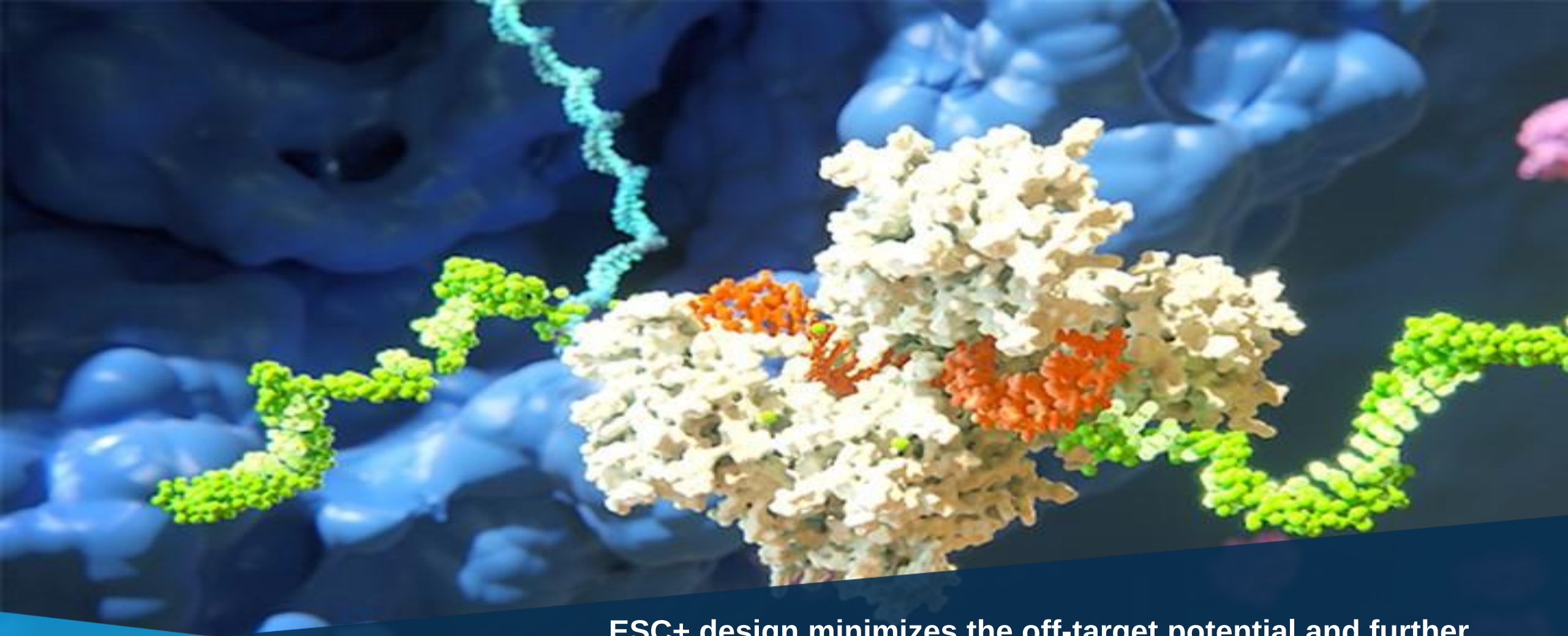


ESC+ design minimizes the off-target potential and further maximizes the therapeutic index of GalNAc-siRNA conjugates

14th Annual Meeting of the Oligonucleotide Therapeutics Society, Oct. 2, *2018*

Martin Maier, PhD
Alnylam Pharmaceuticals

Alnylam® PHARMACEUTICALS

Alnylam Forward Looking Statements

This presentation contains forward-looking statements, within the meaning of Section 27A of the Securities Act of 1933 and Section 21E of the Securities Exchange Act of 1934. There are a number of important factors that could cause actual results to differ materially from the results anticipated by these forward-looking statements. These important factors include our ability to discover and develop novel drug candidates and delivery approaches and successfully demonstrate the efficacy and safety of our product candidates; pre-clinical and clinical results for our product candidates; actions or advice of regulatory agencies; delays, interruptions or failures in the manufacture and supply of our product candidates; our ability to obtain, maintain and protect intellectual property, enforce our intellectual property rights and defend our patent portfolio; our ability to obtain and maintain regulatory approval, pricing and reimbursement for products; our progress in establishing a commercial and ex-United States infrastructure; our ability to successfully launch, market and sell our approved products globally; our ability to successfully expand the indication for ONPATTRO™ (patisiran) in the future; competition from others using similar technology and developing products for similar uses; our ability to manage our growth and operating expenses, obtain additional funding to support our business activities and establish and maintain business alliances; the outcome of litigation; and the risk of government investigations; as well as those risks more fully discussed in our most recent report on Form 10-Q under the caption “Risk Factors.” If one or more of these factors materialize, or if any underlying assumptions prove incorrect, our actual results, performance or achievements may vary materially from any future results, performance or achievements expressed or implied by these forward-looking statements. All forward-looking statements speak only as of the date of this presentation and, except as required by law, we undertake no obligation to update such statements.

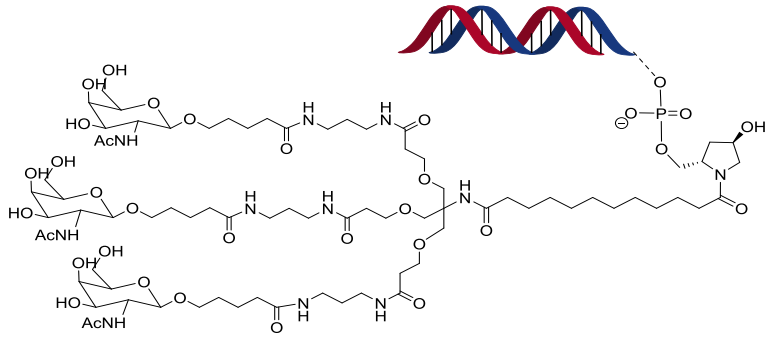
Conflicts of Interest

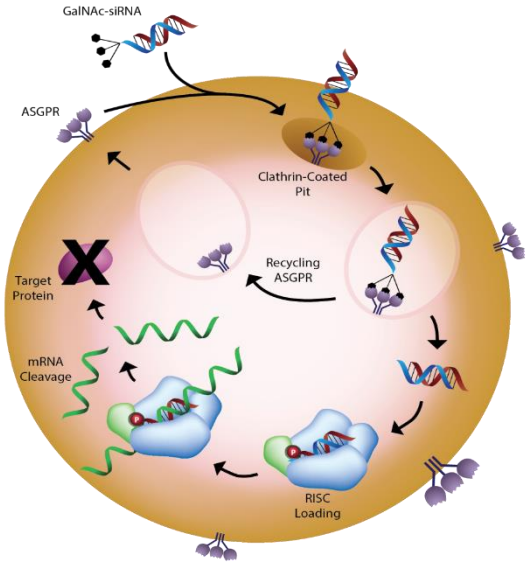
I am an employee of Alnylam Pharmaceuticals

Evolution of GalNAc-Conjugate Designs for Delivery to Liver

GalNAc Conjugates

- Multivalent GalNAc ligand covalently conjugated to siRNA
- Targeted delivery to liver mediated by cell surface receptor (ASGP-R)
- Administered subcutaneously





STC-Conjugate

- Standard Template Chemistry
- SC administration
- Revusiran

- First Generation GalNAc conjugate
- Initial human POC

ESC-Conjugate

- Enhanced Stability Chemistry
- SC administration
- 6 programs in clin. development

- 2nd Gen. GalNAc conjugate
- Human POC
- Greater potency and durability with lower exposures

ESC+ Conjugate

- Enhanced Stability Chemistry
- Increased specificity
- SC administration
- 2018 INDs and CTAs

- Maintained PD (potency/duration)
- Further improvements to specificity and therapeutic index

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Extensive Human Safety Experience

Encouraging Results to Date

Number of Programs	Number of Clinical Studies	Total Patients or Volunteers Dosed	Greatest Duration of Exposure
>10	>25	>1200	>48 months

Minimal platform related findings*

- Low incidence (2.9%) of generally mild, asymptomatic, reversible LFT increases >3x ULN
- Injection site reactions (24%) generally mild, transient and rarely led to discontinuation
  - No events of ulceration, necrosis or tissue damage
- One report of anaphylaxis (<0.05%) in patient with prior history of atopy**
  - No anti-drug antibodies (ADA) detected against GalNAc-siRNA

Revusiran program discontinued in October 2016

- Extensive evaluation showed no clear reason for mortality imbalance
- While possible that imbalance was a chance finding, role for revusiran cannot be excluded
- Revusiran exposure is 12-140 times greater than other pipeline programs

Favorable emerging safety profile for ESC-GalNAc platform

- No evidence of thrombocytopenia, renal toxicity, or systemic inflammatory effects

* Experience as of December 2017 – Data estimated based on available safety data
** Givosiran OLE study, reported April 2018

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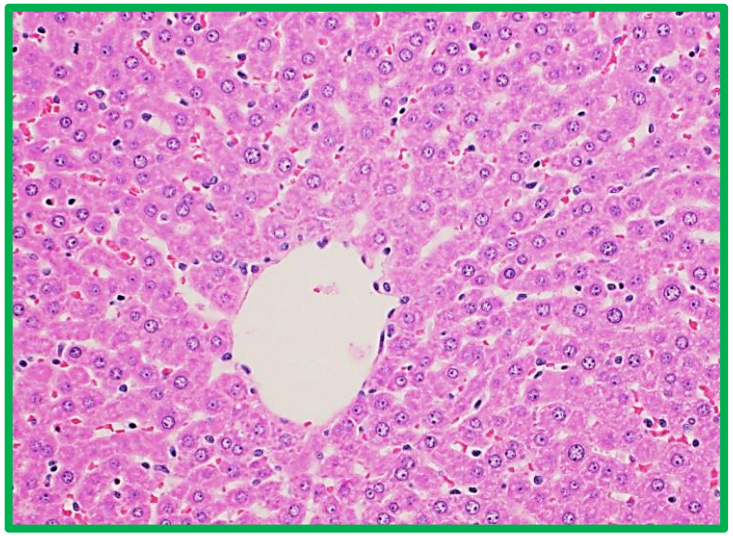
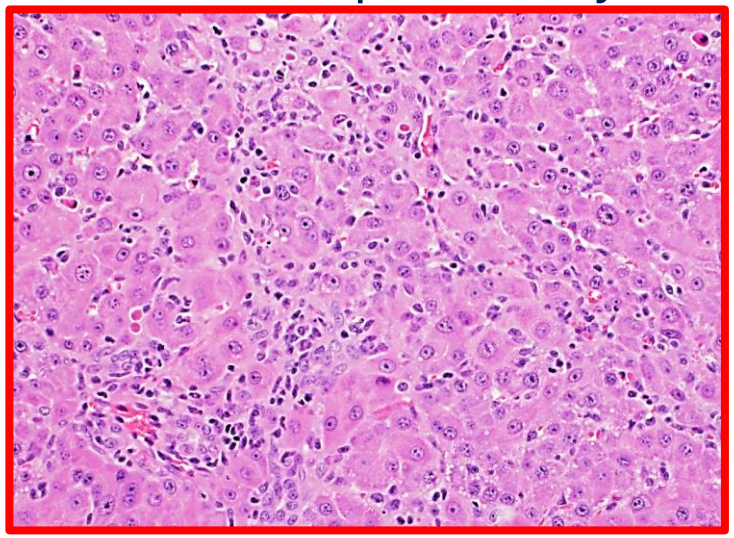
Lead Selection and Conjugate Safety

Target selection → *In silico* prediction *(on-target activity & specificity)* → *In vitro activity & specificity (high homology off-targets)* → Rodent Activity → Rat Tox @ >100x PD dose → NHP Activity → DC

Good Actors (60%):
No hepatotoxicity



Bad Actors (40%):
Show hepatotoxicity



Single cell necrosis and/or hepatocellular degeneration with ↑LFT 2x upper limit of normal

- Subset of conjugates shows rat hepatotoxicity at exaggerated doses and drop out of DC selection process

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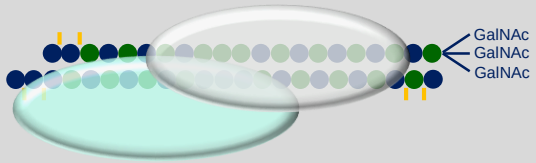
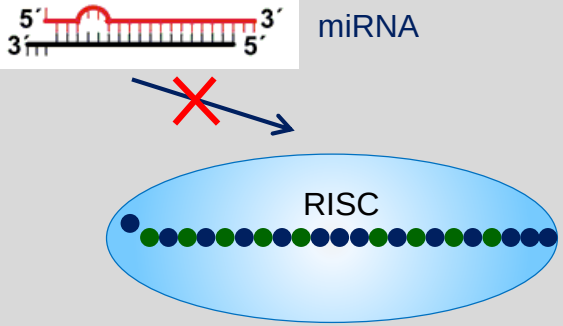
Seed-Based Off-Target Effects Are Important Drivers of Rodent Hepatotoxicity for Subset of Conjugates

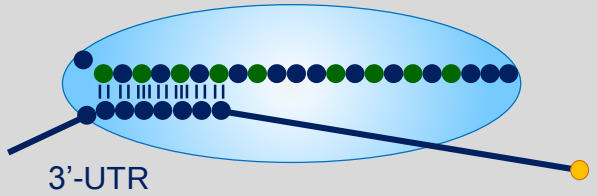
1. Non-RNAi effects
   e.g. siRNA chemistry, metabolites protein binding, drug accumulation



2. Competition for RISC loading with miRNAs



3. Undesired seed-based off-target activity



Off-target binding
Partial sequence match

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, Schlegel et al. *Nat Commun*. **2018**, 9, 723

Poster #015
S. Agrawal et al.
Mechanisms of Rat Hepatotoxicity of GalNAc-siRNA Conjugates

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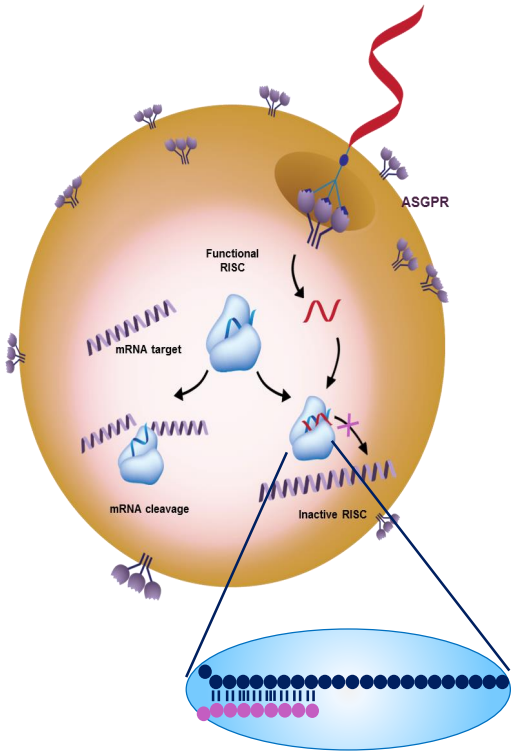
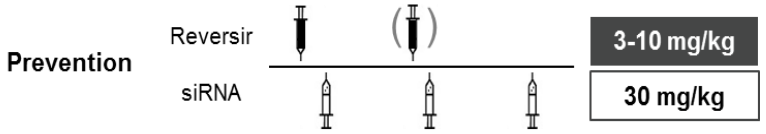
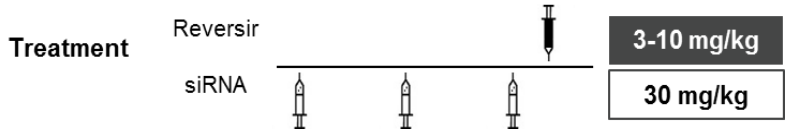
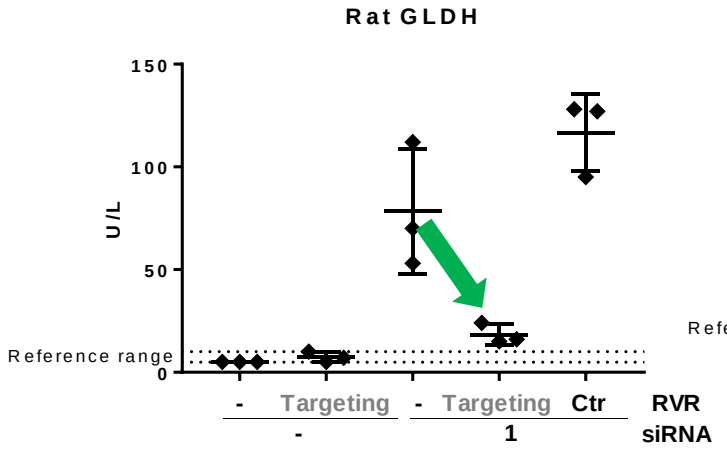
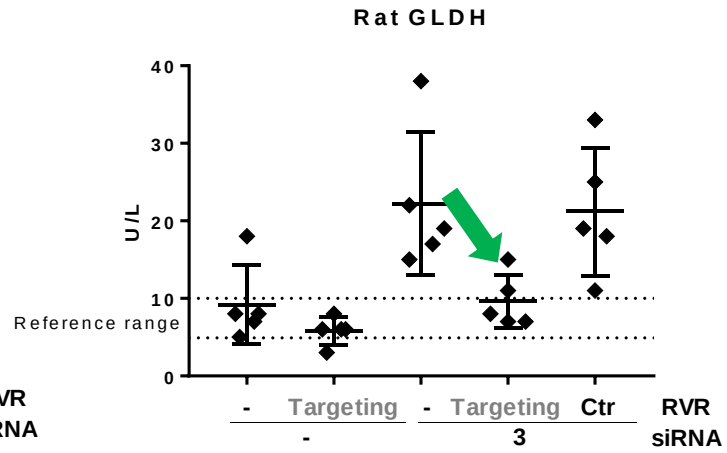
Blocking Activity of RISC-Loaded Antisense Strand Mitigates Hepatotoxicity Without Affecting Liver Exposure or RISC Loading

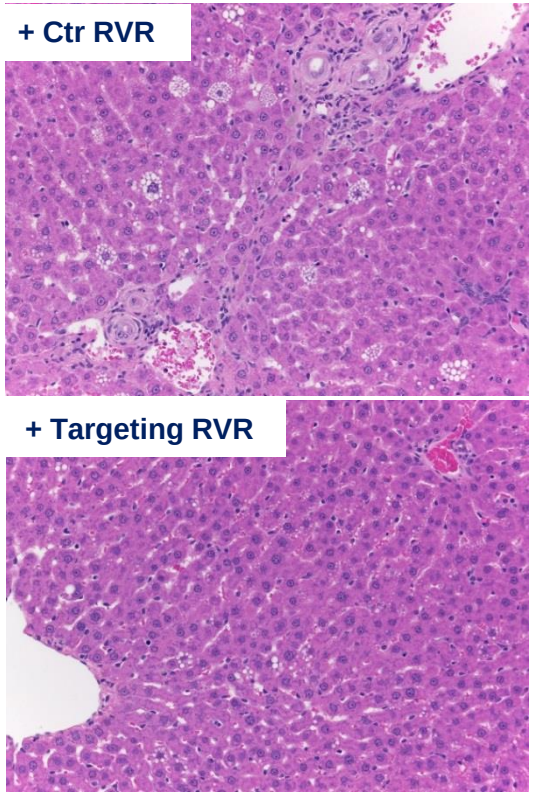












- Competition for RISC loading with endogenous miRNAs does not appear to be a major contributor to the rat hepatotoxicity observed with “bad actors”

Janas, M., Schlegel, M. et al. *Nat Commun*. **2018,** 9,723

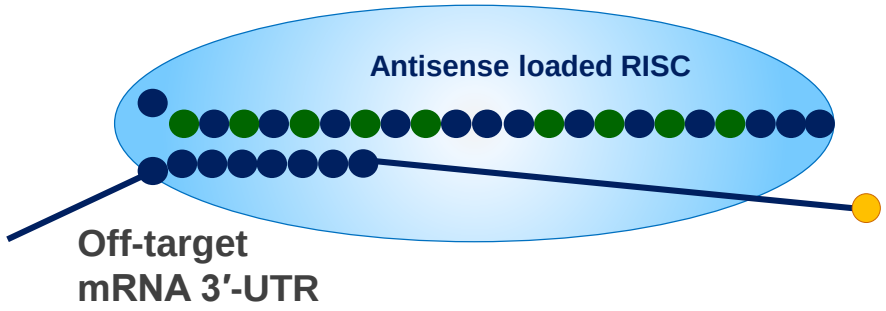
The ESC+ Approach to Improve Specificity and Therapeutic Index

Objective

- Maintain on-target activity (*in vivo*) while minimizing off-target activity

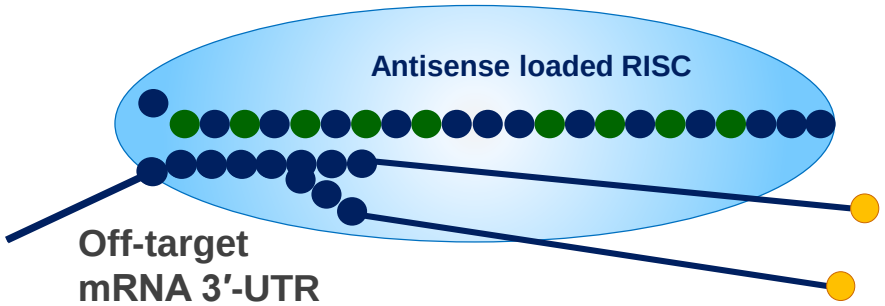
● Thermally destabilizing modification

Off-target binding through partial sequence match



✓ **Off-target effects**

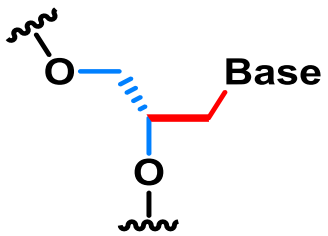
Minimizing off-target binding through seed-pairing destabilization



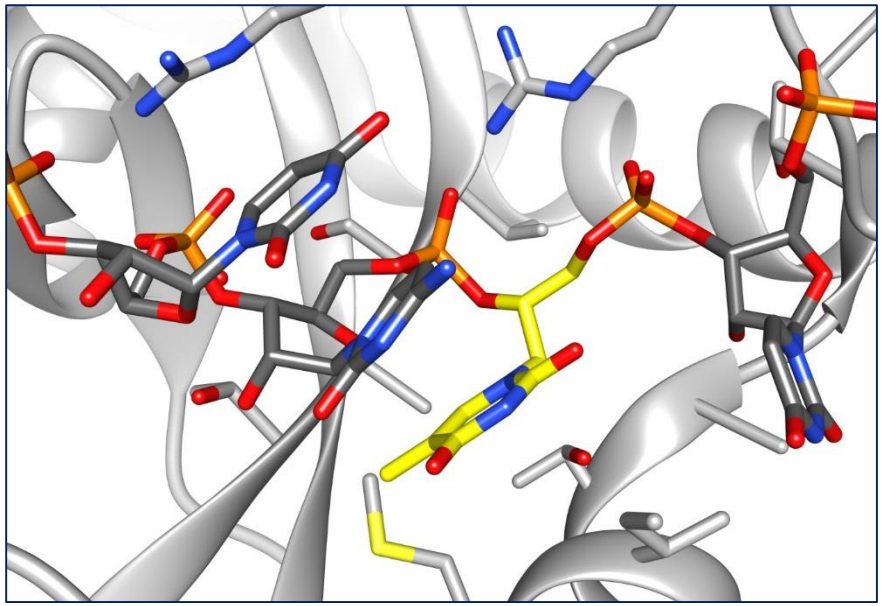
X **Off-target effects**

Bramsen et. al. *Nucleic Acids Res.* 2010, 38, 5761; Vaish et. al. *Nucleic Acids Res.* 2011, 39, 182 3; Lee et. al. *Nat. Comm.* 2015, 6, 10154.

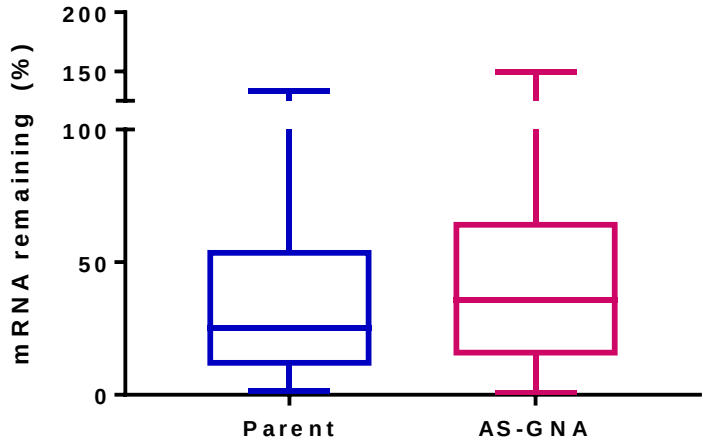
GNA as a Potential Modification for Thermal Destabilization[1]



(*S*)-GNA
(Glycol nucleic acid)



Model obtained from crystal structure of a GNA-modified RNA duplex modeled into structure of miRNA20a:Ago2[2]



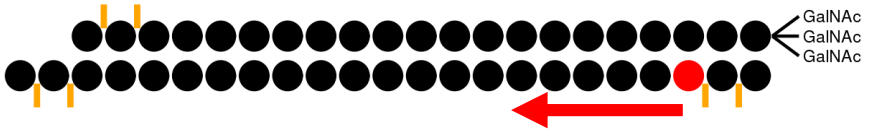
- 315 Sequences, 6 Targets
- Transfection, 10 nM siRNA, 24 hours, PMH

- Consistent with its ability to maintain intrinsic RNAi activity, GNA can adopt a conformation, which is compatible with RISC-loaded guide strand despite shorter phosphate-phosphate and base-backbone distance
- Thermal destabilization (rel. to 2'-OMe) generally ranges between 3-8 K
- Activity screen across a panel of sequences shows varying tolerance ranging from improved to decreased activity; ESC level potency can generally be achieved via individual chemistry optimization

[1]Schlegel et al. *J. Am. Chem. Soc.* **2017**, 139, 8537. [2] Elkayam, E. et al. Cell **2012**, 150, 100-110.

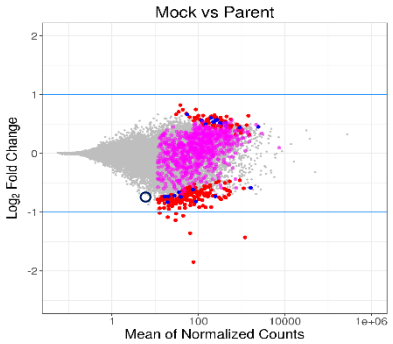
Position-Dependent Impact of GNA on Specificity and Off-Target Mitigation

In Vitro RNASeq



GNA Walk

Parent ESC

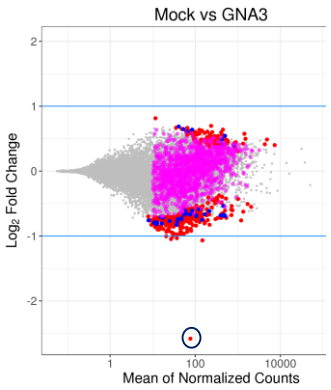


DEGs (Differentially Expressed Genes), significant

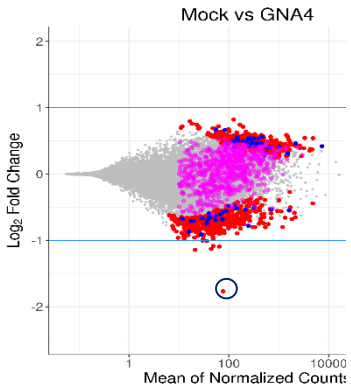
3'UTR seed match, significant

3'UTR seed match, not significant

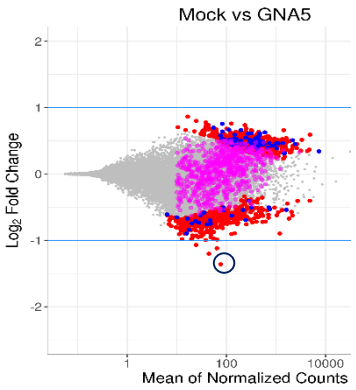
AS3-GNA



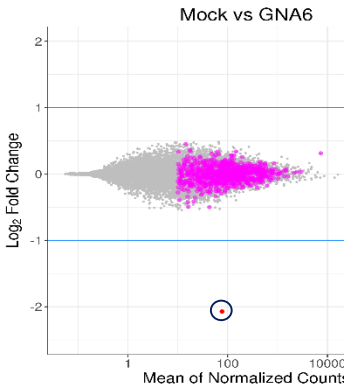
AS4-GNA



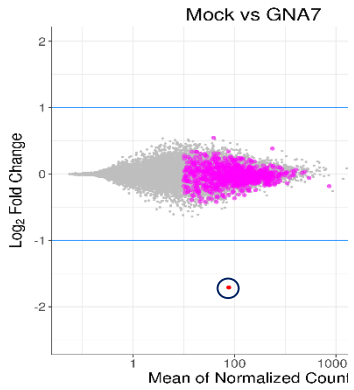
AS5-GNA



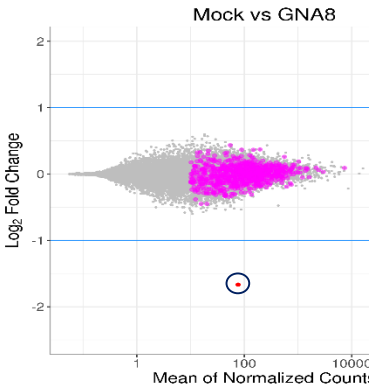
AS6-GNA



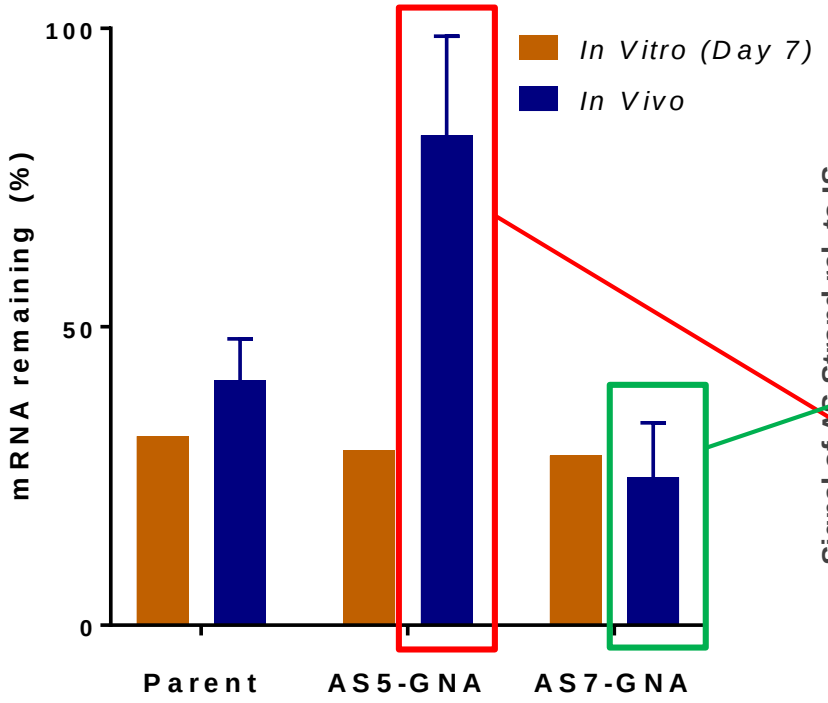
AS7-GNA



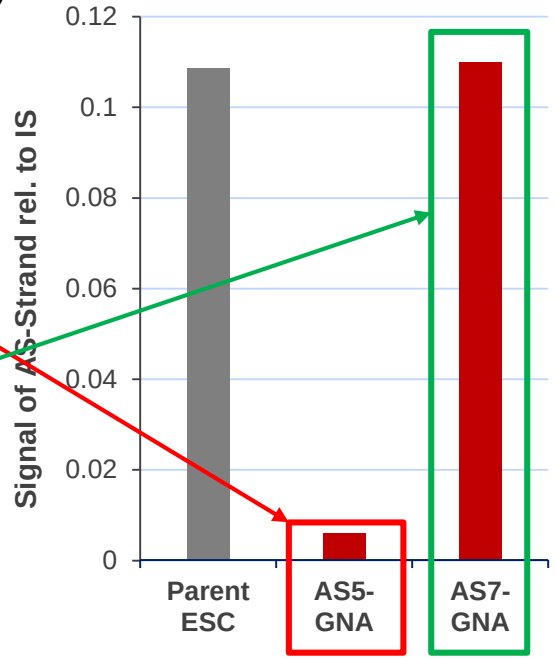
AS8-GNA



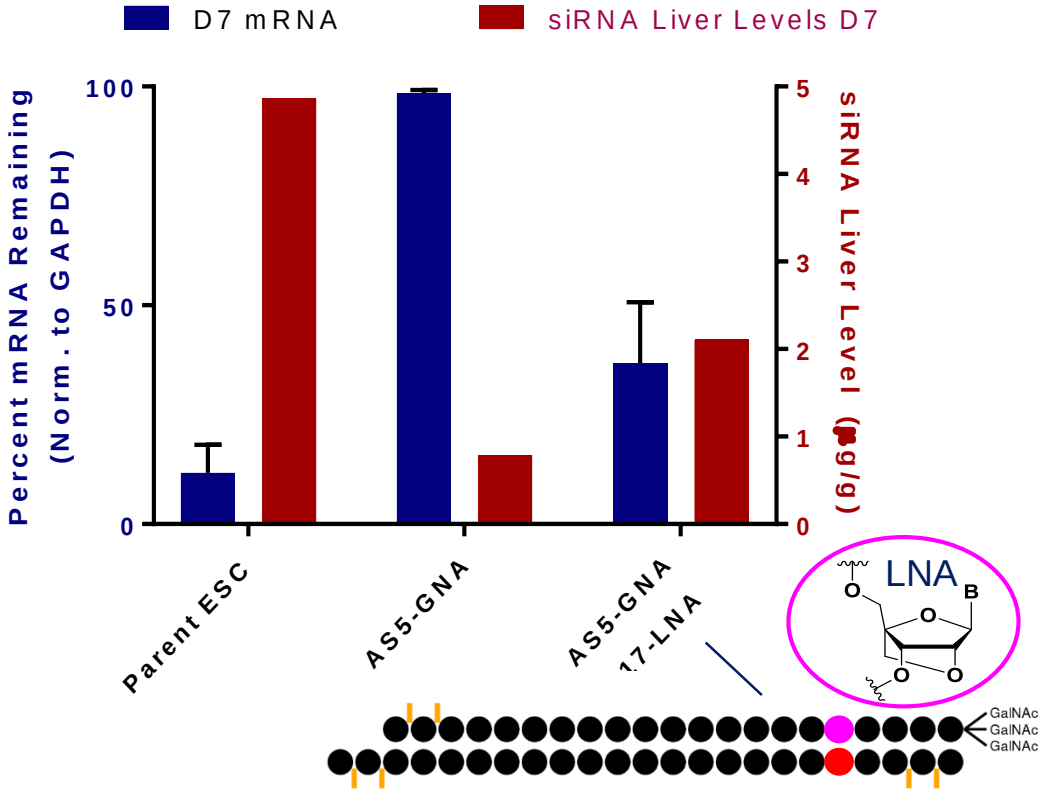
In Vitro-In Vivo Translation



LC/MS-Signal of AS-strand in liver (D7) rel. to internal Std.



Enhancing thermal stability of the siRNA duplex



- Lack of *in vivo* translation can be due to loss in metabolic stability, which can be mitigated through careful design optimization

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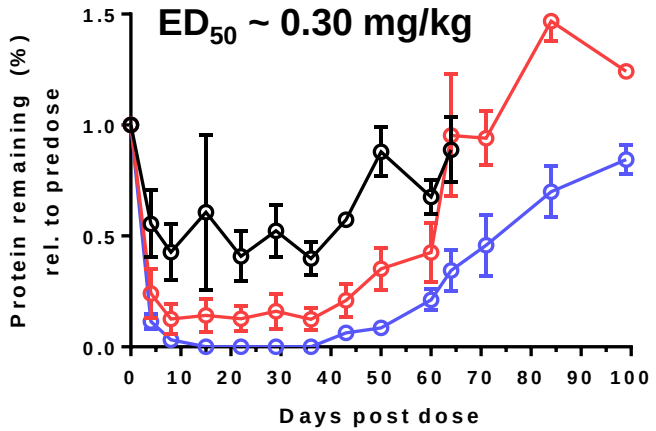
Optimized Designs Show Comparable *In Vivo* Potency in Rodent and NHP

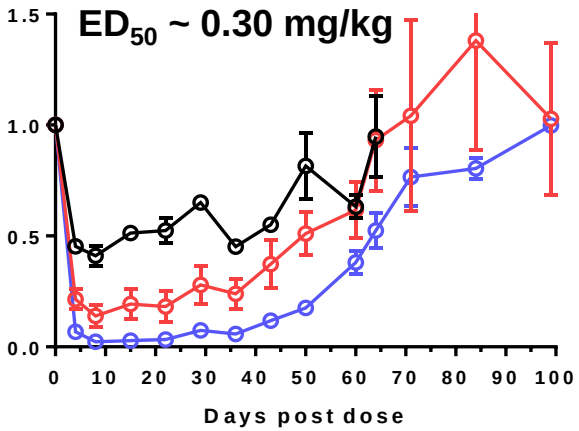
Parent ESC

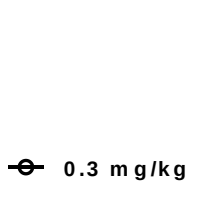
AS7-GNA

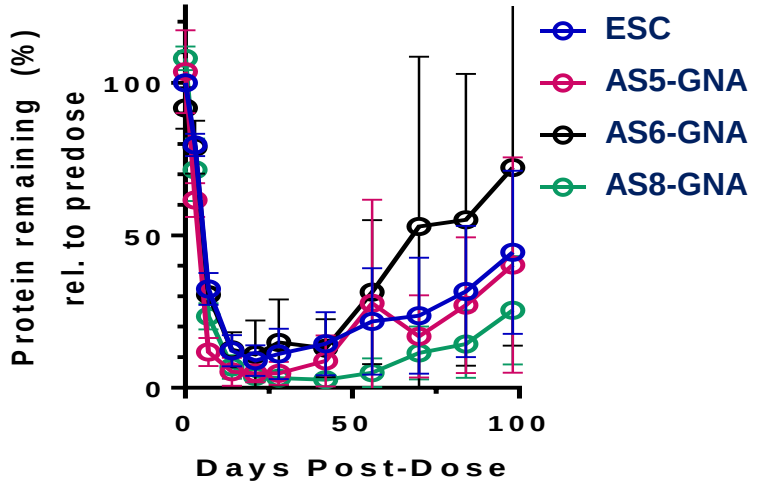
NHP

Mice

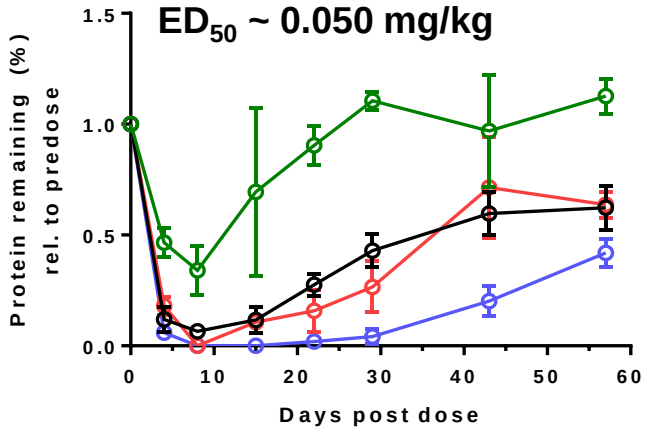


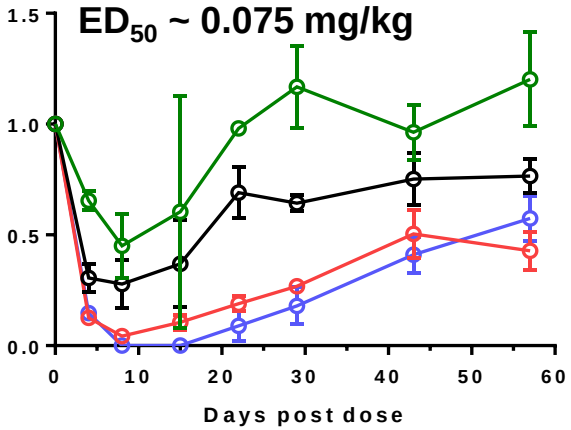


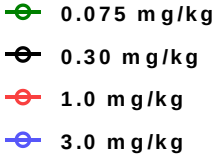


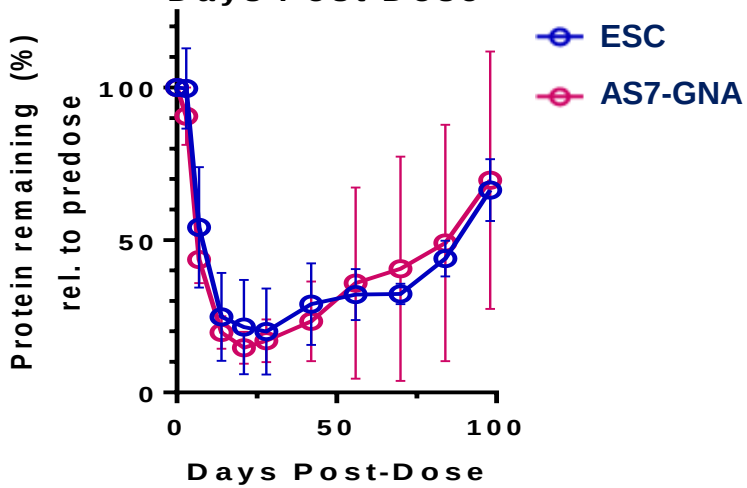


Rats





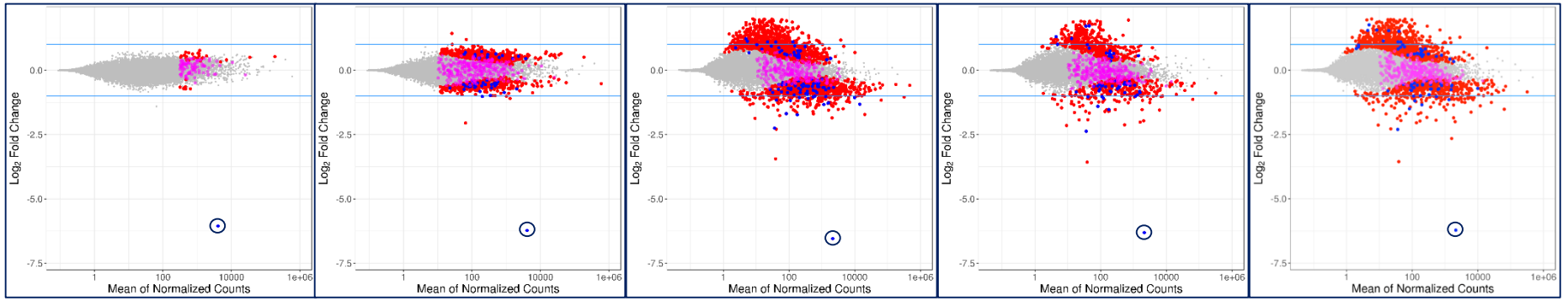




ESC+ Demonstrates More Quiescent Off-Target Signature Across Dose Levels in Rat Liver with Comparable On-Target KD

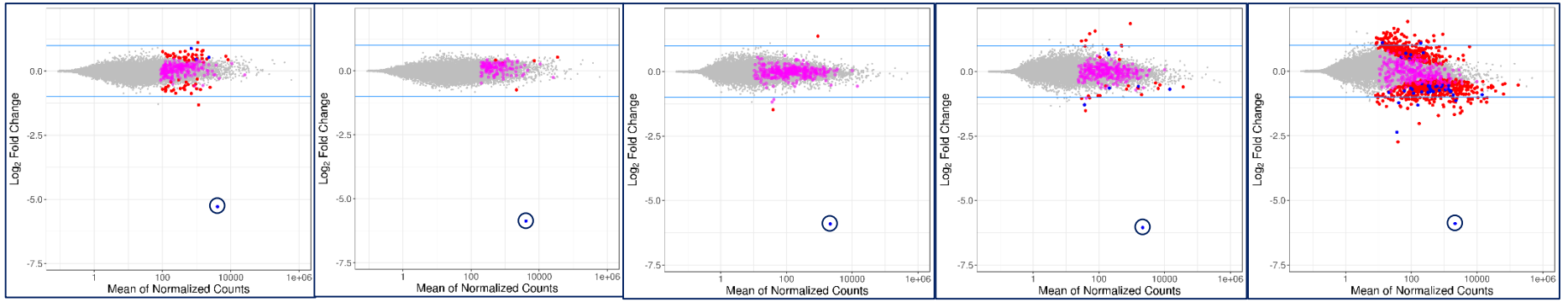
Dose: qw x 3
Necropsy: Day 16

ESC



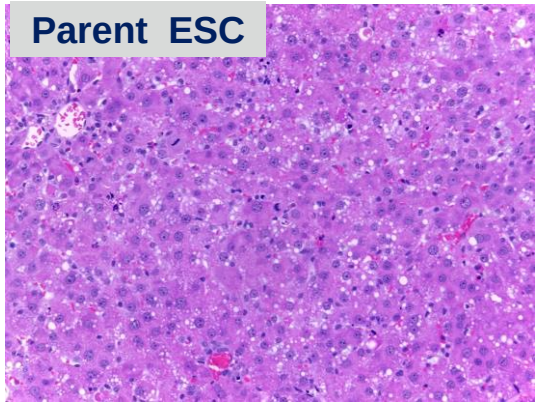
3 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg

ESC+

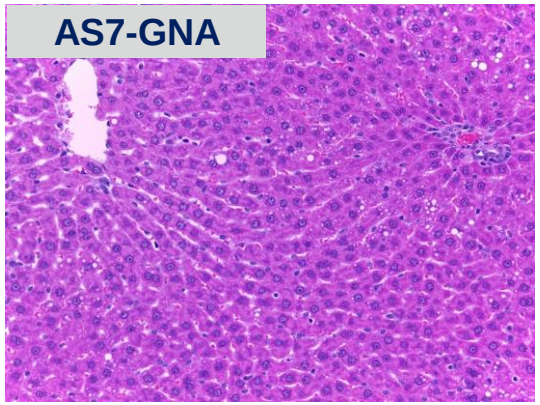


Histopath for 30 mg/kg dose

Parent ESC



AS7-GNA



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

◯ = target mRNA

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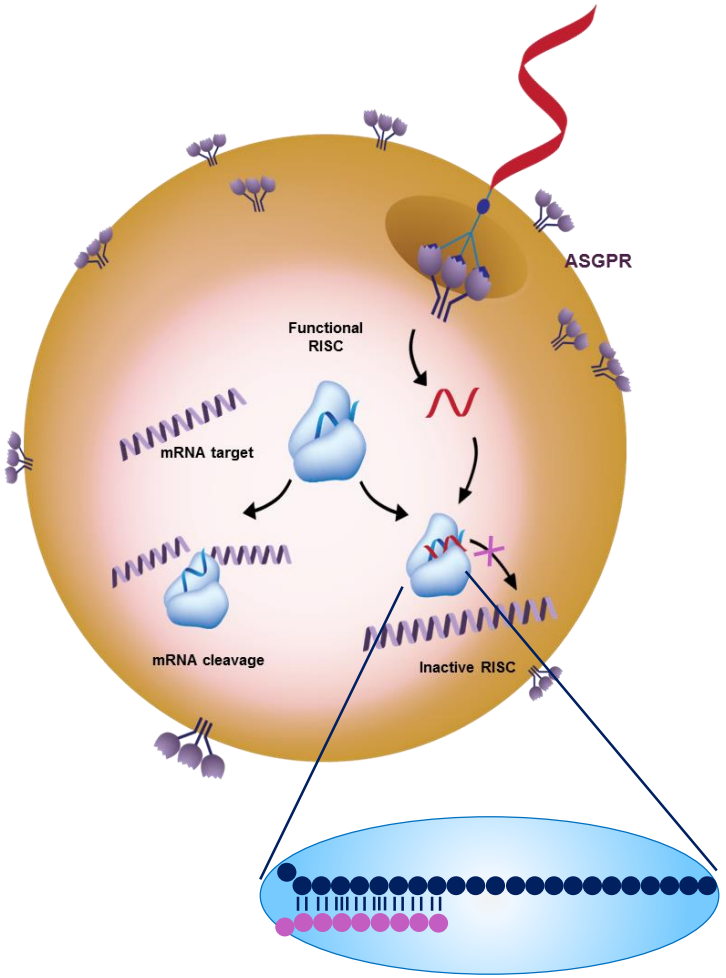
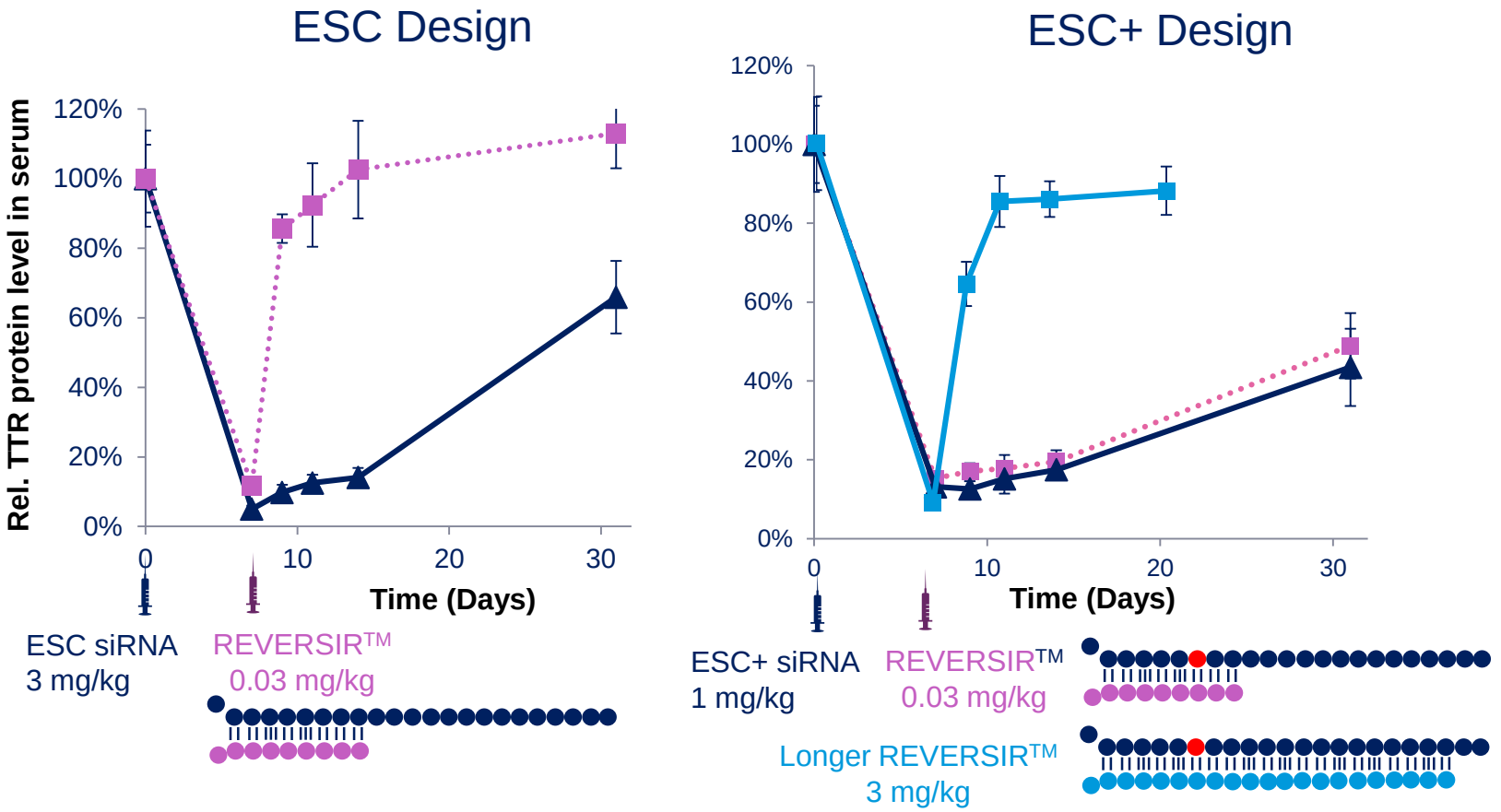
Reduced Off-Target Silencing Potential of ESC+ Confirmed with Reversirs



Reversal of *in vivo on*-target silencing with Reversir™



- Presence of thermally destabilizing GNA prevents short seed region-targeted Reversir™ to bind and block siRNA activity; longer Reversir™ at higher dose required for reversal of activity

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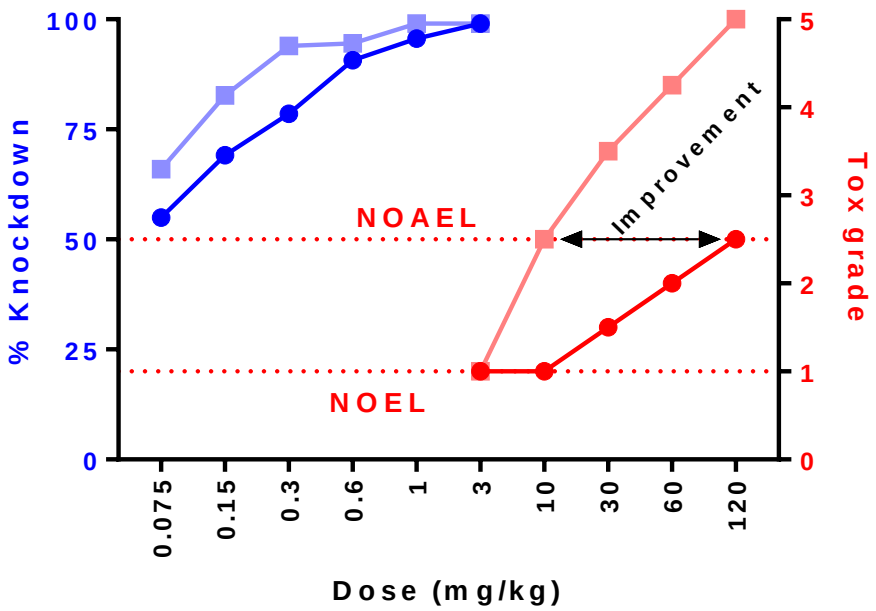
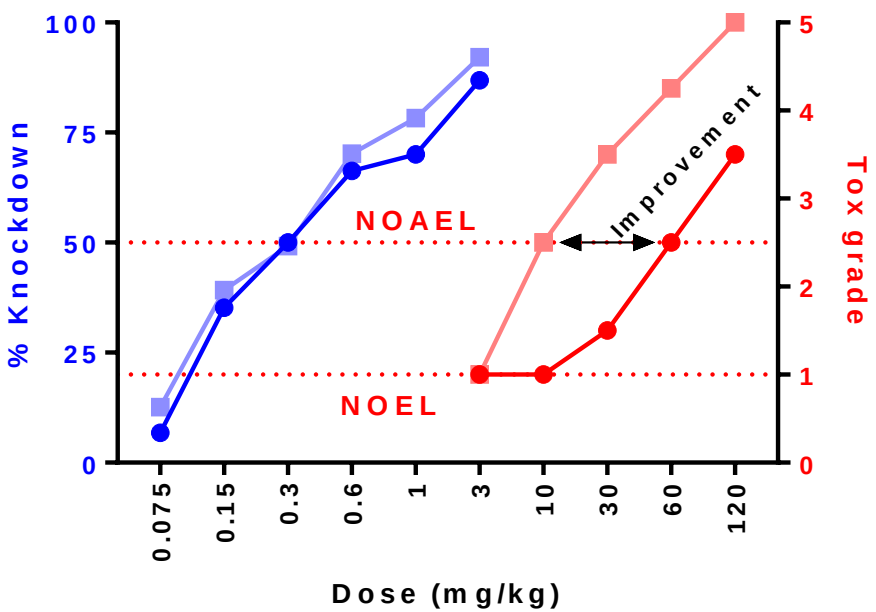
Therapeutic Index Improved Greater Than 5-fold with ESC+ Conjugates

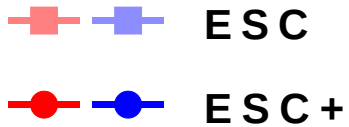
$$\text{Therapeutic Index} = \frac{\text{NOAEL (qw x 3)}}{\text{ED}_{80}\text{(single dose)}}$$

Sequence 1



Sequence 2





NOEL = No observed effect level
NOAEL = No observed adverse effect level

Conclusions

- Evolution of conjugate platform has predominantly been driven by advancements in siRNA design, which has been guided by
  - Continuous improvements in mechanistic understanding
  - Learnings from clinic
- RNAi-mediated off-target effects are important drivers of hepatotoxicity observed for subset of ESC conjugates in rodent
  - No evidence for impact of chemical modifications on observed toxicity – 2'-F safety, Session VII, Preclinical Development (Maja Janas)
- ESC+ strategy mitigates seed-mediated off-target effects, improves specificity and further expands therapeutic window of siRNA conjugates
  - Pharmacodynamics of ESC+ design comparable to ESC
  - Robust translation across species
- Multiple ESC+ conjugates are advancing towards clinical development with first INDs planned for 2018

- Enhanced Stability Chemistry
- Increased specificity
- SC administration
- 2018 INDs and CTAs

- Maintained PD (potency/duration)
- Further improvements to specificity and therapeutic index

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