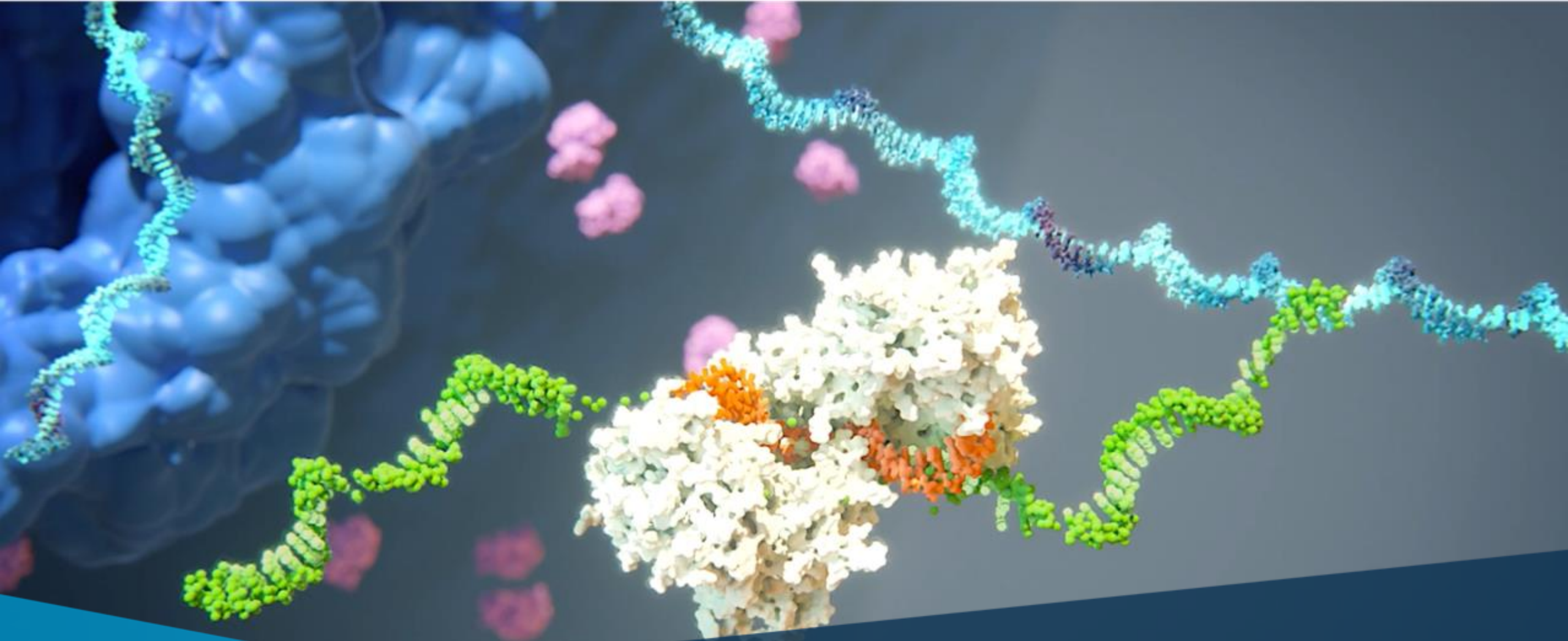


Advancing the Safety and Reach of RNAi Therapeutics

Maja Janas De Angelis, PhD, DABT
Associate Director, Early Development
OTS 2020

Alnylam PHARMACEUTICALS

Thank You Alnylam Team, Past and Present


Martin Maier


Vasant Jadhav


Scott Barros


Natalie Keirstead


Jing-Tao Wu


Pete Smith


Kevin Fitzgerald


Yongfeng Jiang


Saket Agarwal


Lei Johnson


Onur Yilmaz

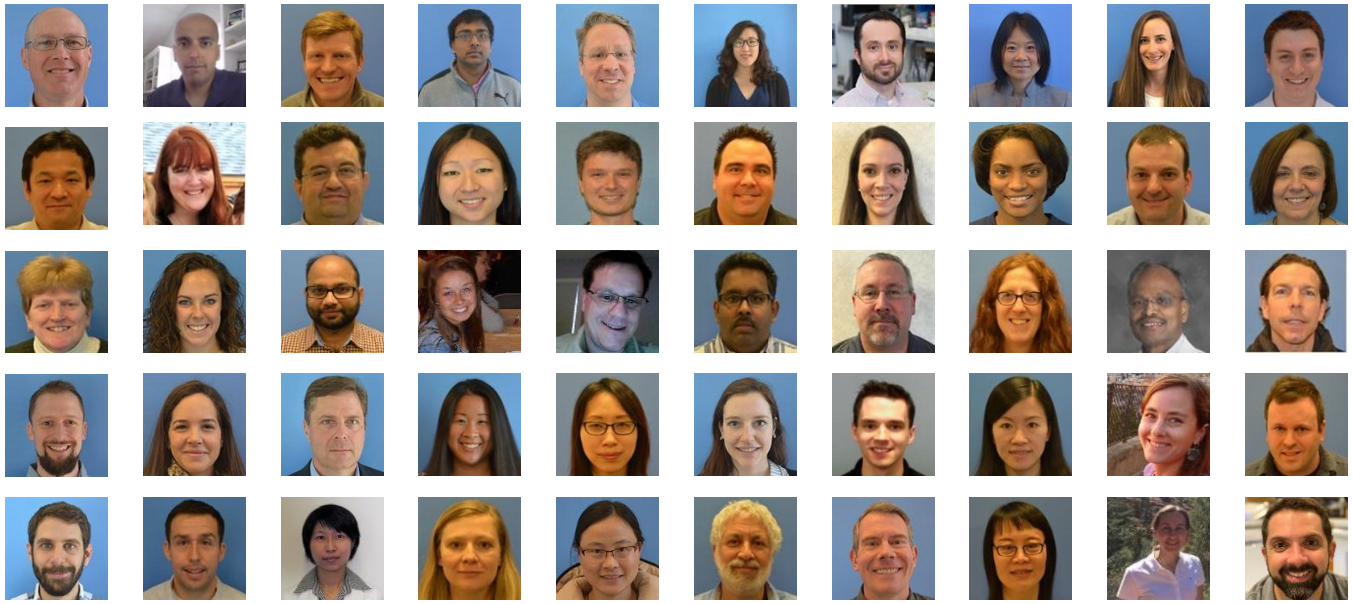

Sam Chigas


Shreya Jadhav


Annabelle Sangree

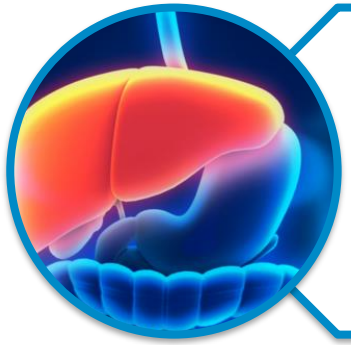

Mark Schlegel


Ivan Zlatev



- Medicinal Chemistry
- RNA Synthesis and Annealing
- RNAi Lead Discovery
- RNAi Biology and Bioinformatics
- In Vivo Sciences
- Pathology
- Bioanalytical Sciences and DMPK

Agenda



Optimizing the safety of liver-targeted GalNAc-siRNA conjugates

Translating liver learnings to CNS-targeted siRNA conjugates

Safety Profile of GalNAc-siRNAs to Date

ORION-10+11 Pooled Data: Safety and Tolerability of Inclisiran

No Evidence of Liver, Kidney, Muscle or Platelet Toxicity

Laboratory tests Safety population[1]		Placebo N = 1,582		Inclisiran N = 1,592	
Liver function	ALT >3x ULN	6	(0.38%)	6	(0.38%)
	AST >3x ULN	9	(0.57%)	6	(0.38%)
	ALP >2x ULN	5	(0.32%)	6	(0.38%)
	Bilirubin >2x ULN[2]	11	(0.70%)	10	(0.63%)
Kidney function	Creatinine >2 mg/dL	41	(2.59%)	35	(2.20%)
Muscle	CK >5x ULN	17	(1.07%)	20	(1.26%)
Hematology	Platelet count <75x10^9/L	1	(0.06%)	1	(0.06%)

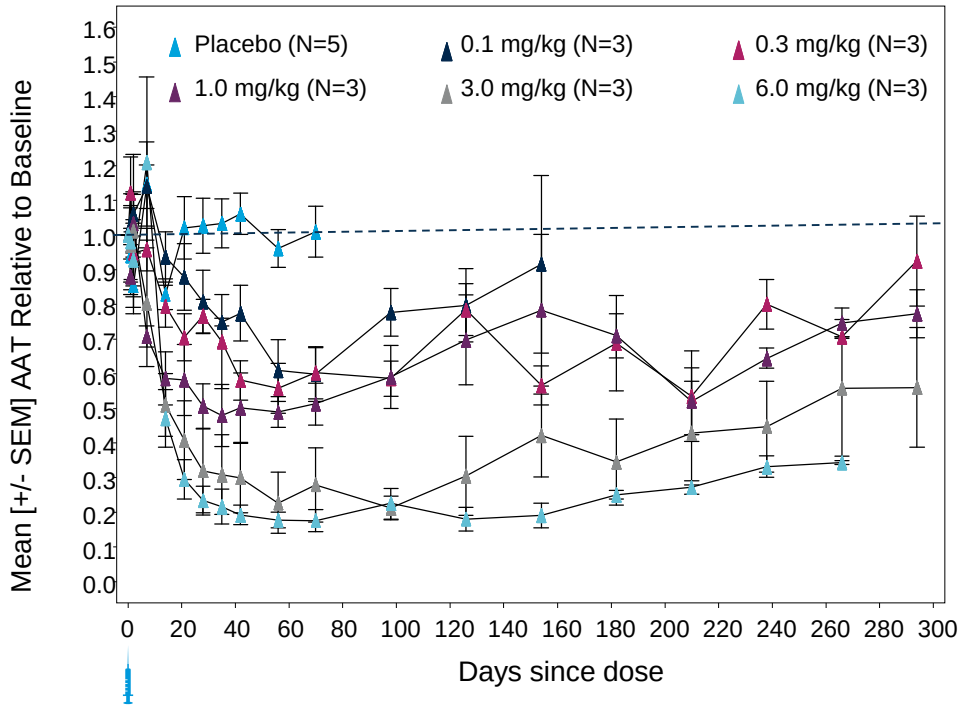
[1] Safety population includes all patients who received at least 1 dose of study medication and individual patients may be counted in more than one category

[2] No cases met Hy's Law

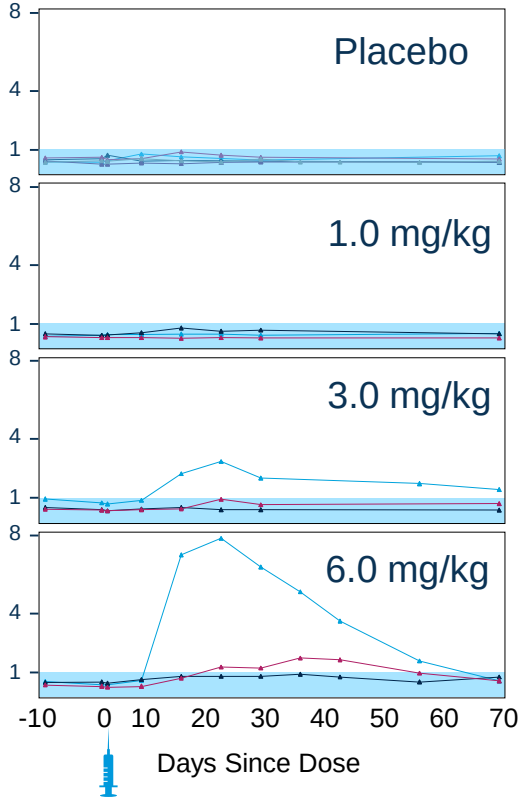
Characteristics of the Human LFT Signal with Subset of GalNAc Conjugates: Only Observed with Few Compounds Suggesting Sequence Specificity

ALN-AAT01 Phase 1/2 Interim Results

PD: Serum AAT Protein Levels

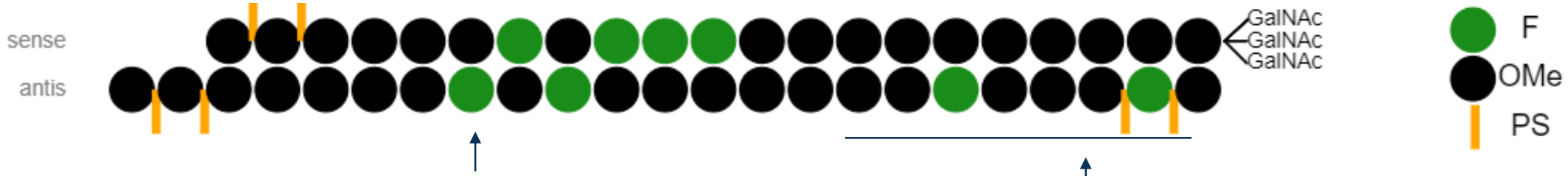


Safety: Serum ALT Levels



- Anecdotal evidence for RISC-mediated mechanism:
  - Onset of LFT elevations coincides with onset of maximum RNAi activity
  - High target knockdown appears necessary (but not sufficient) for LFT elevations
- Similar profile seen in few other programs with sporadic LFT elevations

Safety Considerations for RNAi Therapeutics



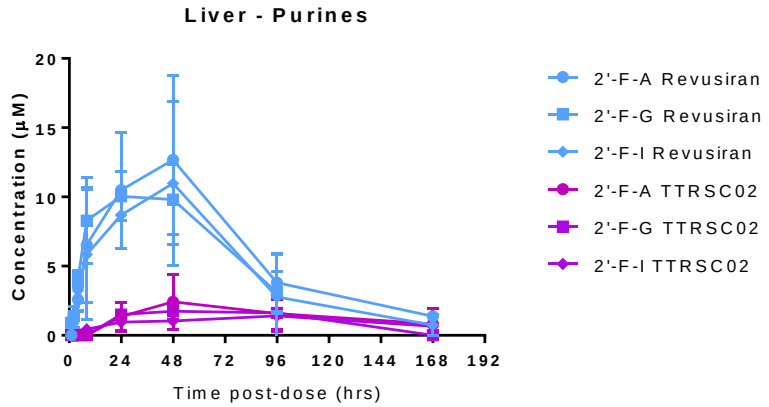
(1) Non-natural chemical modifications, e.g. 2'-fluoro, phosphorothioate

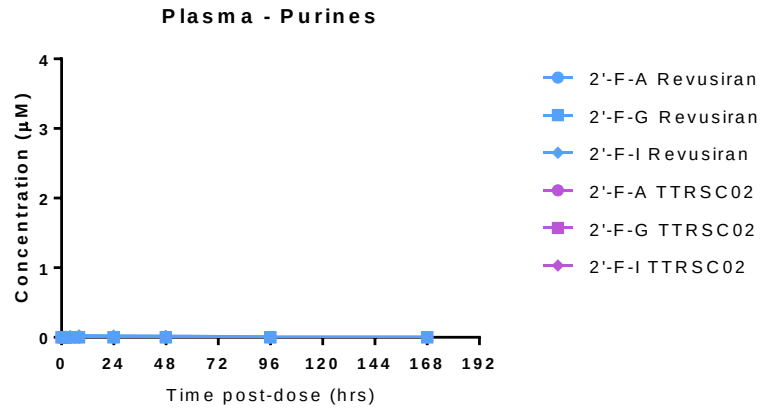
(2) Dysregulation of natural RNAi pathways, e.g. microRNAs

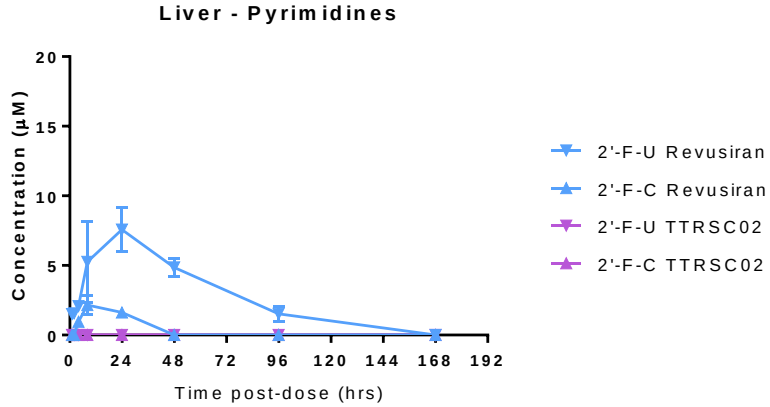
(3) Hybridization-based off-target effects, e.g. microRNA-like seed-based activity

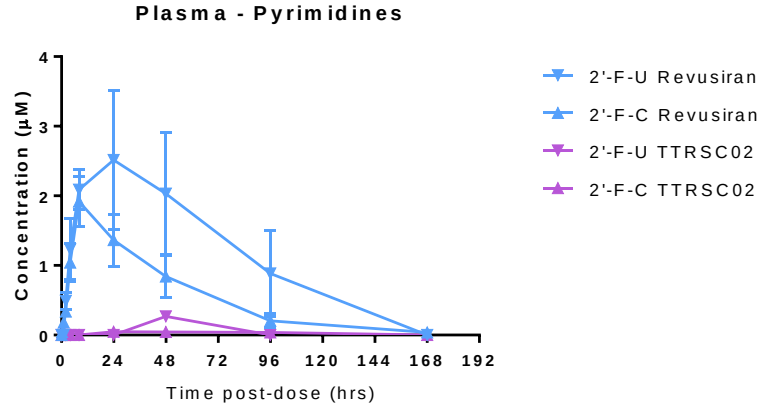
Monomer Generation Is Minimized with the ESC Design *In Vivo*

Rat, 30 mg/kg Single Subcutaneous Dose of **Revusiran** or **ALN-TTRSC02**





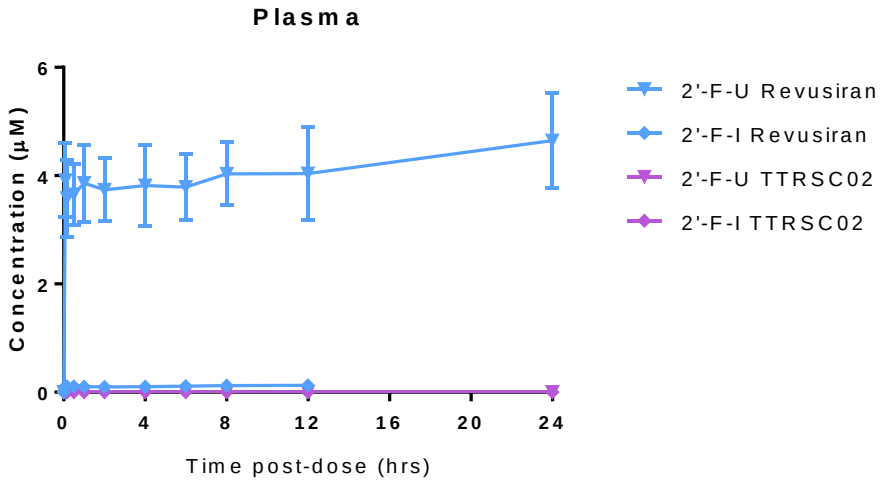


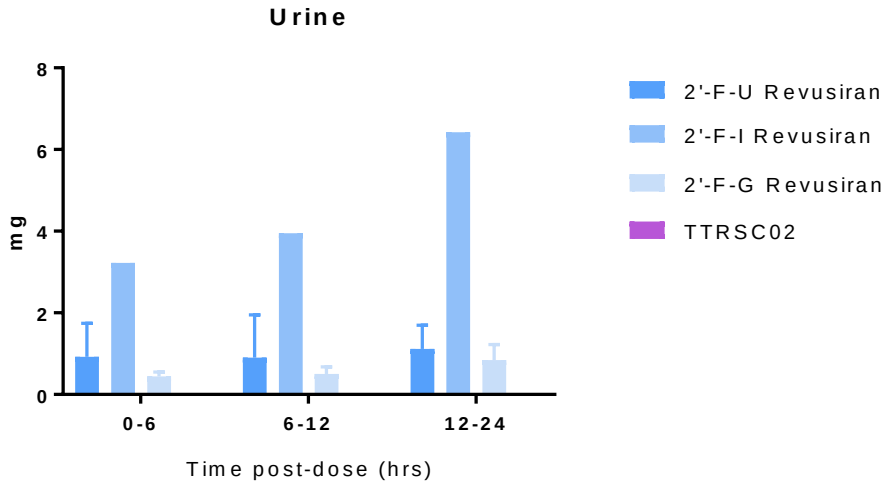


- 2'-F-monomer half-life of 1-2 days indicates that no accumulation is expected with weekly or less frequent dosing
- Only 2'-F-pyrimidines appear to re-distribute systemically in plasma

2'-F-Monomers Are Not Detectable in Human Plasma or Urine at Therapeutically-Relevant Doses of ESC-Conjugate

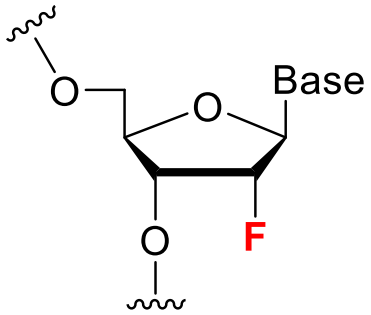
Revusiran: 7.5 mg/kg qd x 5 to healthy volunteers
ALN-TTRSC02: 50 mg (~0.83 mg/kg) single dose to healthy volunteers





- 2'-F-monomers reached steady state in plasma and urine by Day 4, indicating a half-life of ~ 1 day and therefore no accumulation with weekly or less frequent dosing
- Up to ~15 % of total monomer dose is excreted in urine, and therefore even revusiran is likely mainly excreted in oligomeric forms

2'-F-Monomer De-Risking



Nucleic Acids Research, 2019 1
doi: 10.1093/nar/gkz140

Safety evaluation of 2′-deoxy-2′-fluoro nucleotides in GalNAc-siRNA conjugates

Maja M. Janas†, Ivan Zlatev†, Ju Liu, Yongfeng Jiang, Scott A. Barros, Jessica E. Sutherland, Wendell P. Davis, Jingxuan Liu, Christopher R. Brown, Xiumin Liu, Mark K. Schlegel, Lauren Blair, Xuemei Zhang, Biplab Das, Chris Tran, Krishna Aluri, Jing Li, Saket Agarwal, Ramesh Indrakanti, Klaus Charisse, Jayaprakash Nair, Shigeo Matsuda, Kallanthottathil G. Rajeev, Tracy Zimmermann, Laura Sepp-Lorenzino, Yuanxin Xu, Akin Akinc, Kevin Fitzgerald, Akshay K. Vaishnaw, Peter F. Smith, Muthiah Manoharan, Vasant Jadhav, Jing-Tao Wu* and Martin A. Maier*

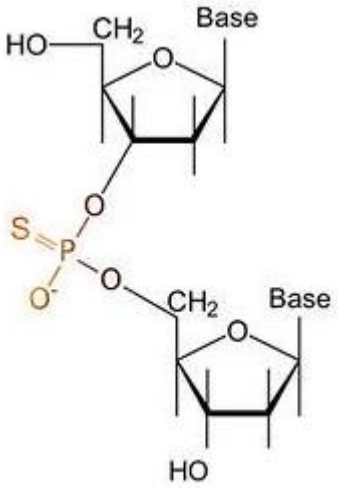
Alnylam Pharmaceuticals, Inc., Cambridge, MA 02142, USA

Received December 27, 2018; Revised February 7, 2019; Editorial Decision February 15, 2019; Accepted February 19, 2019

Monomer generation from STC siRNA in rat and human	**Low**
Monomer generation from ESC siRNA in rat and human	**Mostly undetectable**
Polymerase inhibition (Pol-γ, Pol-α, Pol-β, POLRMT)	**No**
Polymerase substrate (Pol-γ, POLRMT)	**Poor**
Obligate chain termination	**No**
Non-obligate chain termination	**No**
Cytotoxicity and mtDNA effects *in vitro*	**In a subset of cell lines at concentrations > 16-fold higher than generated *in vivo* from STC siRNA**
2-year rat carcinogenicity study with STC siRNAs	**No effects on survival or tumor incidence; no apparent effects on mitochondrial function**

Janas MM, Zlatev, I et al. Nucleic Acids Res. 2019 Apr 23;47(7):3306-3320

Phosphorothioate Modification Is Well-Tolerated in Double-Stranded Oligonucleotides *In Vitro*



NUCLEIC ACID THERAPEUTICS
Volume 00, Number 00, 2016

DOI: 10.1089/nat.2016.0639

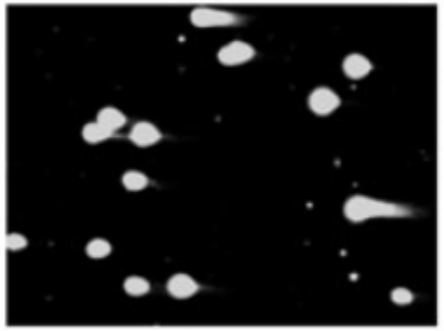
Original Article

Impact of Oligonucleotide Structure, Chemistry, and Delivery Method on *In Vitro* Cytotoxicity

Maja M. Janas,[1] Yongfeng Jiang,[1] Mark K. Schlegel,[1] Scott Waldron,[1] Satya Kuchimanchi,[1] and Scott A. Barros[1,*]

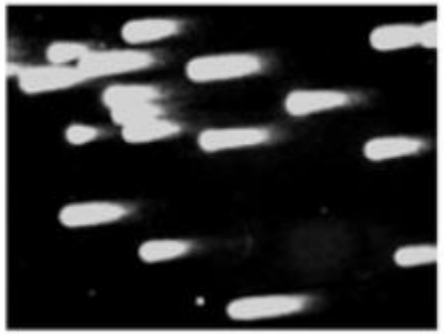
DNA double stranded break assessment after transfection into HeLa cells:

Mock



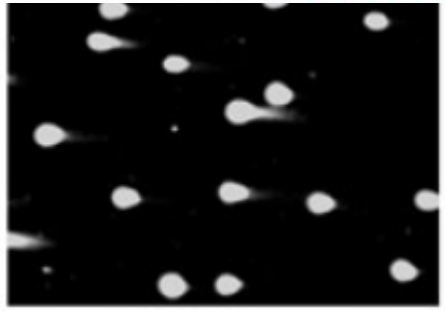
ASO-hiF/hiPSc

CUGCUAGCCTCTGGAUUUGA



siSCR-hiF/hiPSc

GGUUAACACGUUUUAGAUCAA
GACCAAUUGUGCAAAAUCUAGUU



Janas MM et al. Nucleic Acid Ther. 2017 Feb;27(1):11-22

No Evidence of a Class Effect of siRNAs on microRNA Activity

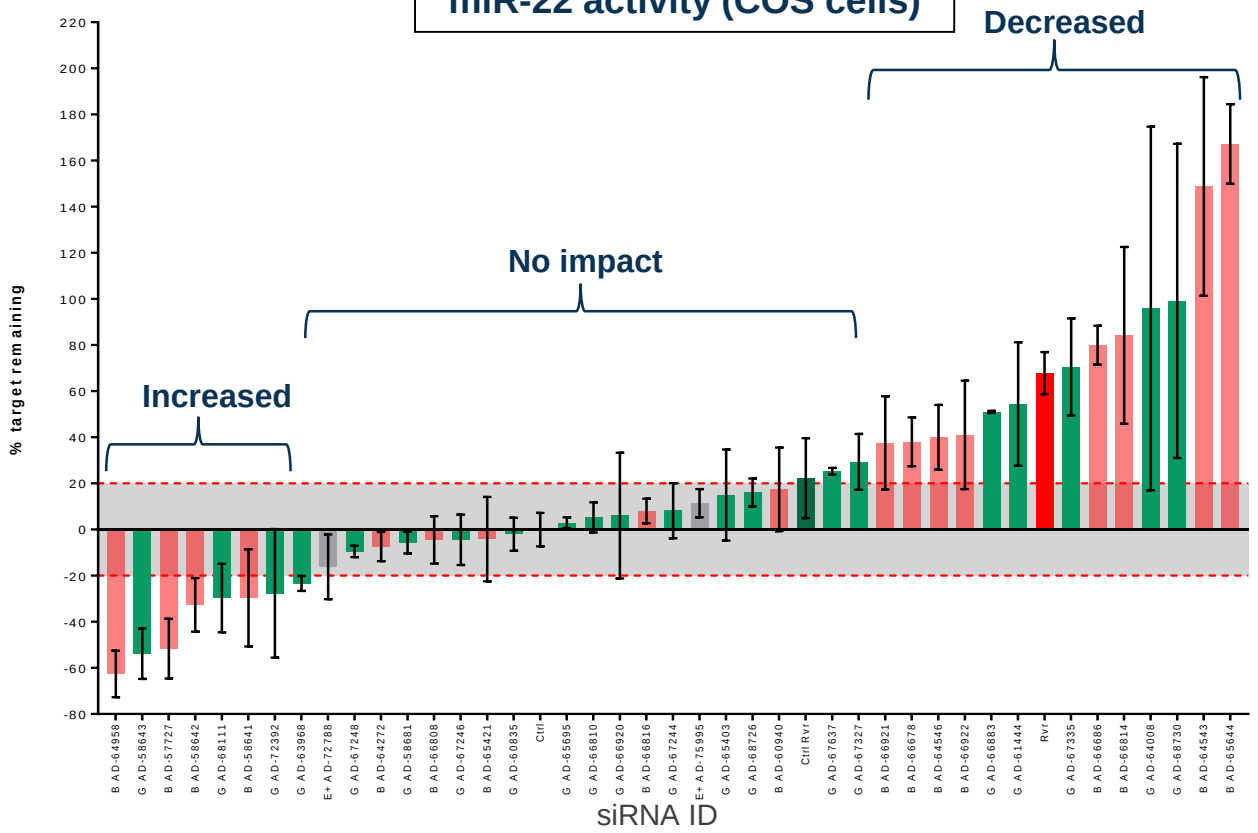
Potential Reasons:

- Relatively low % of cellular RISC pool occupied by siRNA
- Stabilization of additional RISC complexes in the presence of siRNA*

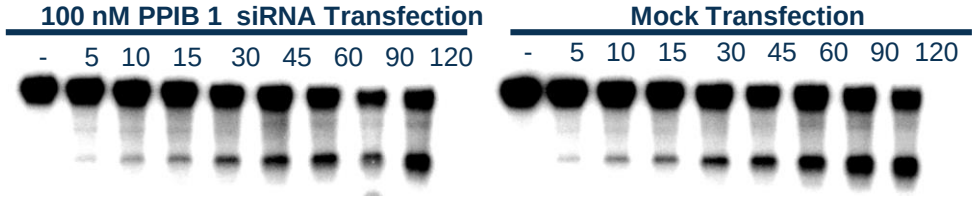
*Nat Struct Mol Biol. 2013 Jul;20(7):789-95; Mol Biol Cell. 2010 May 1;21(9):1462-9; RNA. 2013 May;19(5):605-12

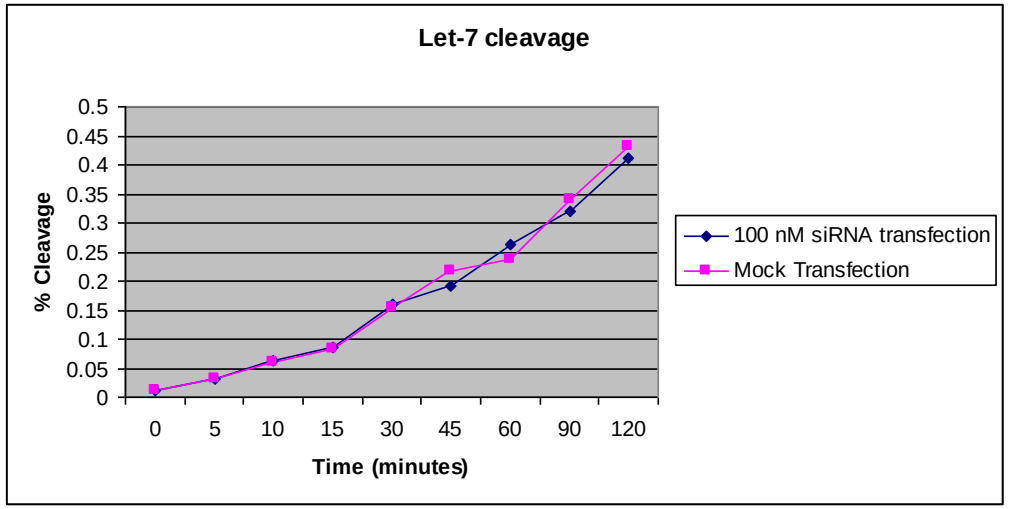
Endogenous microRNA activity assessment after siRNA transfection:

miR-22 activity (COS cells)



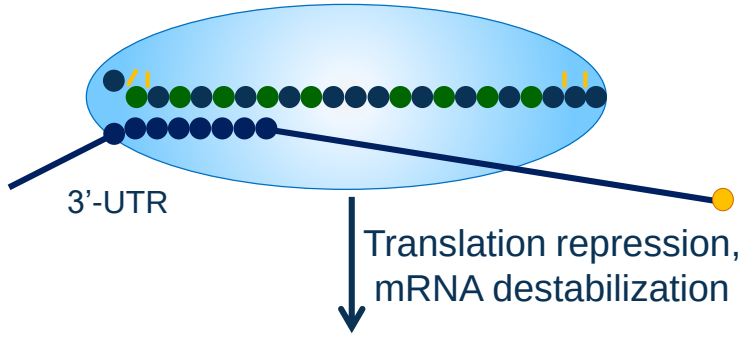
let-7 activity (HeLa cells)

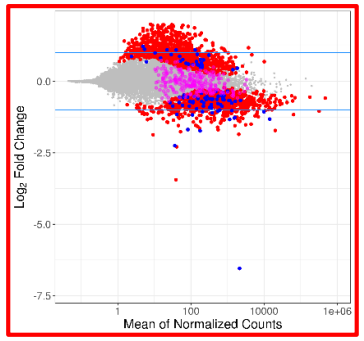




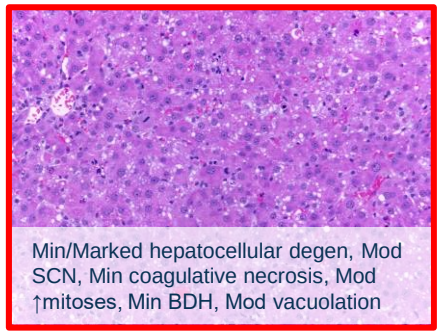
Seed-Based Off-Target Effects Are Important Drivers of Rodent Hepatotoxicity for Subset of GalNAc-siRNAs

miRNA-like seed mediated binding to off-targets

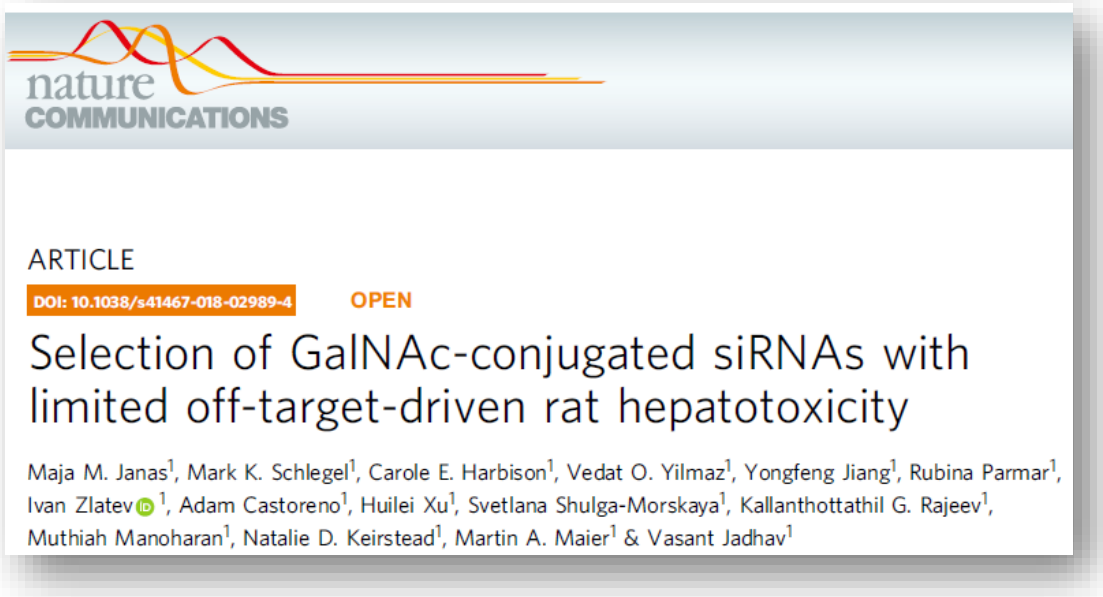




Seed enriched off-target effects observed



Rat NOAEL: 10 mg/kg


nature COMMUNICATIONS

ARTICLE

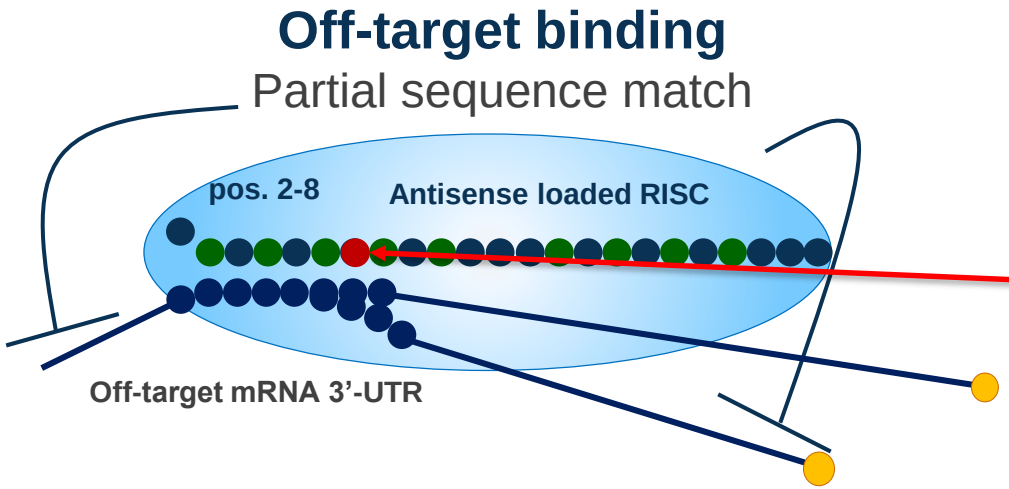
DOI: 10.1038/s41467-018-02989-4 OPEN

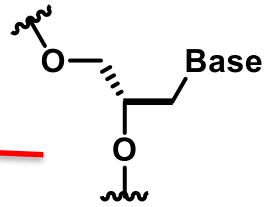
Selection of GalNAc-conjugated siRNAs with limited off-target-driven rat hepatotoxicity

Maja M. Janas[1], Mark K. Schlegel[1], Carole E. Harbison[1], Vedat O. Yilmaz[1], Yongfeng Jiang[1], Rubina Parmar[1], Ivan Zlatev[1], Adam Castoreno[1], Huilei Xu[1], Svetlana Shulga-Morskaya[1], Kallanthottathil G. Rajeev[1], Muthiah Manoharan[1], Natalie D. Keirstead[1], Martin A. Maier[1] & Vasant Jadhav[1]

Janas MM, Schlegel, MK et al. Nat Commun. 2018 Feb 19;9(1):723

ESC+ Seed Pairing Destabilization Strategy to Improve Specificity and Therapeutic Index





(*S*)-GNA

Utilize seed-pairing destabilization via novel chemical modifications to selectively destabilize off-target binding

~~Undesired off-target activity~~

Important Considerations

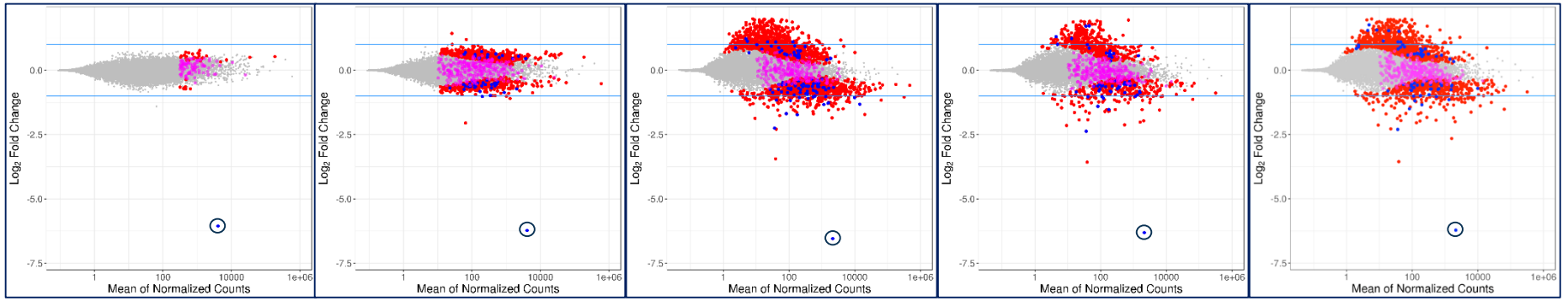
1. On-target potency must be maintained *in vivo*
2. Off-target activity should be minimized

Bramsen et. al. *Nucleic Acids Res*. **2010**
Vaish et. al. *Nucleic Acids Res.* **2011**
Lee et. al. *Nat. Comm.* **2015**
Janas, Schlegel et al. *Nat. Comm.* **2018**

ESC+ Demonstrates More Quiescent Off-Target Signature Across Dose Levels in Rat Liver with Comparable On-Target KD

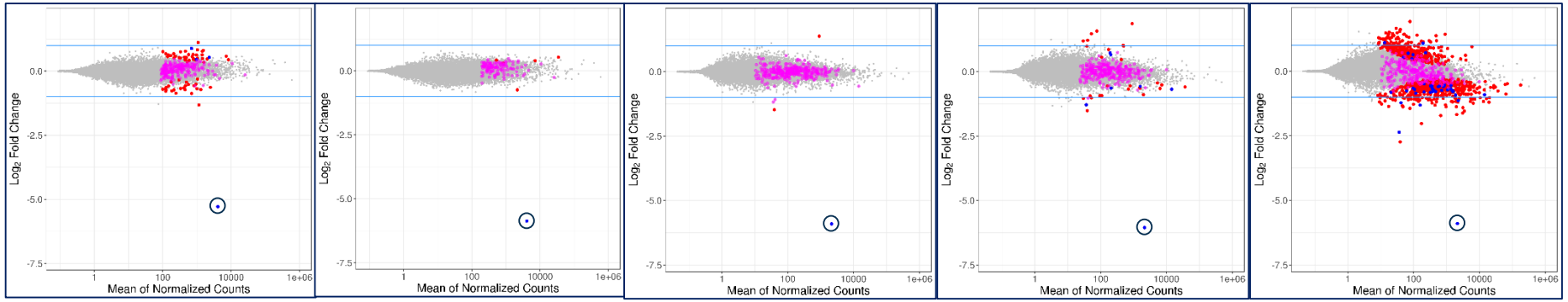
Dose: qw x 3
Necropsy: Day 16

ESC



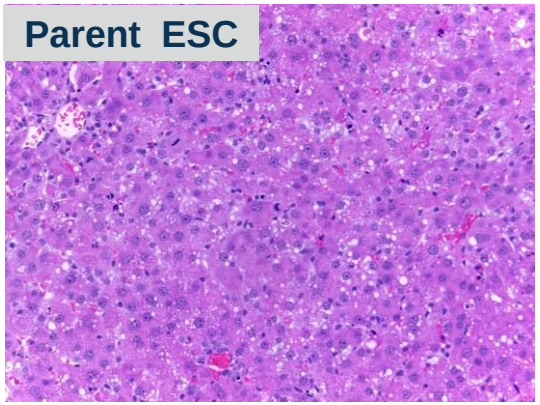
3 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg

ESC+

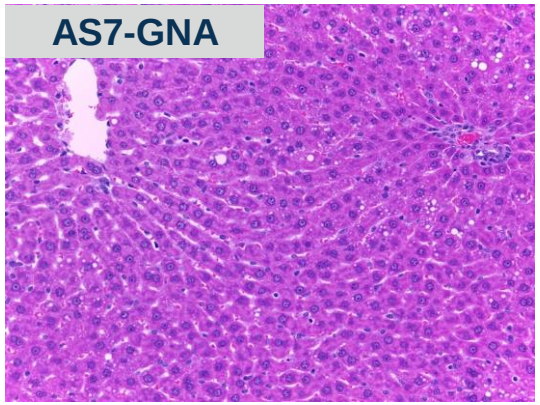


Histopath for 30 mg/kg dose

Parent ESC



AS7-GNA

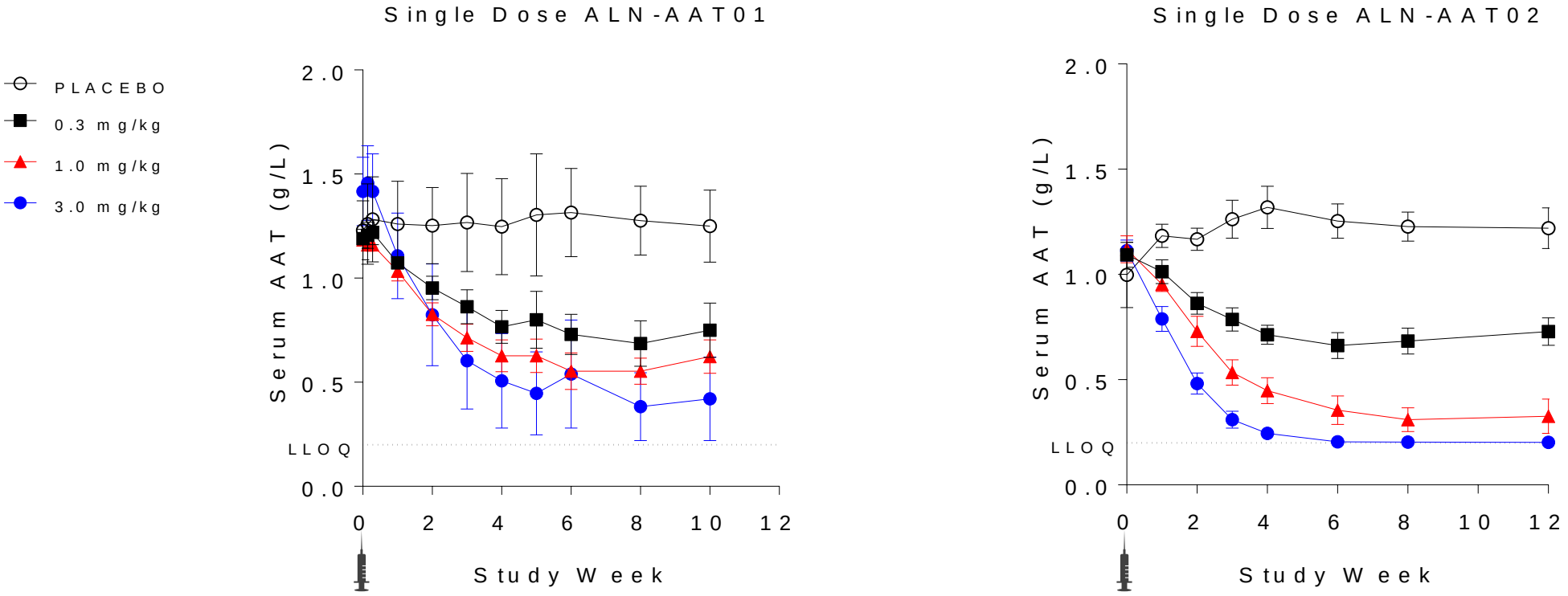


DEGs (Differentially Expressed Genes), significant, 3'UTR match, significant, 3'UTR match, not significant

◯ = target mRNA

Positive ESC+ Human POC

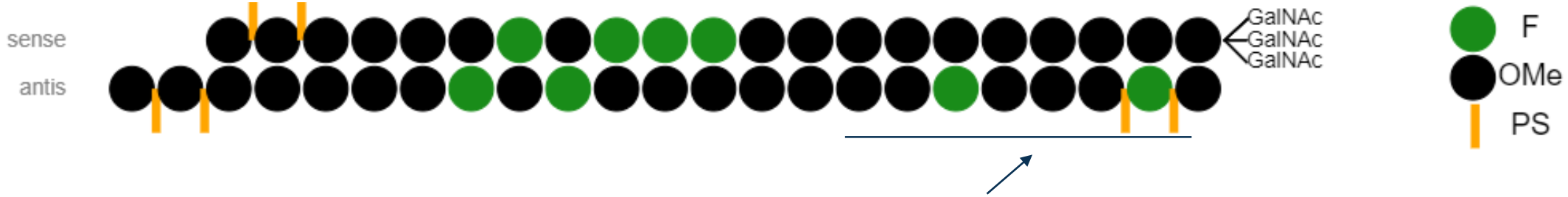
ALN-AAT02 Clinical Activity and Safety



	ALN- AAT01	ALN-AAT02
Structure*		
Efficacy	Up to **89% KD**	Up to **89% KD**
Liver Safety (ALT >3x ULN)	**1/15** (up to 6 mg/kg dose)	**0/18** (up to 6 mg/kg dose)

* Images are representative

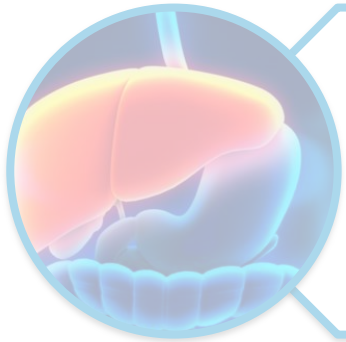
GalNAc-siRNA Safety Summary



Hybridization-based off-target effects, e.g. microRNA-like seed-based activity

- Antisense strand-driven RNAi-mediated off-target effects are important drivers of hepatotoxicity
  - No evidence for impact of chemical modifications on observed toxicity
  - No evidence for microRNA competition
- ESC+ strategy mitigates seed-mediated off-target effects, improves specificity and further expands therapeutic window of siRNA conjugates
  - Utilizes thermally destabilizing nucleotide, such as glycol nucleic acid (GNA), in seed region of antisense strand
  - Multiple ESC+ conjugates have advanced into clinical development
  - Positive human POC achieved
  - Some sequences show an inherent ESC+ profile and may not require further chemical modification

Agenda



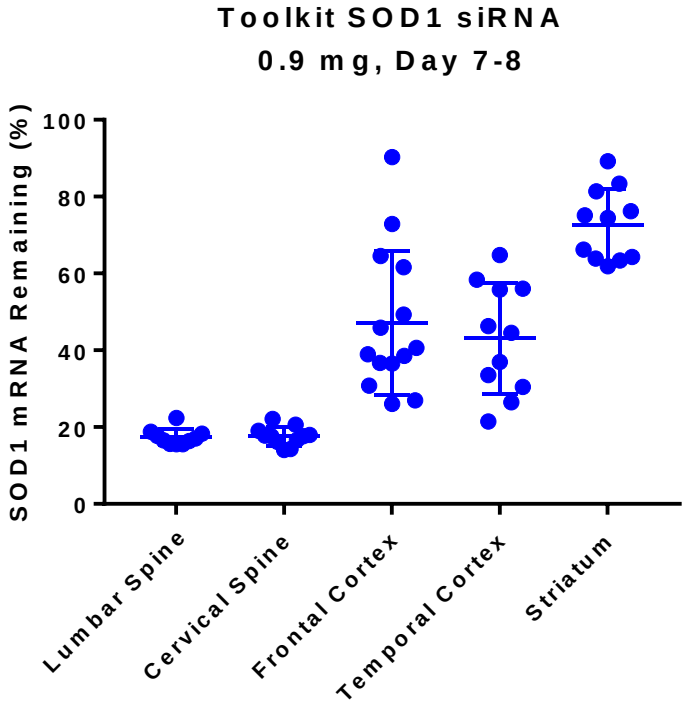
Optimizing the safety of liver-targeted GalNAc-siRNA conjugates

Translating liver learnings to CNS-targeted siRNA conjugates

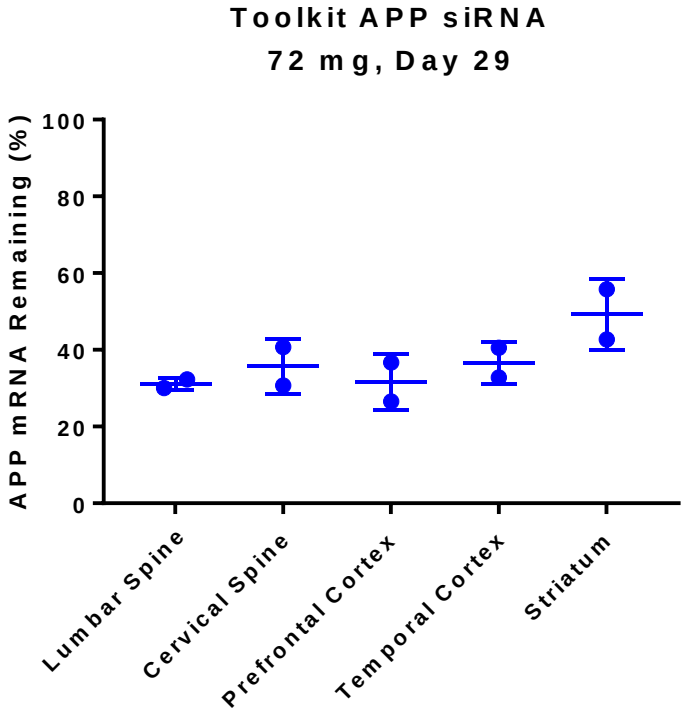
siRNA Conjugate Is Active Across CNS Regions

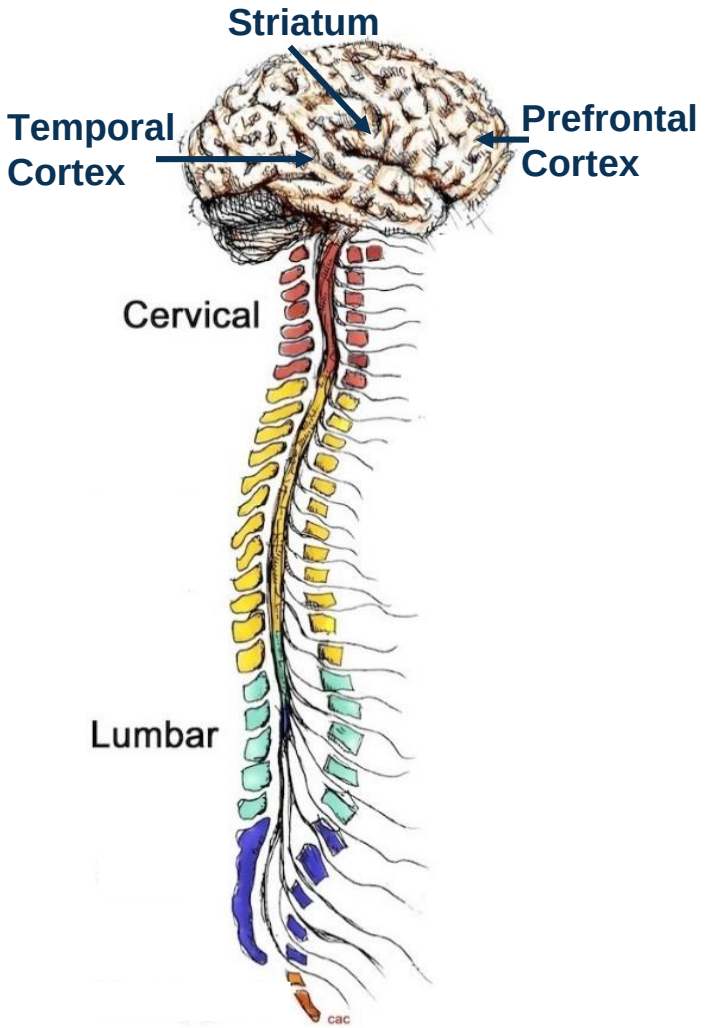
Knockdown Observed in Deep Brain Regions after a Single IT Injection

Rat



NHP



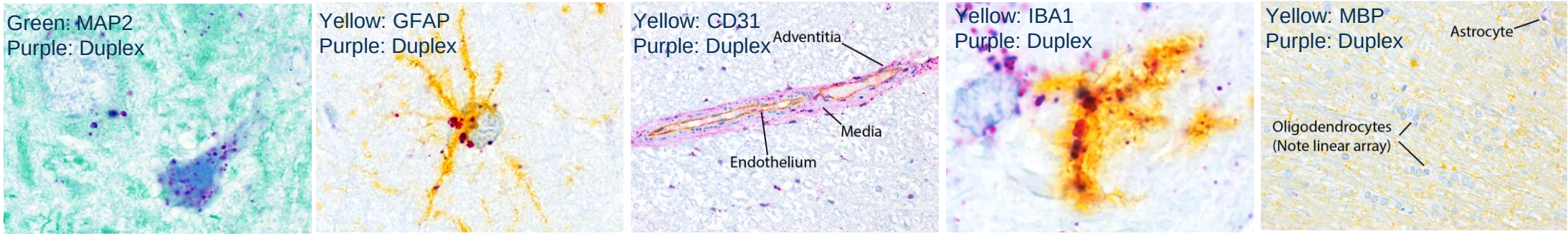


siRNA Conjugate Is Active in Most CNS Cell Types in Rat

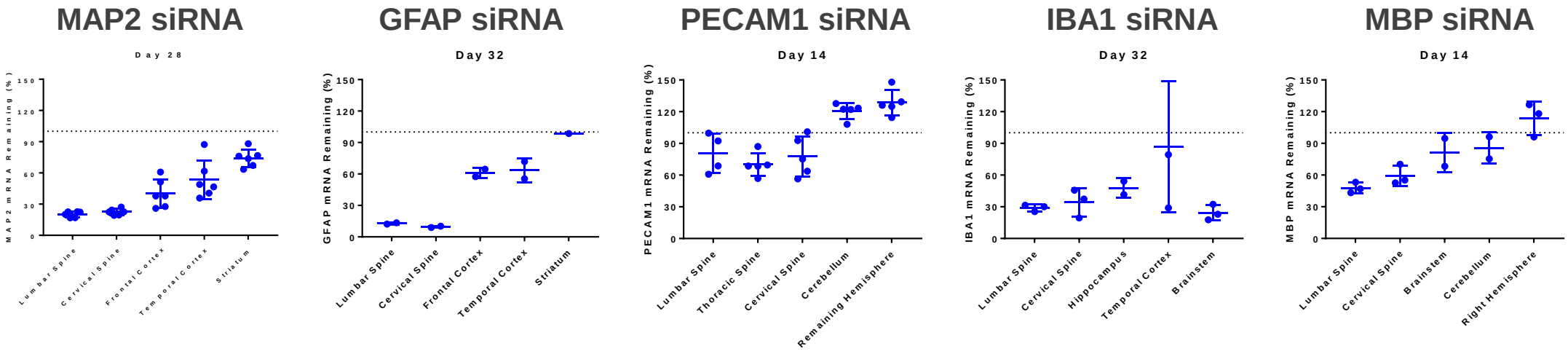
Lowest Activity in the Endothelium

siRNA accumulation

Neuron | Astrocyte | Endothelium | Microglia | Oligodendrocyte

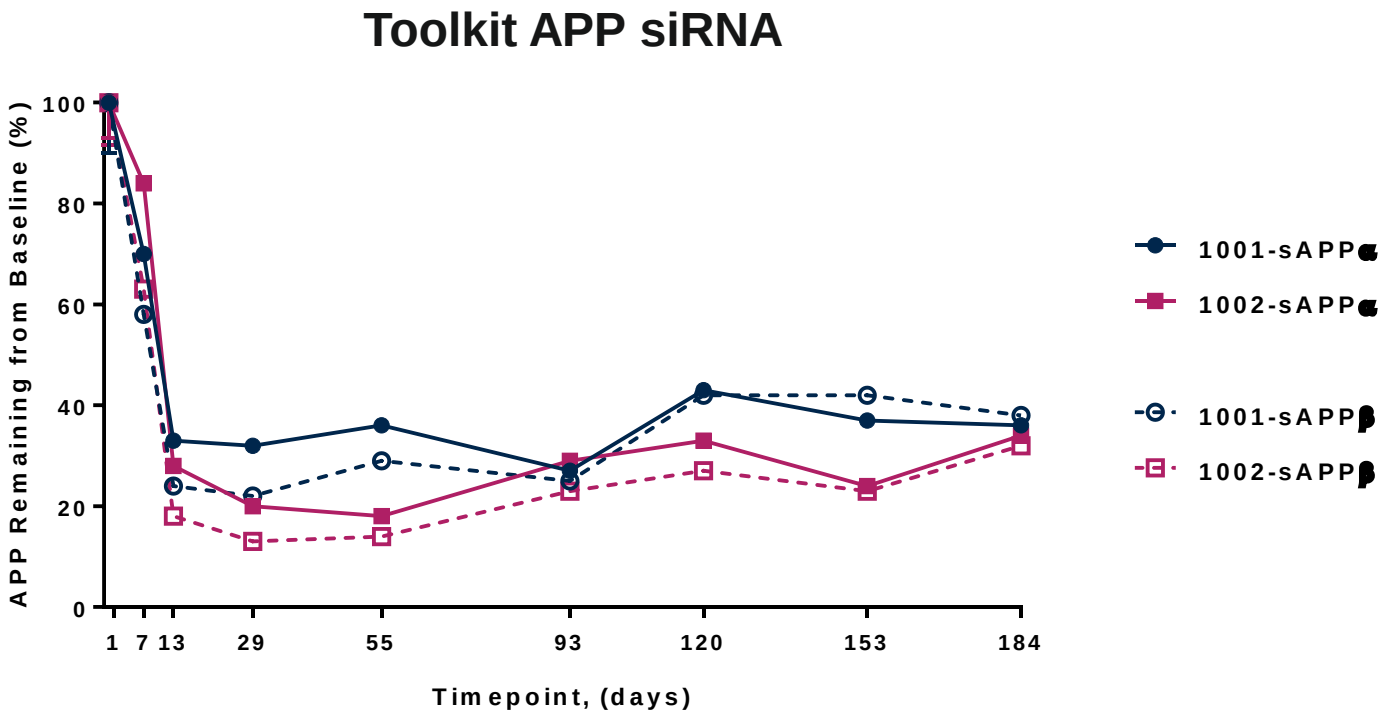


siRNA activity



siRNA Conjugate Is Durable for > 6 Months in NHP

Single IT Injection, 60 mg



CNS Summary

- siRNA conjugates are active across CNS regions, including deep brain structures
- siRNA conjugates are active in most CNS cell types, including neurons, microglia, astrocytes, and oligodendrocytes
- siRNA conjugates have long half-life in the CNS, resulting in durable activity (> 6 months in monkey)
- **No test article-related safety findings to date in rodent or monkey nonclinical studies**

Our Innovative Work Continues…with Physical Distancing