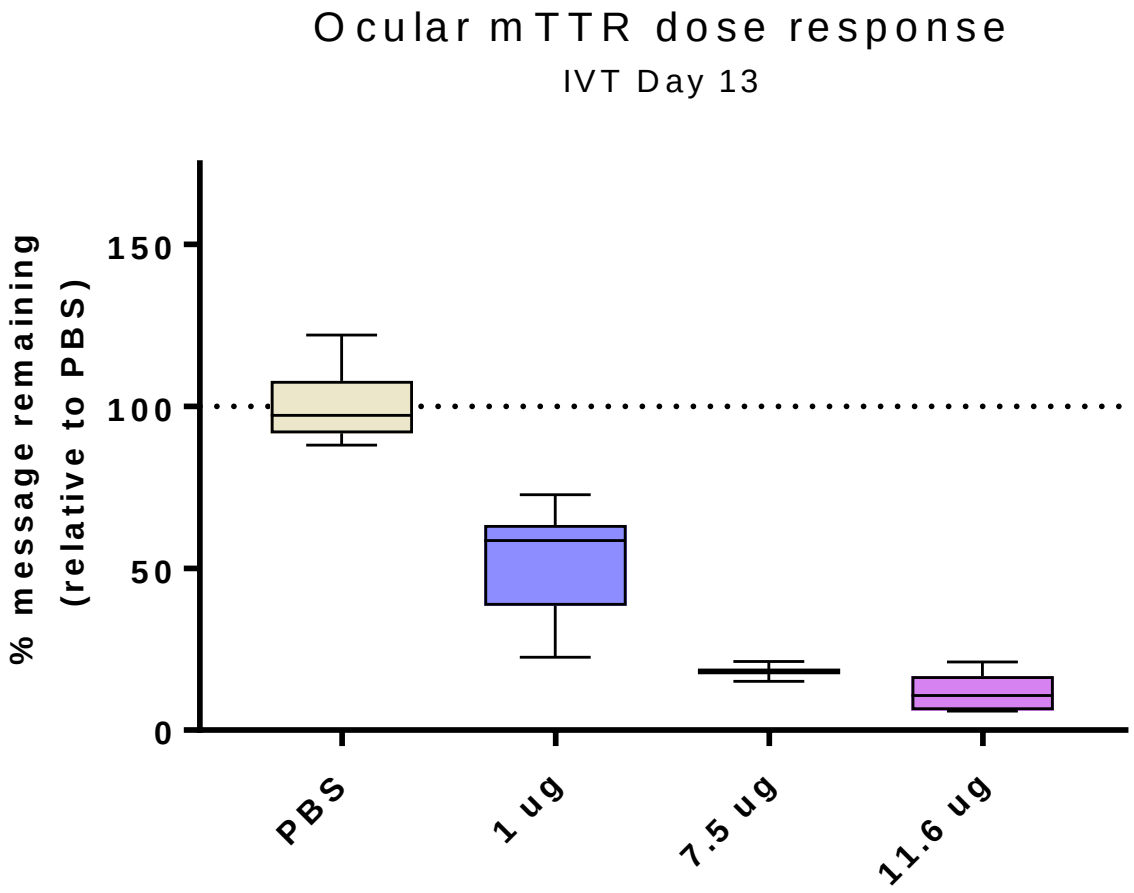




Dose Response and Duration of Activity of Ocular siRNA Conjugates in Mice

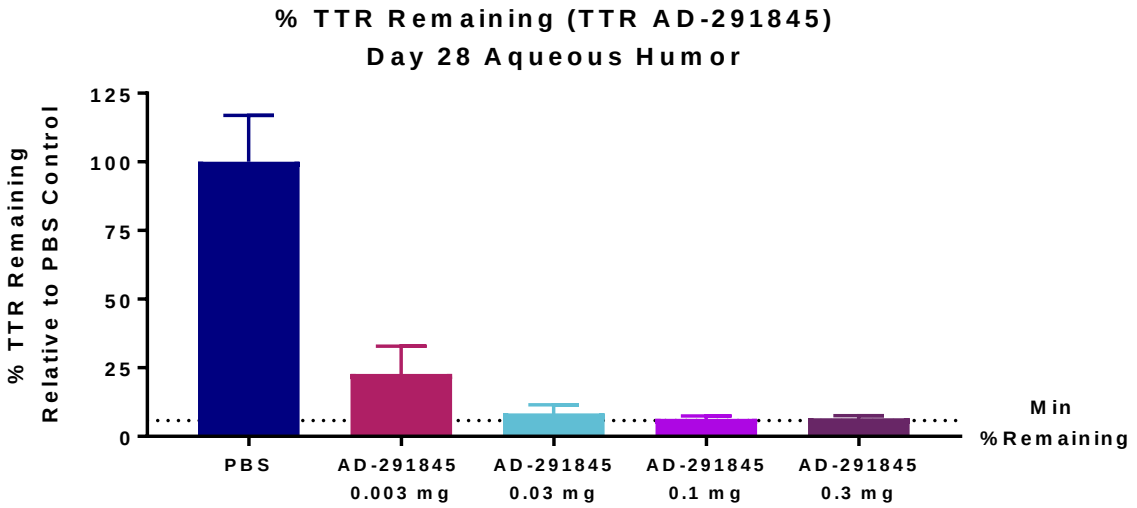
After Single Intravitreal Injection

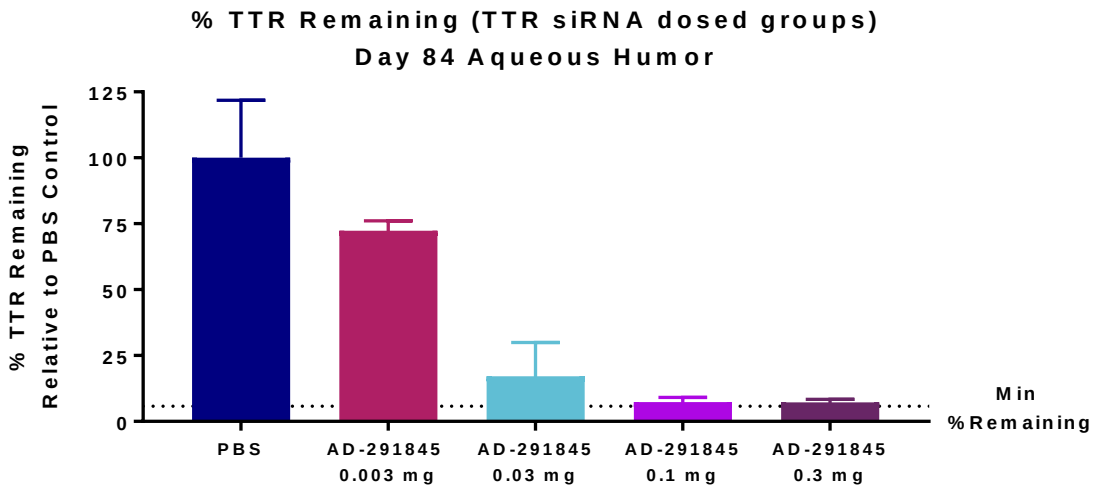




Excellent duration observed for siRNA conjugates in eye

Current Ocular Design Shows Impressive Potency and Duration in NHP





Excellent duration observed for siRNA conjugates in NHP eye

Alnylam-Regeneron Alliance*

Landmark Alliance Focused on CNS & Ocular RNAi Therapeutics

- Partnership of two leading biopharmaceutical companies committed to innovation
  - Alnylam R&D expertise and scientific excellence in RNAi therapeutics with emerging global commercial presence
  - Regeneron scientific excellence, world-leading capabilities in human genetics, and industry-leading commercial presence in ophthalmology and other large markets
- Broad, multi-product alliance across CNS, ocular, and select liver targets
  - Both companies fully participate in value creation with 50-50 structure in CNS and select liver programs
  - Milestone/royalty structure for ocular disease programs
- Accelerates Alnylam CNS and ocular programs, driving significant pipeline expansion
  - Robust, highly durable, and widely distributed RNAi knockdown of key targets in CNS/ocular pre-clinical models
  - Adds 1-2 new planned INDs/year toward CNS or ocular targets to previously planned 1-2 new INDs/year in liver beginning in 2020
- Significantly bolsters Alnylam balance sheet to >$2B *pro forma* for increased pipeline investment and future growth

* Alliance and equity agreements with Regeneron expected to close during Q2 2019

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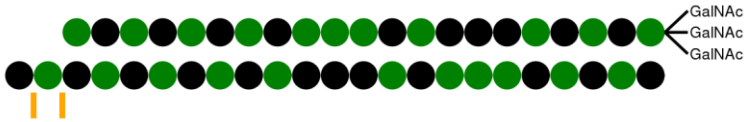
Extended Duration of Activity by ESC Conjugates

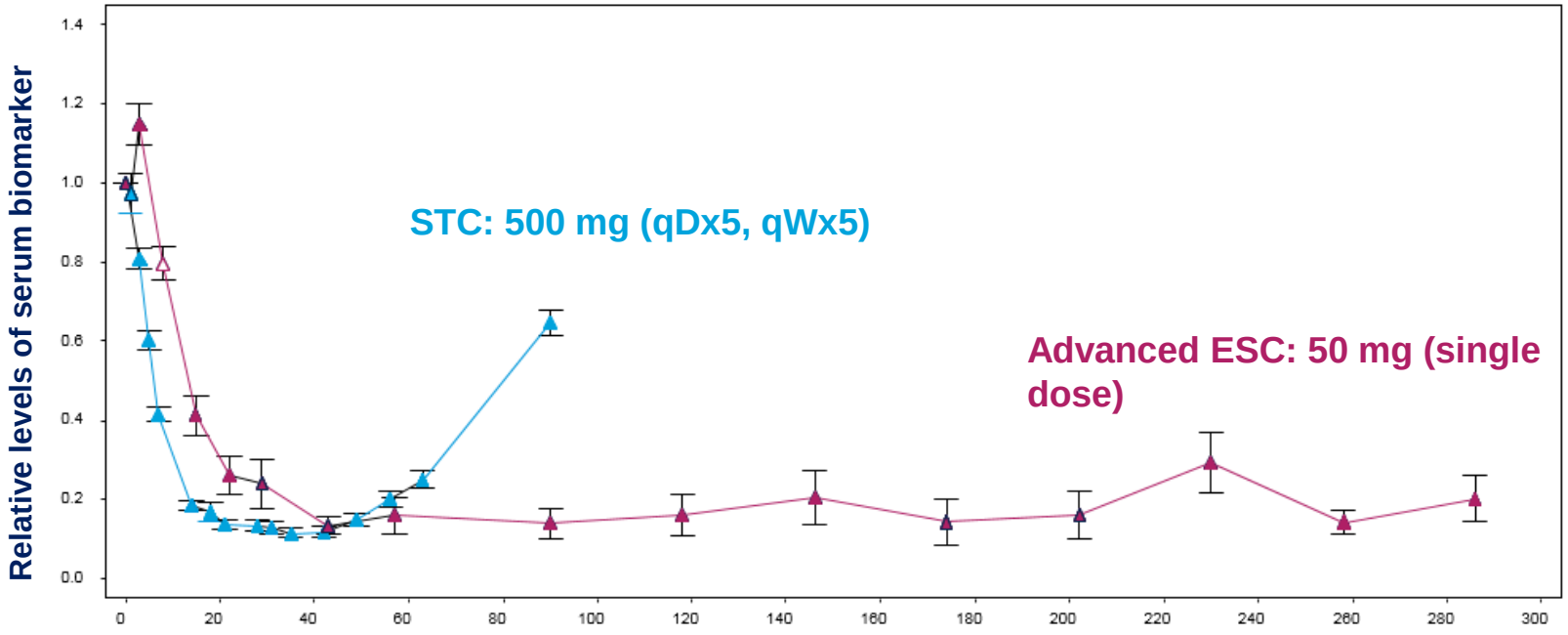
Human pharmacodynamic response* of two siRNAs with the same sequence, different chemistry

STC: Standard Template Chemistry



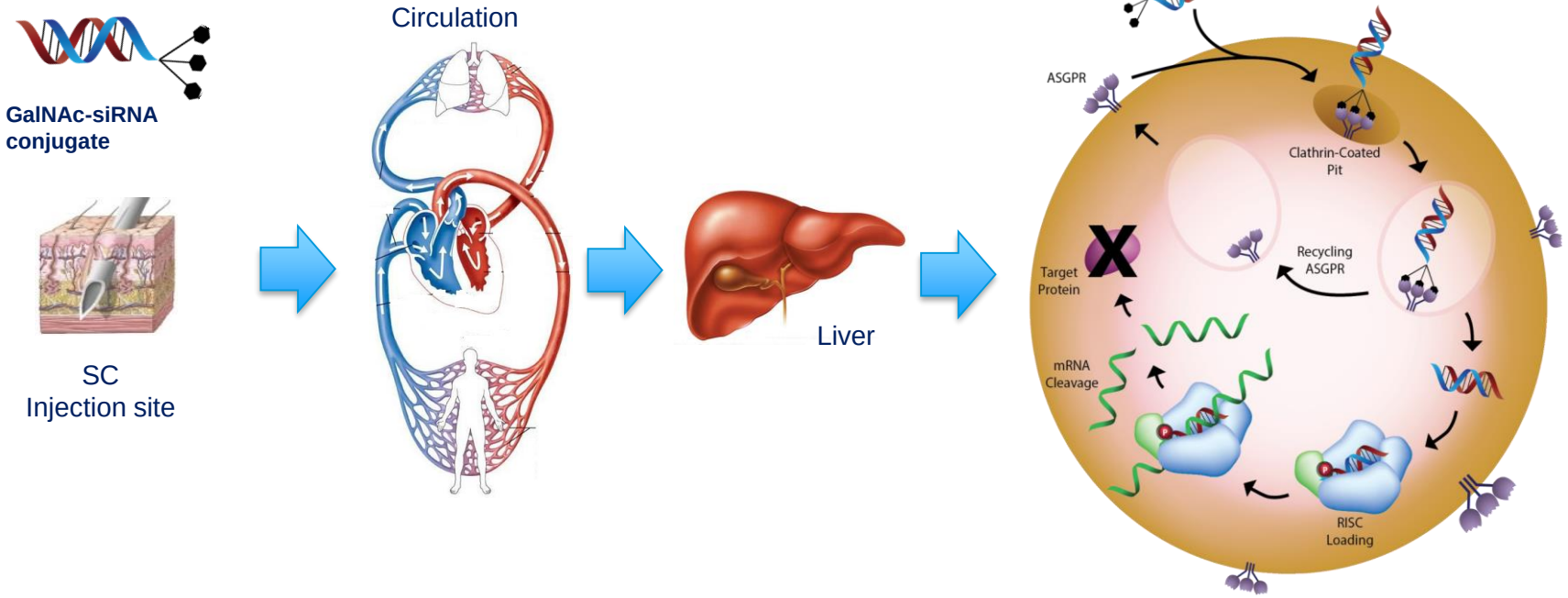
Advanced ESC: Enhanced Stability Chemistry

2'-Fluoro **2'-OMe** **PS- Phosphorothioate**



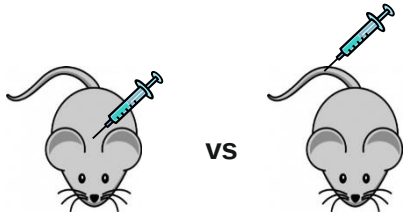
*Phase 1 data in healthy volunteers from separate studies

Depot Effect Hypothesis for Conjugate Extended Duration of Effect



- Sustained release of conjugate from SC injection site to liver?
- Increased half-life of siRNA-loaded RISC?
- Continuous supply of siRNA from an intracellular depot?

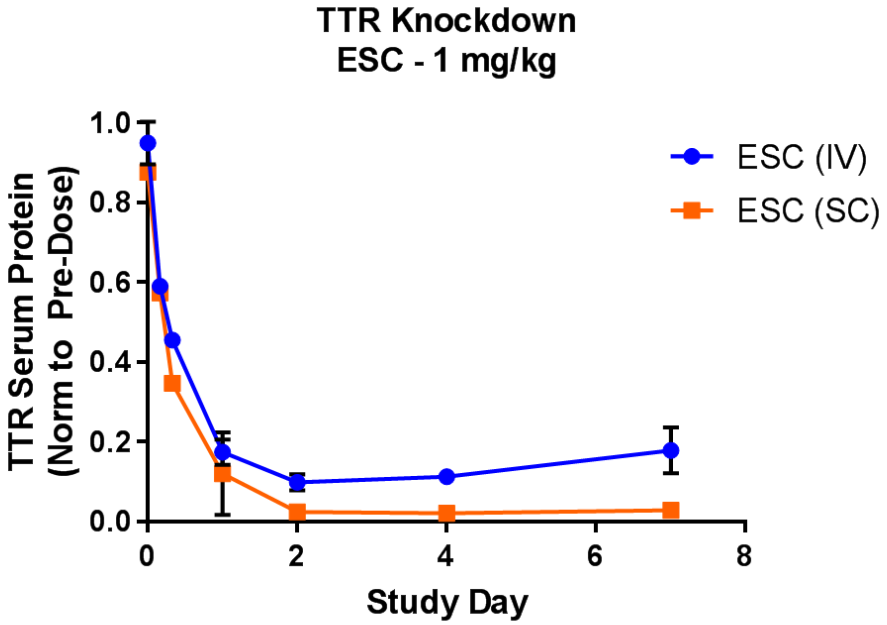
The SC Injection Site Is Not A Depot For GalNAc-siRNA - IV Dosing Of Potent Compounds Shows Similar Profile



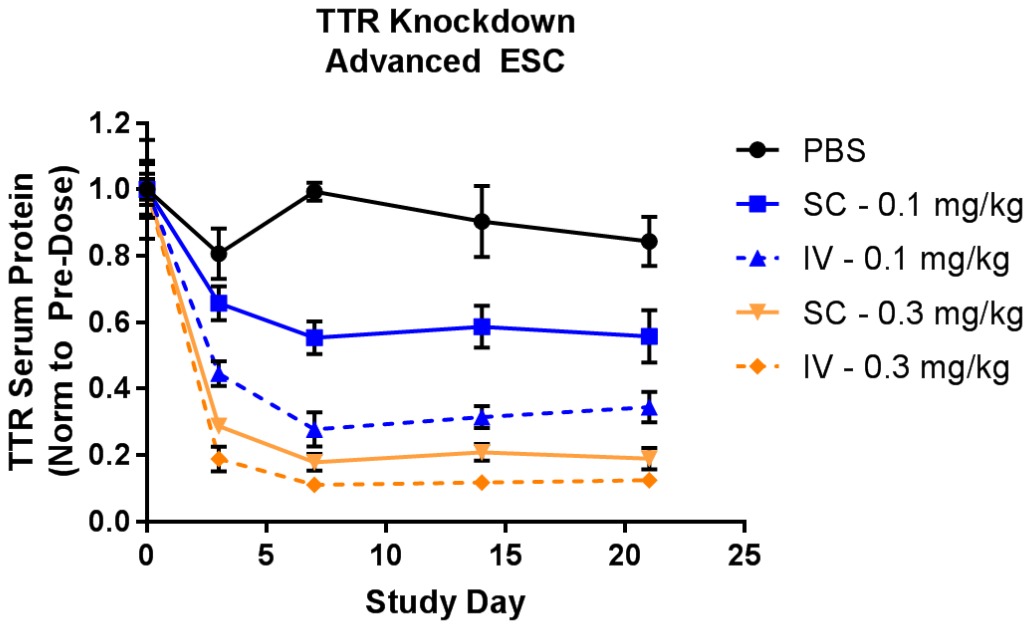
vs

SC
Depot?

IV
No potential SC depot



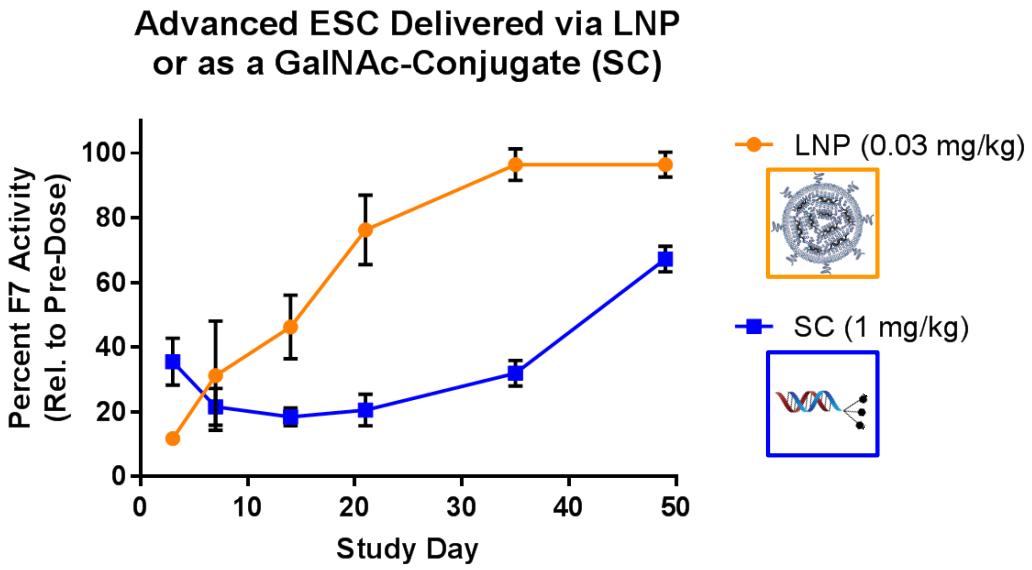
Increase stability

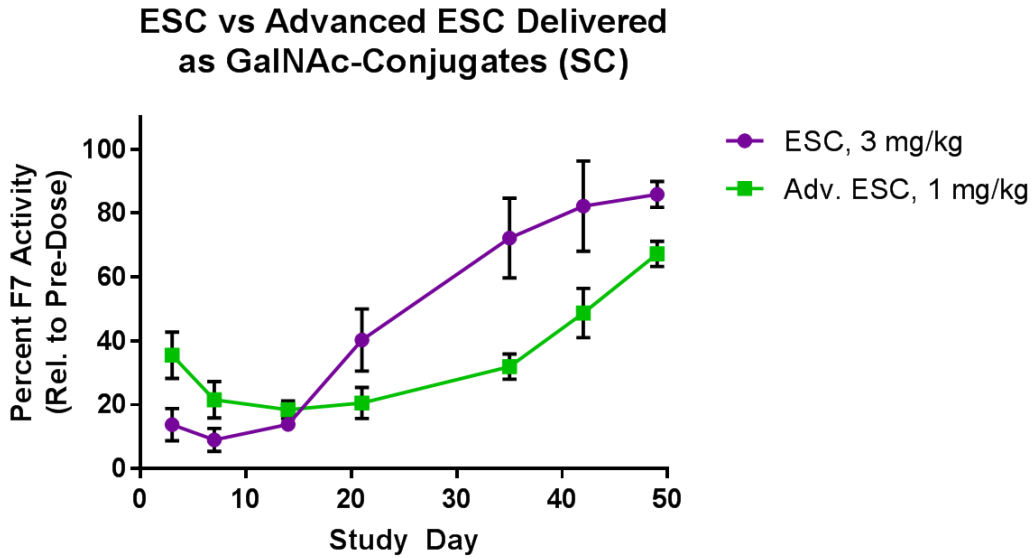


Nair *et al., NAR,* 2017

In Vivo Duration Of Silencing In Mice Is Dependent On Delivery Modality And Stability

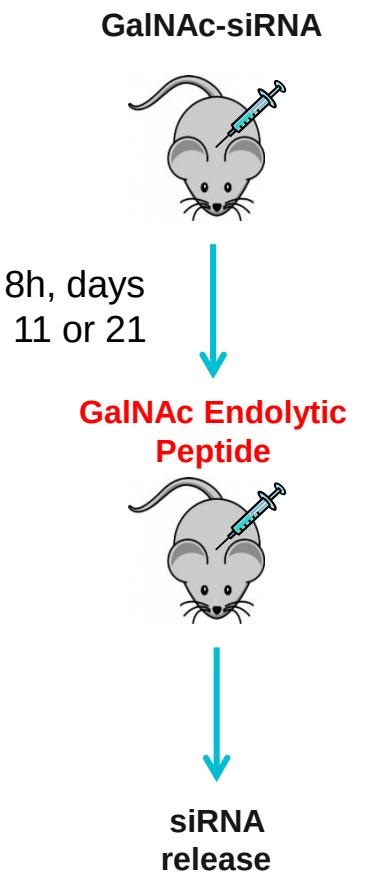
- Unlike GalNAc-siRNA conjugates, LNP designed to promote efficient endosomal escape of siRNAs

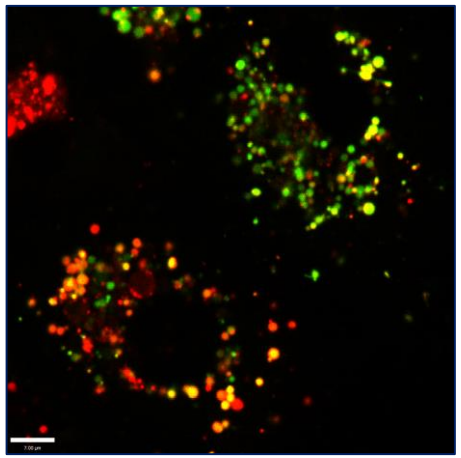




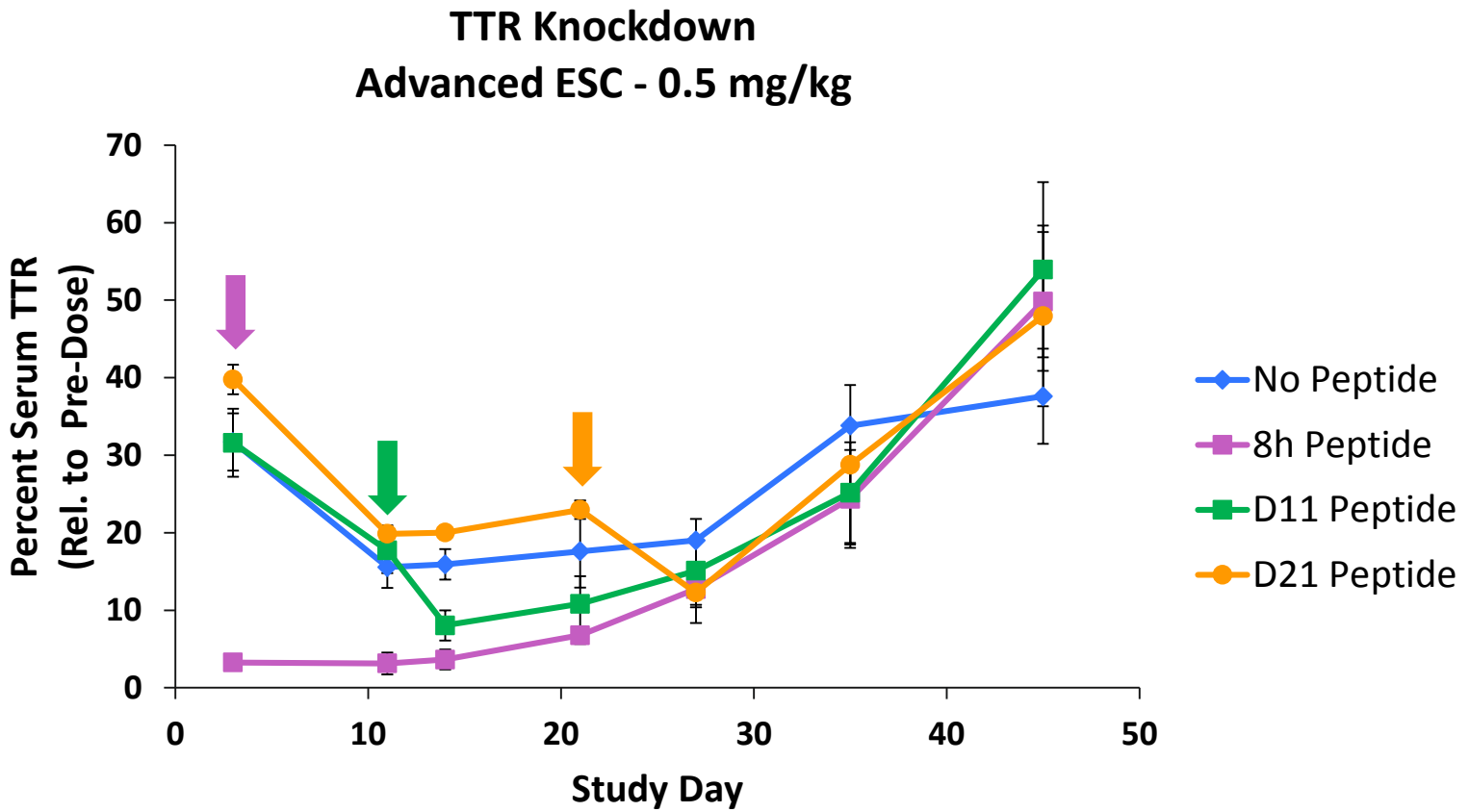
- Doses selected to get similar level of KD and thus similar level of RISC loading expected
- Faster onset and recovery of activity with LNP
- Slower onset but substantially extended duration with GalNAc-conjugate
- Overall data suggests that RISC half-life alone can not explain the duration of activity for conjugates

Functional siRNA Released From Acidic Compartments Up To Three Weeks Post-Dose

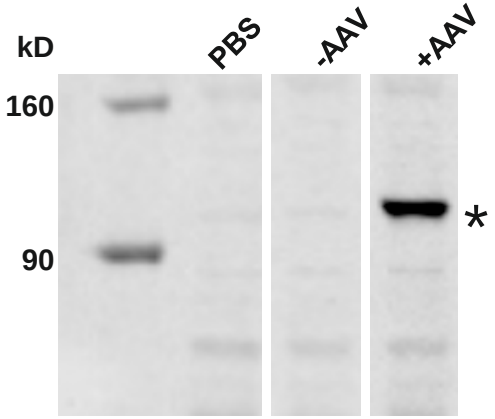


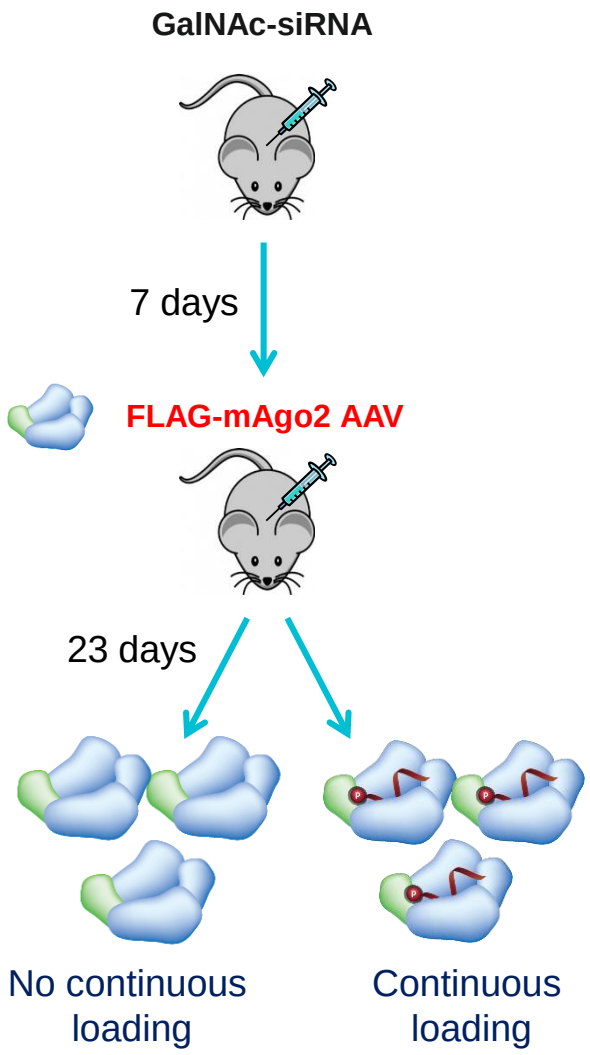


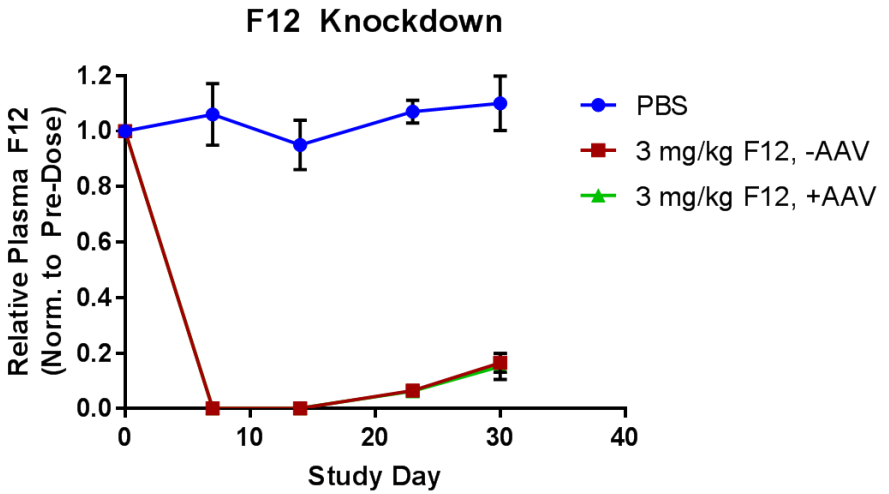
siRNA
Lysopainter

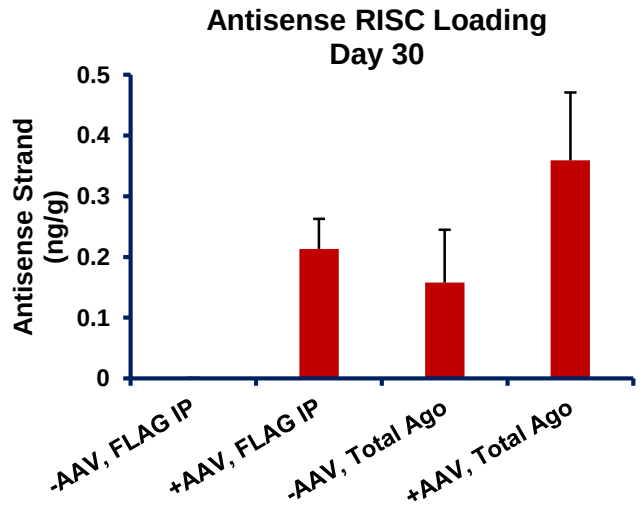


Weeks After Conjugate Dosing, Ectopically Expressed Tagged Ago2 Continues To Load siRNA









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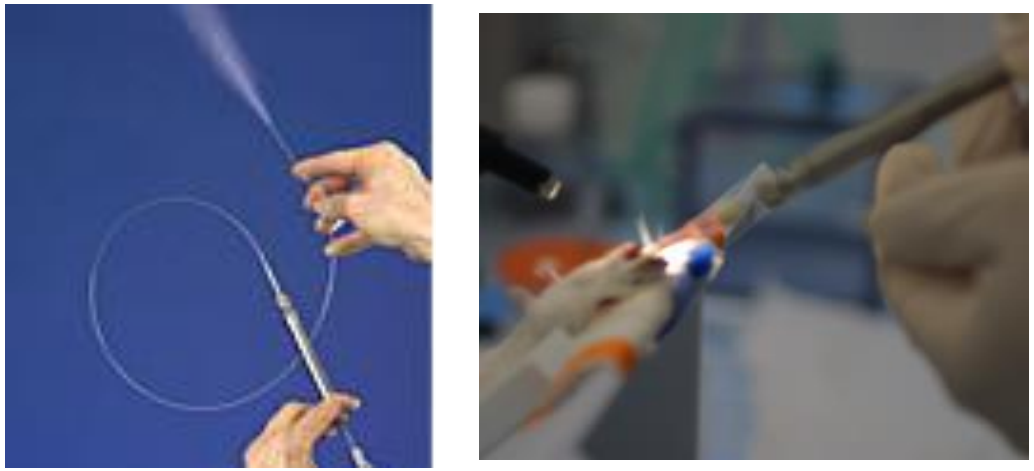
Needle Free Delivery of GalNAc-siRNA via Lung

PoC Demonstrated in Mice Using Microsprayer®

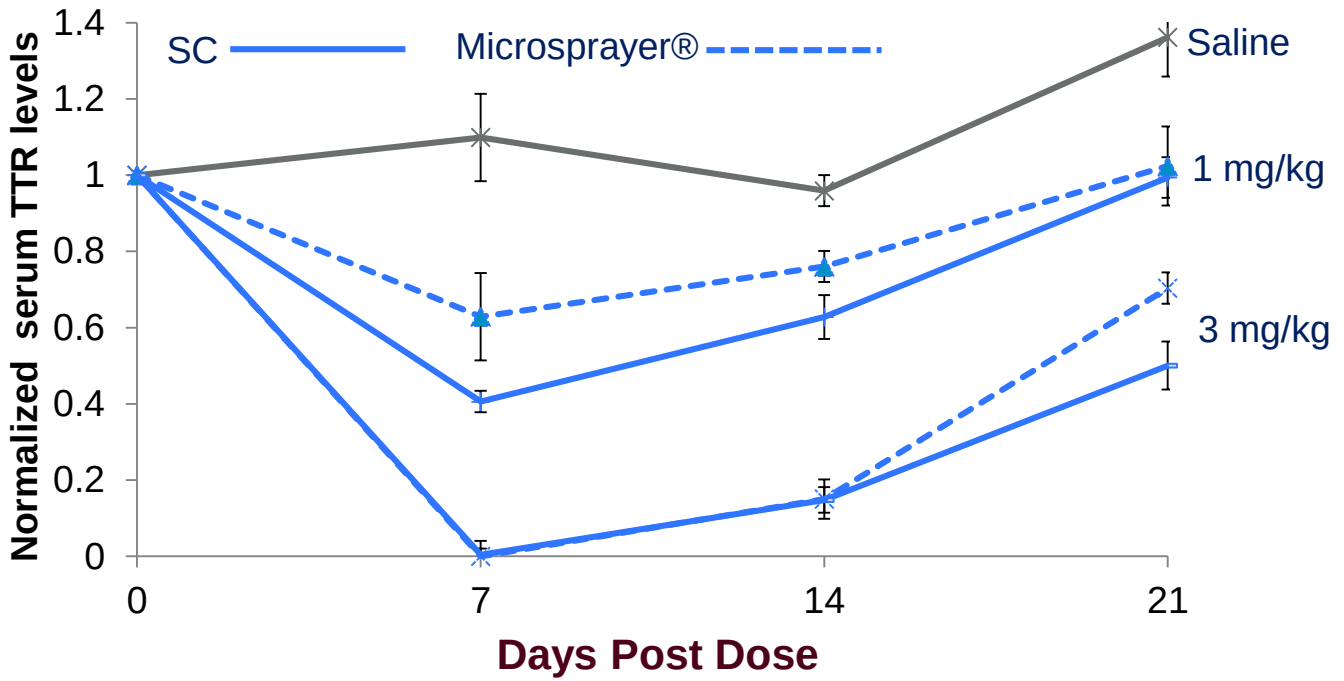
Presented at OTS 2015

Microsprayer® - A high pressure syringe for direct administration of aerosol at the junction of trachea for delivery in lung



Microsprayer® developed by PennCentury

Microsprayer® Mediated Dosing Achieves Comparable Potency and Duration of Activity to SC Delivered ESC Conjugates in Mouse Liver



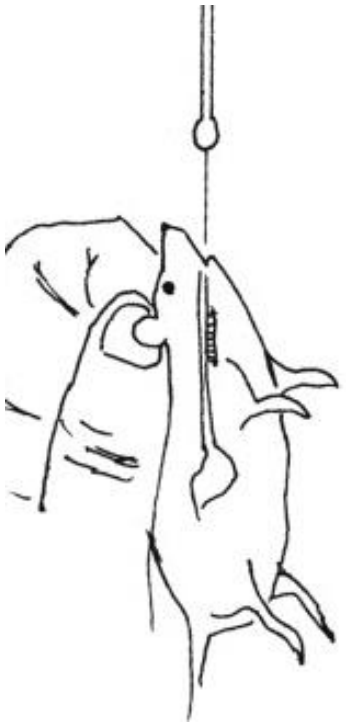
Given the superior potency, metabolic stability and durable activity of GalNAc-siRNA conjugates, would they also work via **Oral Dosing- The least invasive method**?

Systemic exposure of siRNA and LNA-antisense oligos by intra-tracheal dosing. *Molecular Therapy* 2011 **19** (12), 2163–2168

Alnylam PHARMACEUTICALS

PoC for Oral Dosing of GalNAc-siRNA in Mice Delivered via Gavage Tube

The feeding tube is passed gently through the mouth and pharynx into the esophagus to deposit solution in stomach

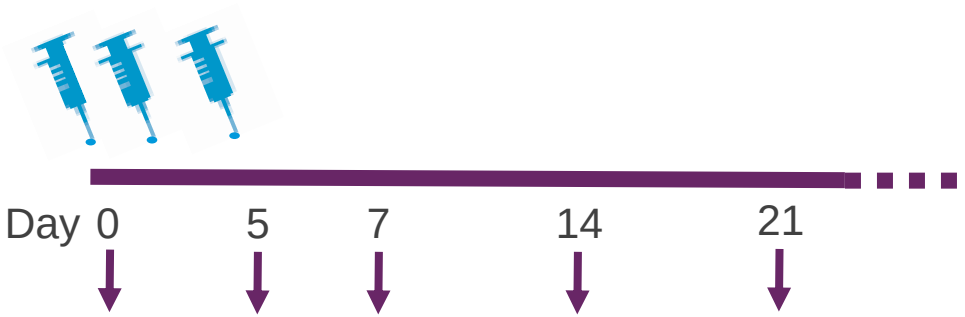


Flexible Plastic Feeding Tubes
Instech's plastic gavage tubes are flexible to reduce trauma

The Laboratory Mouse (2nd Edition)
2012, Pages 709-725

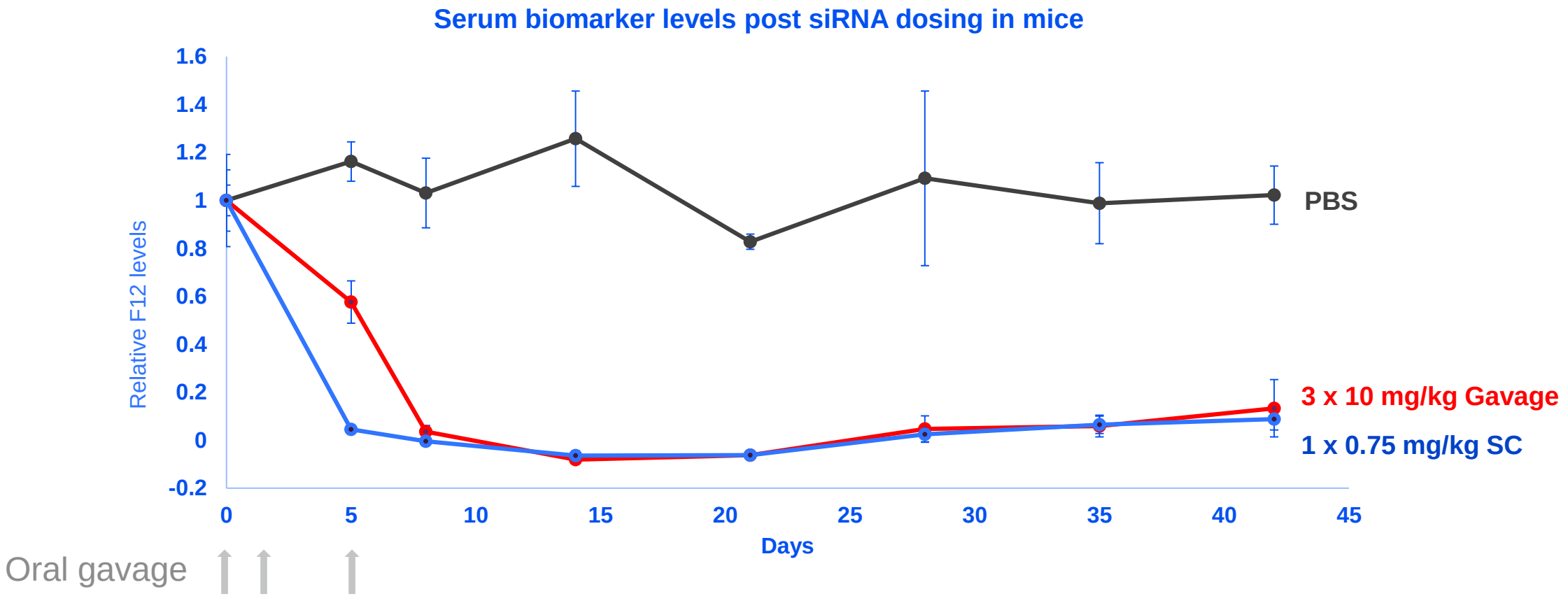
Study design

- ESC siRNA +/- GalNAc
- Formulation containing permeation enhancer
- Single or 3 doses at Day 1, 2 and 5

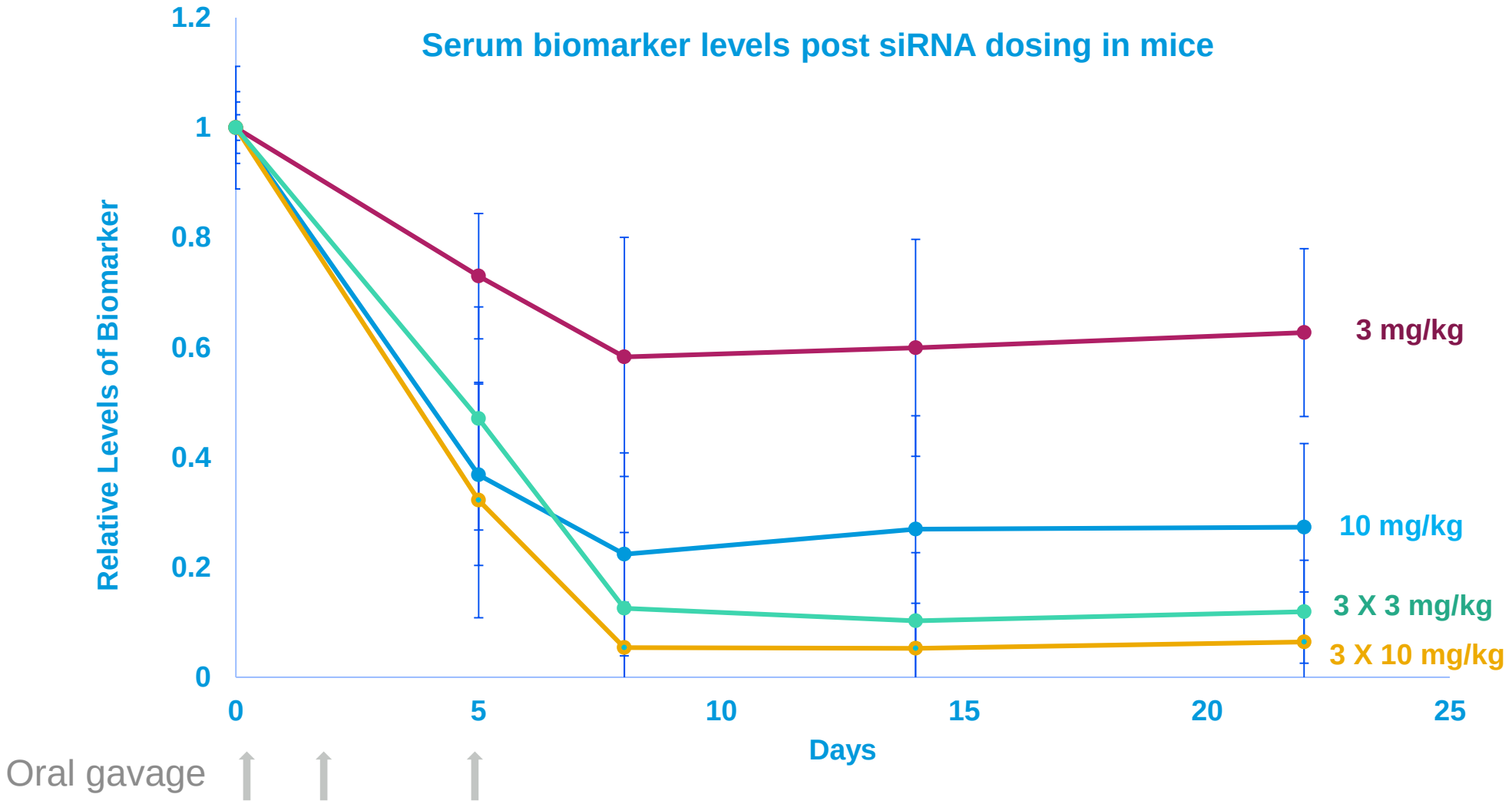


Serum collection for biomarker analysis

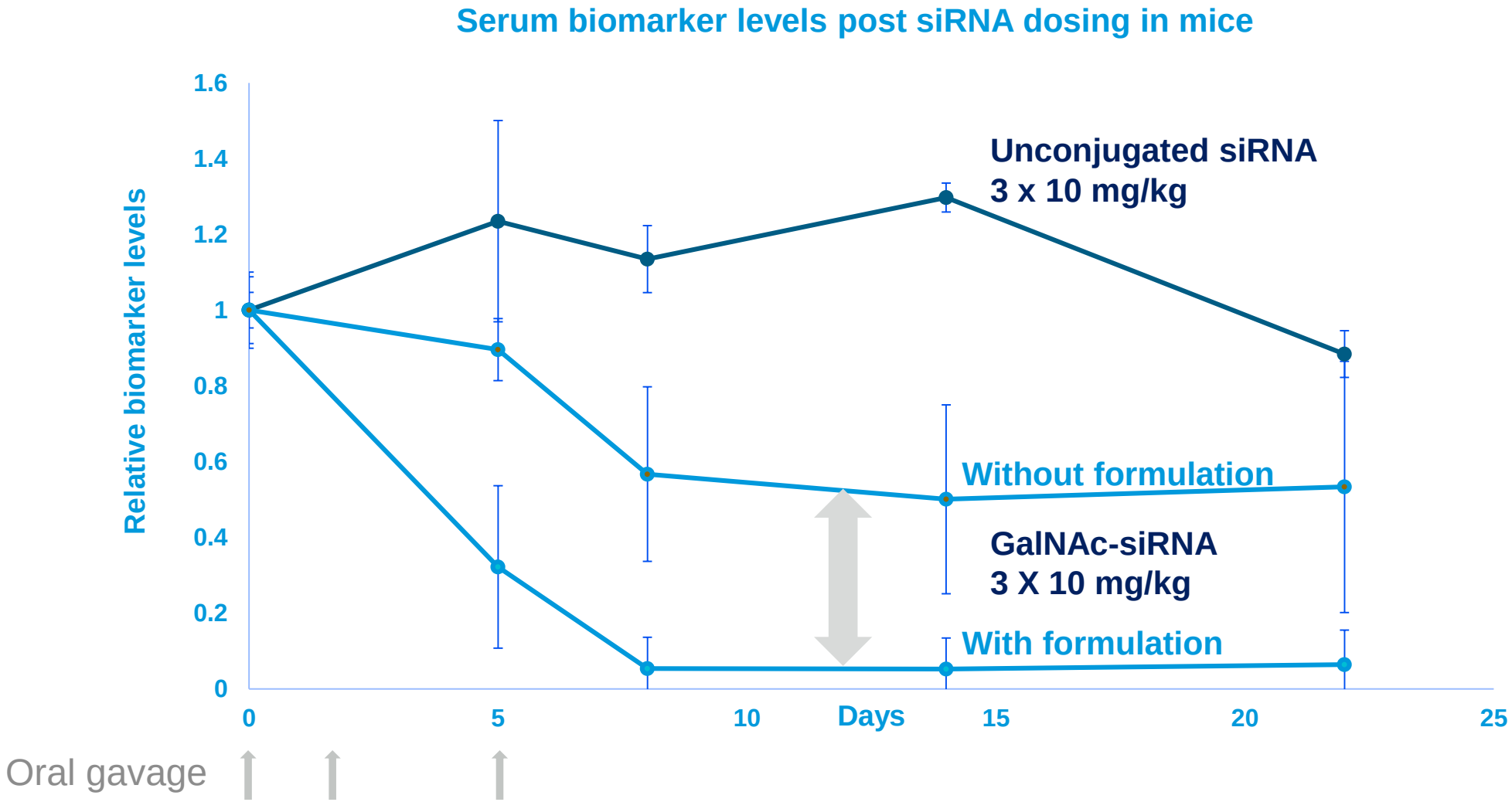
Robust and Durable Activity Seen by Oral Dosing of GalNAc-siRNA in Mice



Dose Dependent Activity Seen by Oral Dosing of GalNAc-siRNA in Mice



GalNAc Conjugation and Formulation are Important for siRNA Activity via Oral Dosing



Summary

RNAi therapeutics emerging as high impact, transformational medicines

- ONPATTRO® as 1st RNAi therapeutic is now in market serving patients
- Multiple RNAi therapeutics are in advanced stages of clinical development

New frontiers for future expansion of RNAi therapeutics opportunity

- Delivery of RNAi therapeutics to CNS and eye achieved
- Our learnings in the liver apply!!

Preclinical data suggests durability of GalNAc-siRNAs likely from continuous supply of siRNA from intracellular depot

Achieved PoC for oral dosing of GalNAc-siRNA conjugates- the least invasive method of drug administration

- Convenience of conventional dosing for modern medicine

Acknowledgements

Participating volunteers, patients and their families

Alnylam colleagues:
- Research Department
- Early Development
- RNAi Platform

MGH:
- Dr. Brown lab

Thank you!