Reversir™ Platform for Rapid and Potent Reversal of siRNA Silencing Activity

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Outline

- Introduction
- Design considerations
- Reversir activity *in vivo* and *in vitro*
- Summary

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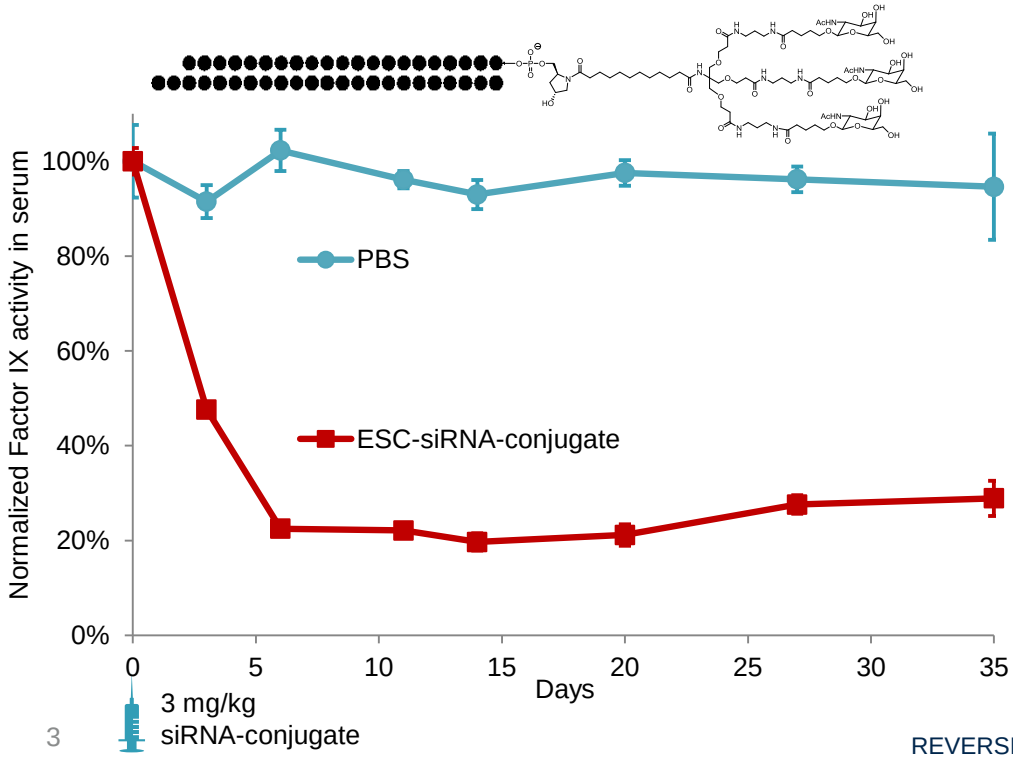
ESC-siRNA-Conjugates and Reversir™ Platform

ESC-siRNA-conjugates Exhibit Prolonged Duration of Activity

Example: Factor IX silencing after single SC dose in mice



Reversir™ Platform Objective

- Enable rapid and complete reversal of siRNA silencing activity to expand the utility of ESC-conjugates displaying prolonged duration of silencing
- Allows tailored control of RNAi pharmacology

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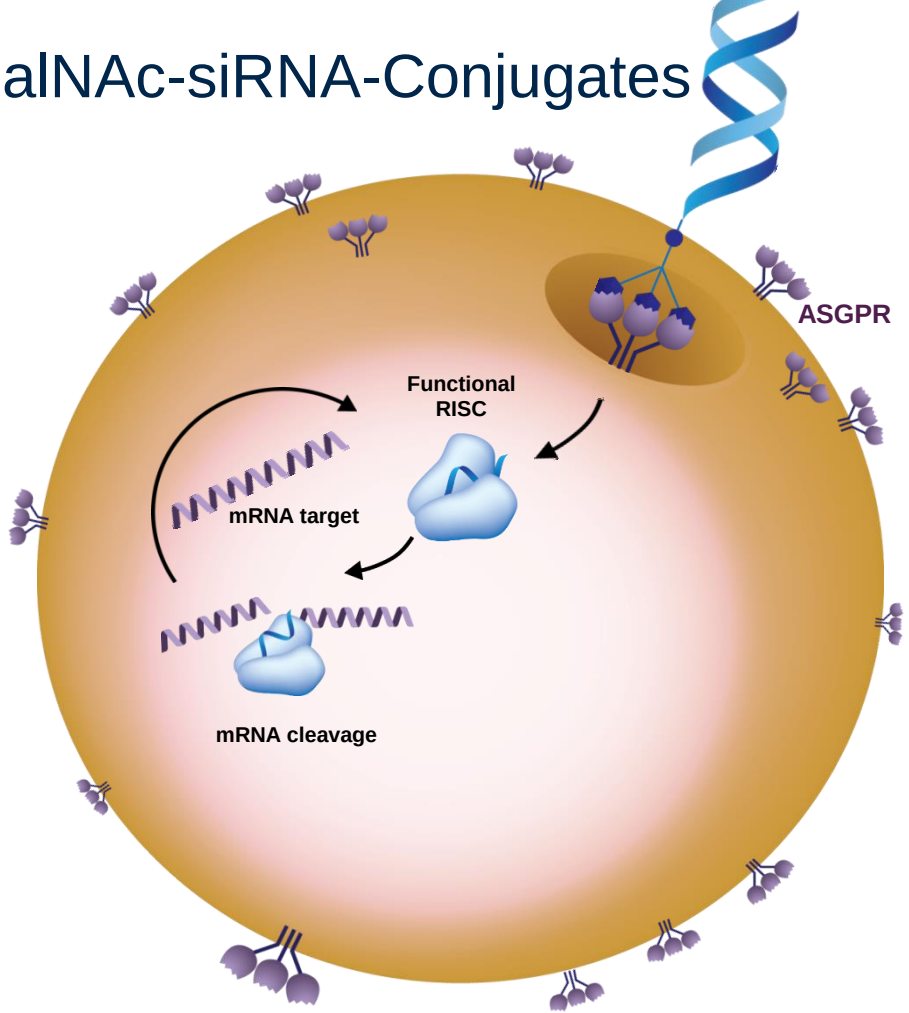
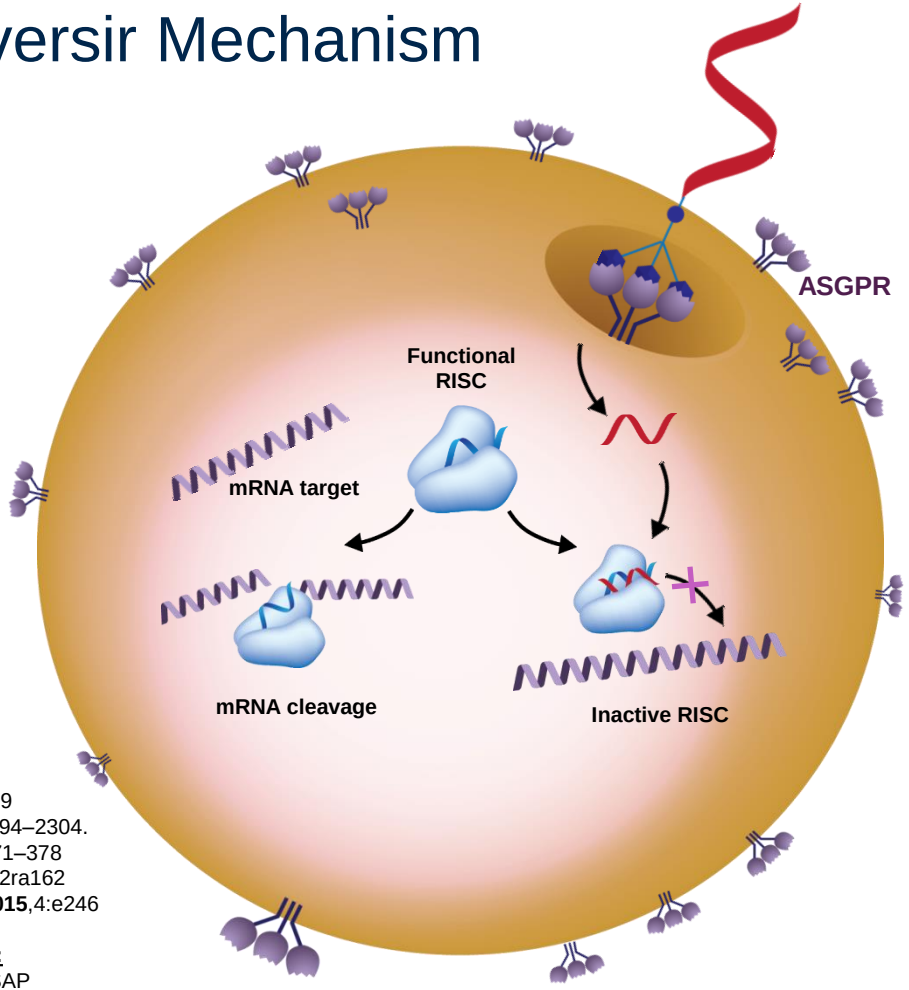
Silencing by GalNAc-siRNA-Conjugates



Simplified schematic of a hepatocyte

1. Binding and internalization of siRNA-conjugate by ASGPR
2. RISC loading and formation of functional RISC
3. mRNA target recognition and cleavage
4. Catalytic process

Proposed Reversir Mechanism



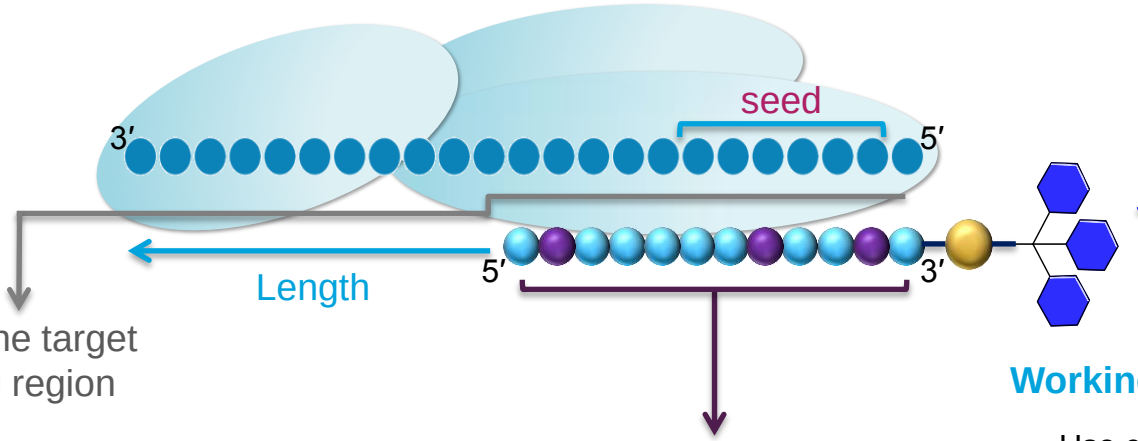
1. Binding and internalization of Reversir conjugate by ASGPR
2. Irreversible binding of Reversir as a “synthetic target” to AS-strand in functional RISC
3. Abrogation of mRNA target recognition and cleavage

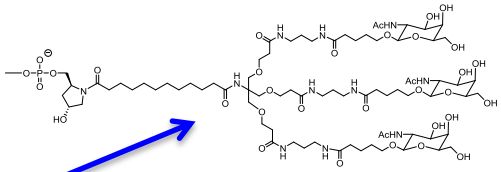
Anti-miRs/Antagomirs:
Meister G *et al*. RNA **2004**,10:544-550
Krützfeldt J *et a*l. Nature **2005**,438:685-689
Davis S *et al.* Nucl. Acids Res **2006**,34:2294–2304.
Obad S *et al*. Nature Genetics **2011**,43:371–378
Rottiers V *et al*. Sci Transl Med **2013**,5:212ra162
Staedel C *et al*. Mol Ther Nucleic Acids **2015**,4:e246

ASO – Sense Oligonucleotide Antidote:
Crosby J *et al.* Nucleic Acid Ther **2015**,ASAP

Reversir Design Considerations

RISC-loaded siRNA Antisense Strand – Target of Reversir





Same ligand as in siRNA-conjugate

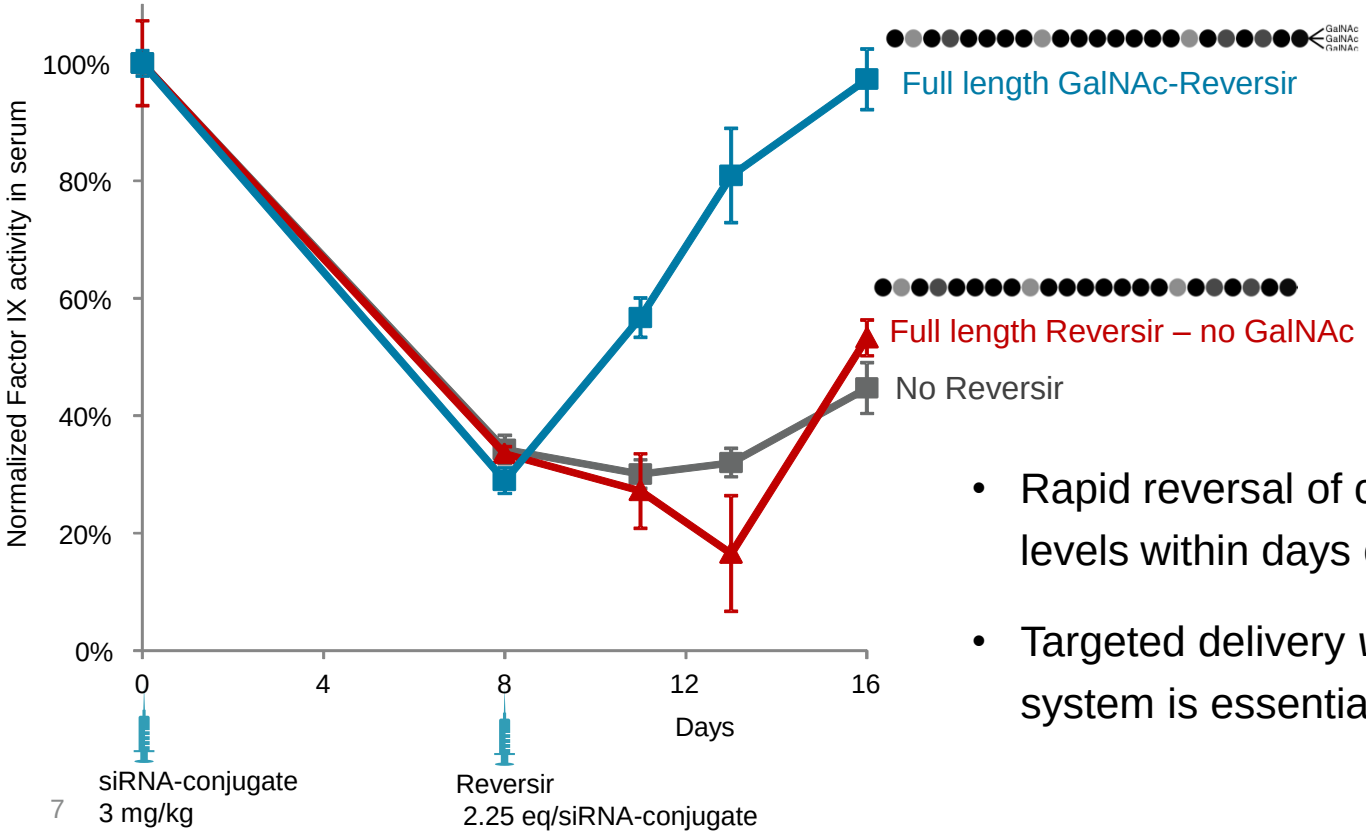
Tiling the target binding region

High-affinity/sugar-phosphate backbone modifications for thermal and nuclease stability

Working Hypothesis and Strategy

- Use complementary high affinity oligonucleotides as a “synthetic target” or decoy to abrogate silencing activity of antisense-loaded RISC
- Identify critical design parameters and optimize design for maximal potency

Rapid and Full Reversal of Silencing Activity Achieved by Single Reversir Dose in Mice



- Rapid reversal of conjugate activity from nadir levels within days of Reversir dosing
- Targeted delivery *via* GalNAc:ASGPR system is essential for the Reversir activity

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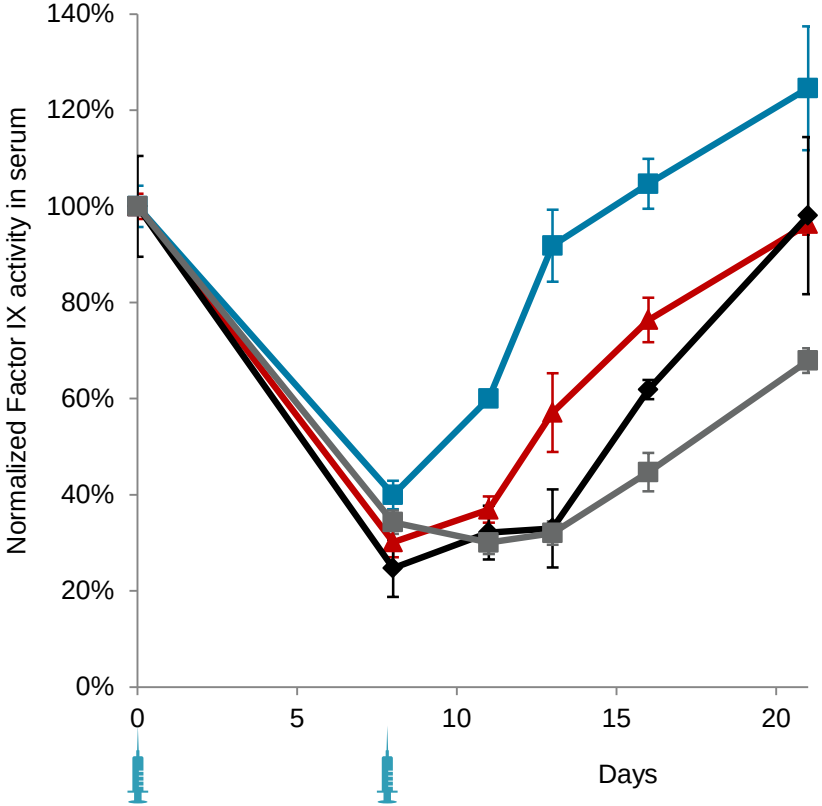
Reducing Reversir Length Improves Potency

Reversal of Factor IX siRNA-Conjugate Activity in Mice



8-mer

15-mer

22-mer

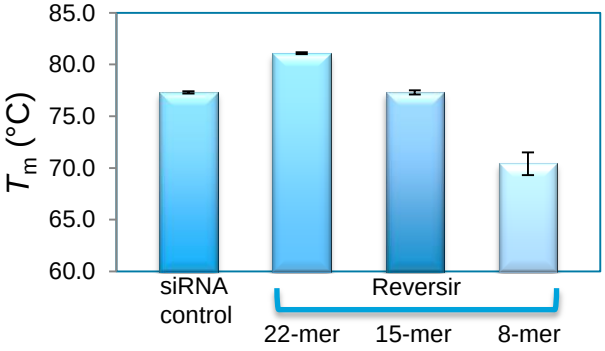
No Reversir

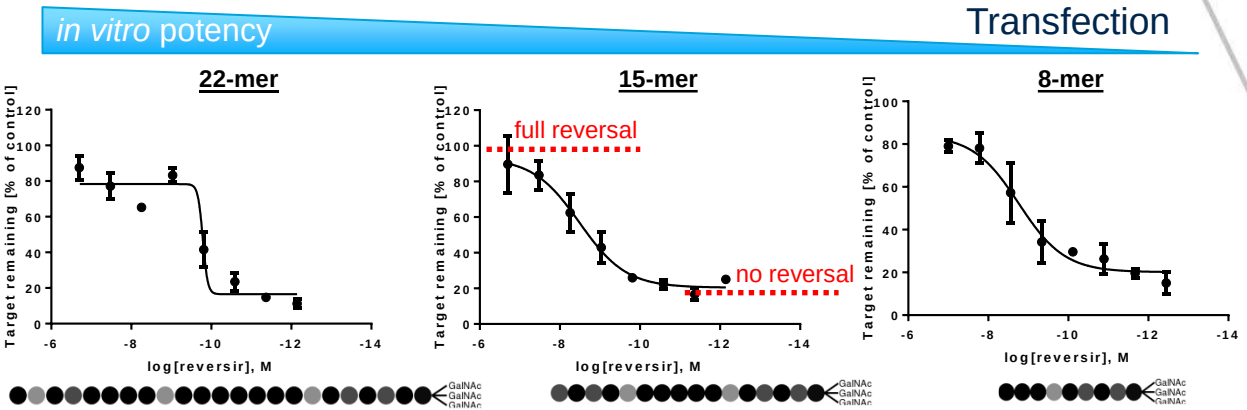
- Clear length-dependent effect on potency
- Is this due to enhanced inherent potency or improved functional delivery?

Why Are Shorter Reversir Molecules More Potent?

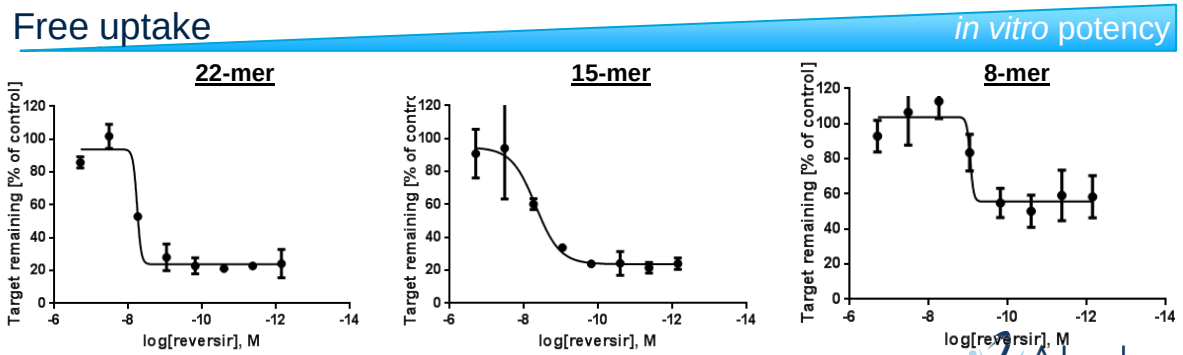
Probing the Mechanism

- Improved thermal stability and inherent potency (*in vitro* transfection) with longer (full length) Reversir – opposite of *in vivo* trend





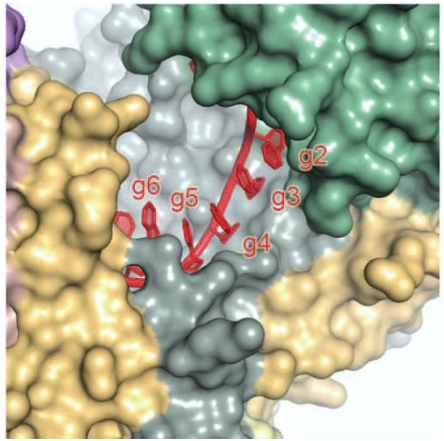
- Free uptake in primary hepatocytes (uptake by ASGPR) show improved activity by shorter Reversir - mimicking the *in vivo* results
- **Data suggestive of improved functional delivery**



Optimal Reversir Activity Requires Targeting of the Full Seed Region

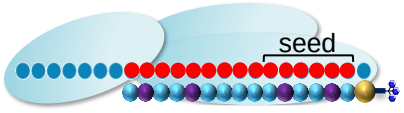
Structure of hAgo2-Antisense

Schirle N *et al.* Science **2014**,346:608

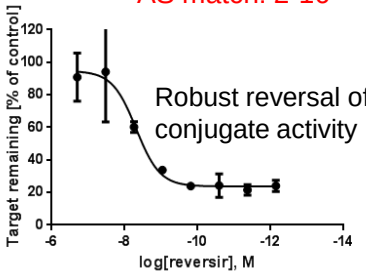


Nucleotides 2-5 exposed for initial target recognition

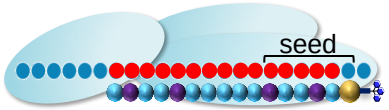
Free uptake assay in primary mouse hepatocytes



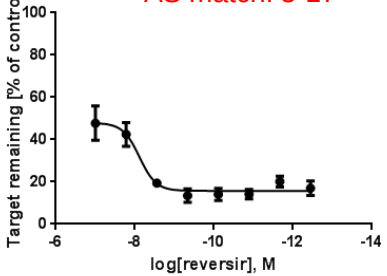
AS match: 2-16

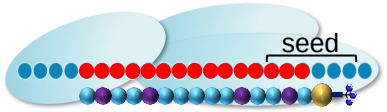


Robust reversal of conjugate activity

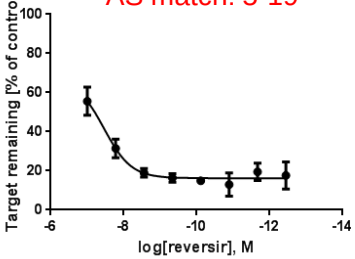


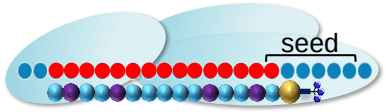
AS match: 3-17



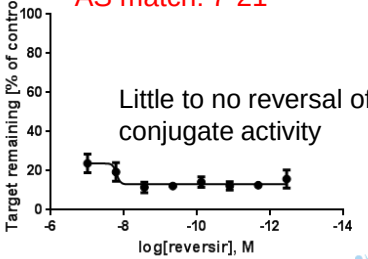


AS match: 5-19





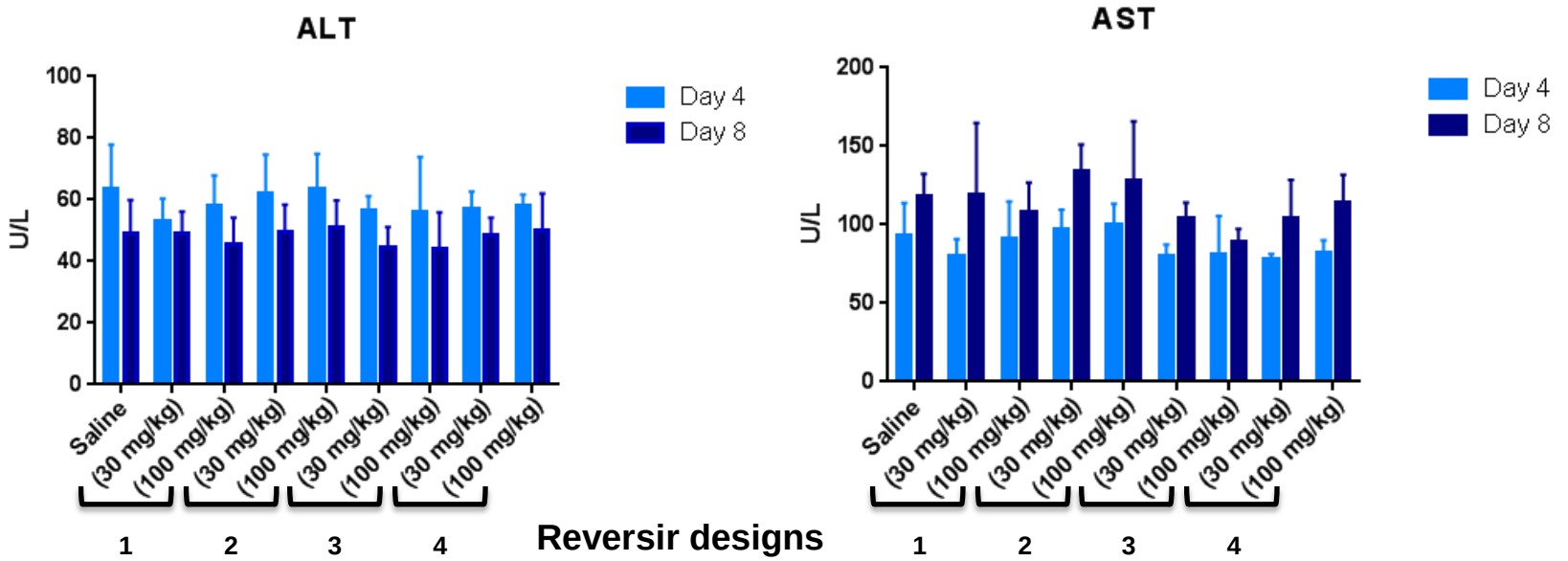
AS match: 7-21



Little to no reversal of conjugate activity

Reversir Designs Show Good Tolerability in Rat

Single SC dose of 30 or 100 mg/kg



- Promising safety profile observed for 4 different Reversir designs
  - » Lack of changes in body weight gain
  - » No liver enzyme elevation observed across doses (30 and 100 mg/kg) and time points (Day 4 and 8)

Summary

- Developed a Reversir technology that allows rapid, potent and full reversal of conjugate silencing activity in preclinical studies
  - » Reversir design includes the use of high-affinity modifications and same ASGPR/GalNAc-mediated delivery as for siRNA-conjugates
  - » Promising safety profile observed to date in rats
- Ability to quickly reverse the silencing activity allows tailored control of RNAi pharmacology for ESC-siRNA-conjugates
- Modular nature of Reversir platform provides quick adaptation to any siRNA-conjugate in our pipeline

Acknowledgements

- Mark Schlegel
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Thank you!