SILENCE THERAPEUTICS

EuroTIDES Nov 2018

Novel hepatocyte-targeted siRNA conjugates for the treatment of liver-related disorders

Marie Wikström Lindholm, PhD,
VP, Head of Technology Innovation

Disclaimer

This Presentation is being supplied to you solely for your information and may not be reproduced, further distributed to any other person or published, in whole or in part, for any purpose. Subject to certain exceptions, this Presentation is not for distribution in the United States, Australia, Canada or Japan or any other jurisdiction where its distribution may constitute a violation of the laws of such jurisdiction.

The information contained in this document ("Presentation") has been prepared by Silence Therapeutics plc (the "Company") and neither this Presentation, nor the information contained in it should be considered a recommendation by the Company or any of its shareholders, directors, officers, agents, employees or advisers in relation to any purchase of the Company's securities, including any purchase of or subscription for any shares (or securities convertible into shares) in the capital of the Company. This Presentation has not been fully verified and is subject to material updating, revision and further amendment. Any person who receives this Presentation should not rely or act upon it. This Presentation should not be re-distributed, re-published, reproduced or disclosed by recipients, in whole or in part.

While the information contained herein has been prepared in good faith, neither the Company nor any of its shareholders, directors, officers, agents, employees or advisers give, have given or have authority to give, any representations or warranties (express or implied) as to, or in relation to, the accuracy, reliability or completeness of the information in this Presentation, or any revision thereof, or of any other written or oral information made or to be made available to any interested party or its advisers (all such information being referred to as "Information") and liability therefor is expressly disclaimed. Accordingly, neither the Company nor any of its shareholders, directors, officers, agents, employees or advisers take any responsibility for, or will accept any liability whether direct or indirect, express or implied, contractual, tortious, statutory or otherwise, in respect of, the accuracy or completeness of the Information or for any of the opinions contained herein or for any errors, omissions or misstatements or for any loss, howsoever arising, from the use of this Presentation.

This Presentation may contain forward-looking statements that involve substantial risks and uncertainties, and actual results and developments may differ materially from those expressed or implied by these statements and past performance is no guarantee of future performance. These forward-looking statements are statements regarding the Company's intentions, beliefs or current expectations concerning, among other things, the Company's results of operations, financial condition, prospects, revenue generation, growth, strategies and the industry in which the Company operates. By their nature, forward-looking statements involve risks and uncertainties because they relate to events and depend on circumstances that may or may not occur in the future. These forward-looking statements speak only as of the date of this Presentation and the Company does not undertake any obligation to publicly release any revisions to these forward-looking statements to reflect events or circumstances after the date of this Presentation.

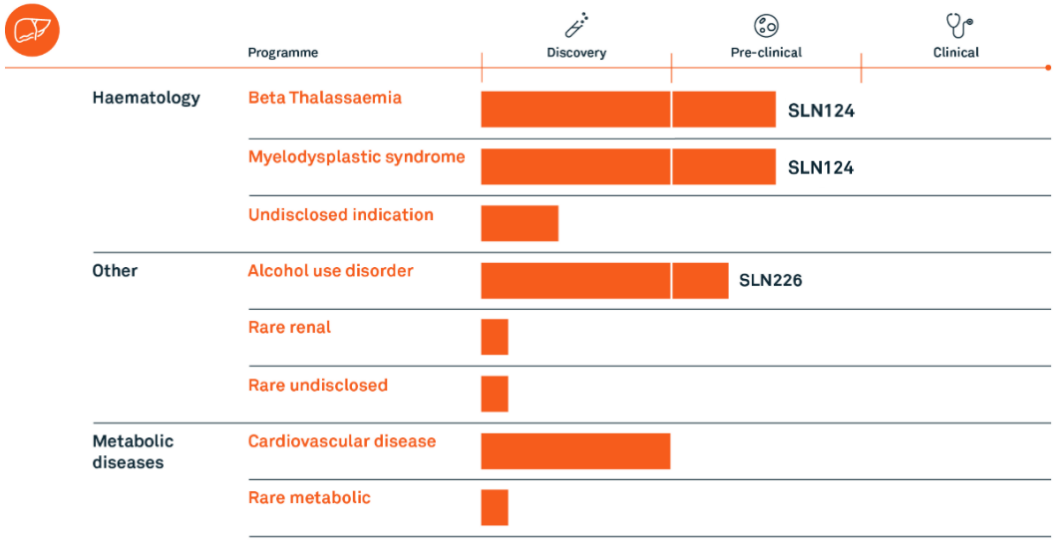
This Presentation has not been approved by an authorised person in accordance with Section 21 of the Financial Services and Markets Act 2000.

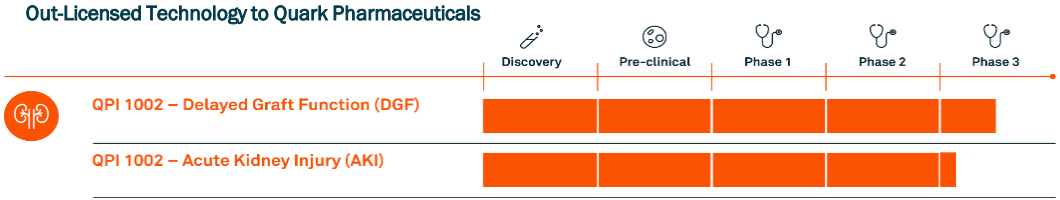
In no circumstances will the Company be responsible for any costs, losses or expenses incurred in connection with any appraisal or investigation of the Company. In furnishing this Presentation, the Company does not undertake or agree to any obligation to provide the recipient with access to any additional information or to update this Presentation or to correct any inaccuracies in, or omissions from, this Presentation which may become apparent. This Presentation does not constitute an offer or invitation to subscribe for or purchase any securities and neither this Presentation nor anything contained herein shall form the basis of any contract or commitment whatsoever. In particular, this Presentation is for information purposes and does not constitute an offer or invitation to subscribe for or purchase any securities in the United States. The securities of the Company have not been and will not be registered under the US Securities Act of 1933, as amended (the "US Securities Act") or the securities laws of any state or other jurisdiction of the United States and may not be offered, sold, resold, pledged, delivered, distributed or transferred, directly or indirectly, into or in the United States except pursuant to an exemption from, or in a transaction not subject to, the registration requirements of the US Securities Act and in accordance with any applicable state securities laws. There will be no public offering of the securities of the Company in the United States.

By participating in and/or accepting delivery of this Presentation you agree to be bound by the foregoing restrictions and the other terms of this disclaimer.

© Silence Therapeutics 2018

Our pipeline





© Silence Therapeutics 2018

SLN124 for the treatment of iron overload disorders

© Silence Therapeutics 2018

Treatment of iron overload disorders

GOAL

> Provide an effective and safe novel treatment option for patients with iron overload conditions, such as β-Thalassemia and Myelodysplastic syndrome

RATIONALE

> Target TMPRSS6, a key modulator in iron regulation with a GalNAc-siRNA molecule providing a highly specific, effective & safe option through inhibition of a disease relevant target gene expressed in hepatocytes

CURRENT STAGE

> Preclinical development with plans to enter the clinic in 2019

© Silence Therapeutics 2018

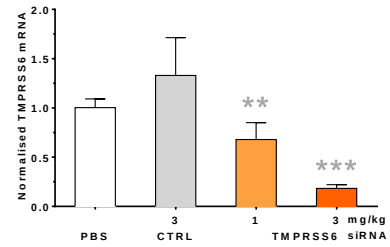
Therapeutic activity of SLN124 in a disease model for hereditary haemochromatosis ($HFE^{-/-}$ mice)

Collaboration with
Prof. Dr. Martina Muckenthaler
Heidelberg University, Germany

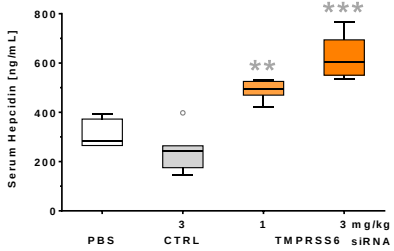
Study design



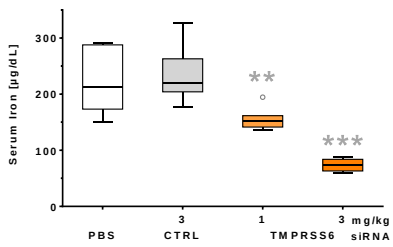
TMPRSS6 mRNA (liver)



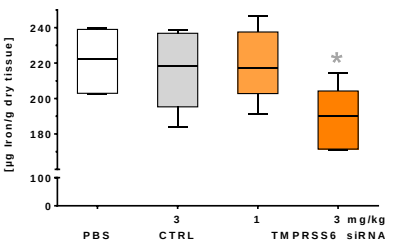
Hepcidin (serum)



Iron (serum)



Iron (kidney)



Kruskal-Wallis test with uncorrected Dunn's test against non-targeting control CTRL

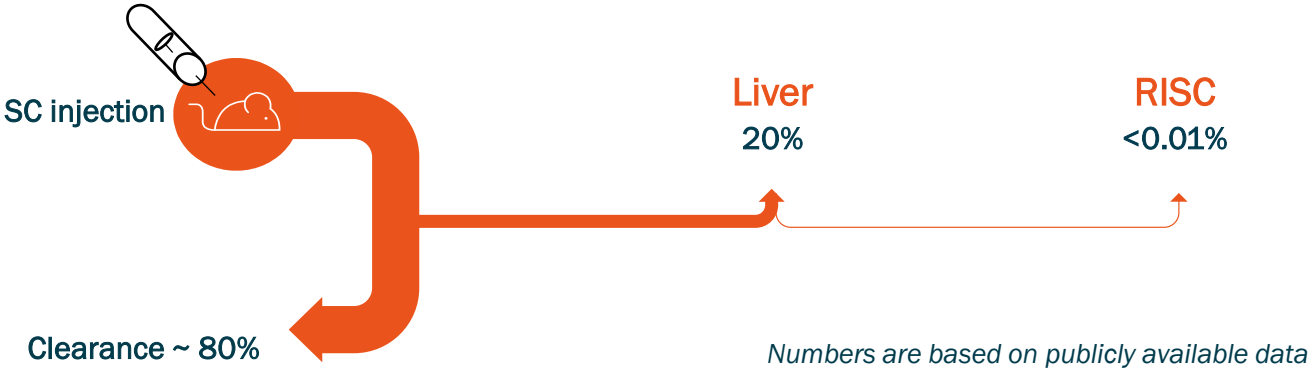
- Dose-dependent and robust silencing of TMPRSS6 mRNA in the liver
- Increase in serum hepcidin levels
- Reduction of serum and kidney iron levels to physiological values

© Silence Therapeutics 2018

Making a good thing better:
when less is more

© Silence Therapeutics 2018

GalNAc conjugates work, so why spend time on further improvements?



Numbers are based on publicly available data

There should be room for improvement if we:

> Increase blood circulation time

> Create intracellular depot

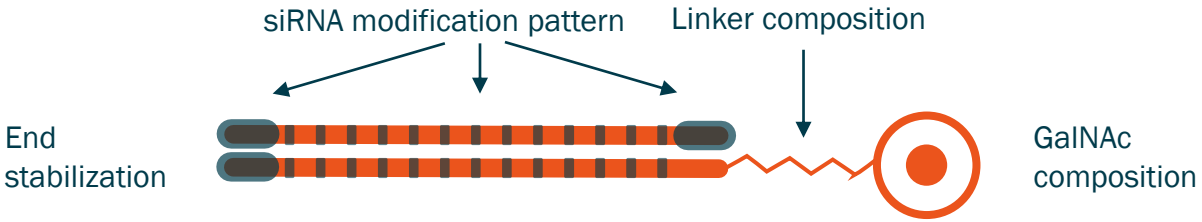
> Improve crossing over cellular membranes

> Improve RISC formation & target access

> Increase and extend RISC activity

© Silence Therapeutics 2018

Fine-tuning molecular design to enhance performance



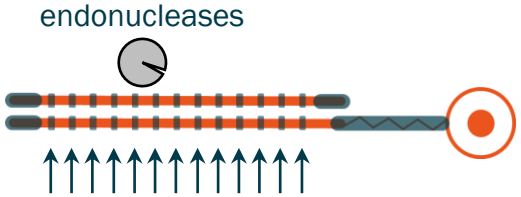
- Modification pattern: reduced number of non-natural modifications resulting in retained or improved activity
- End stabilization: increased circulation half-life, increased activity and duration of action
- Linker: simplified and flexible synthesis, increased activity, and option to control circulation and intracellular half-life
- GalNAc: optimization of number and placement of GalNAc units for increased activity and duration of action with a reduced dose vs. “traditional design”

© Silence Therapeutics 2018

Reducing the amount of non-natural nucleotides

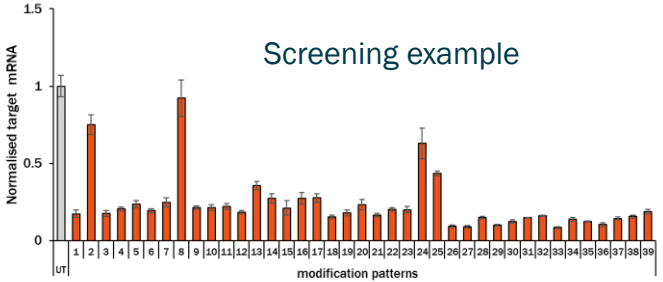
© Silence Therapeutics 2018

Screening strategy for modification pattern

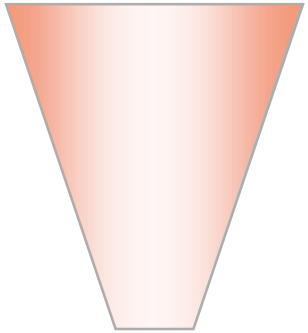


Internal siRNA modifications

Screening example



Hundreds of chemical modification patterns
~50% non-natural



Unique chemical modification patterns
<15% non-natural

***In vitro* activity**: without GalNAc, with various modifications, patterns, sequences

Serum stability: with or without GalNAc

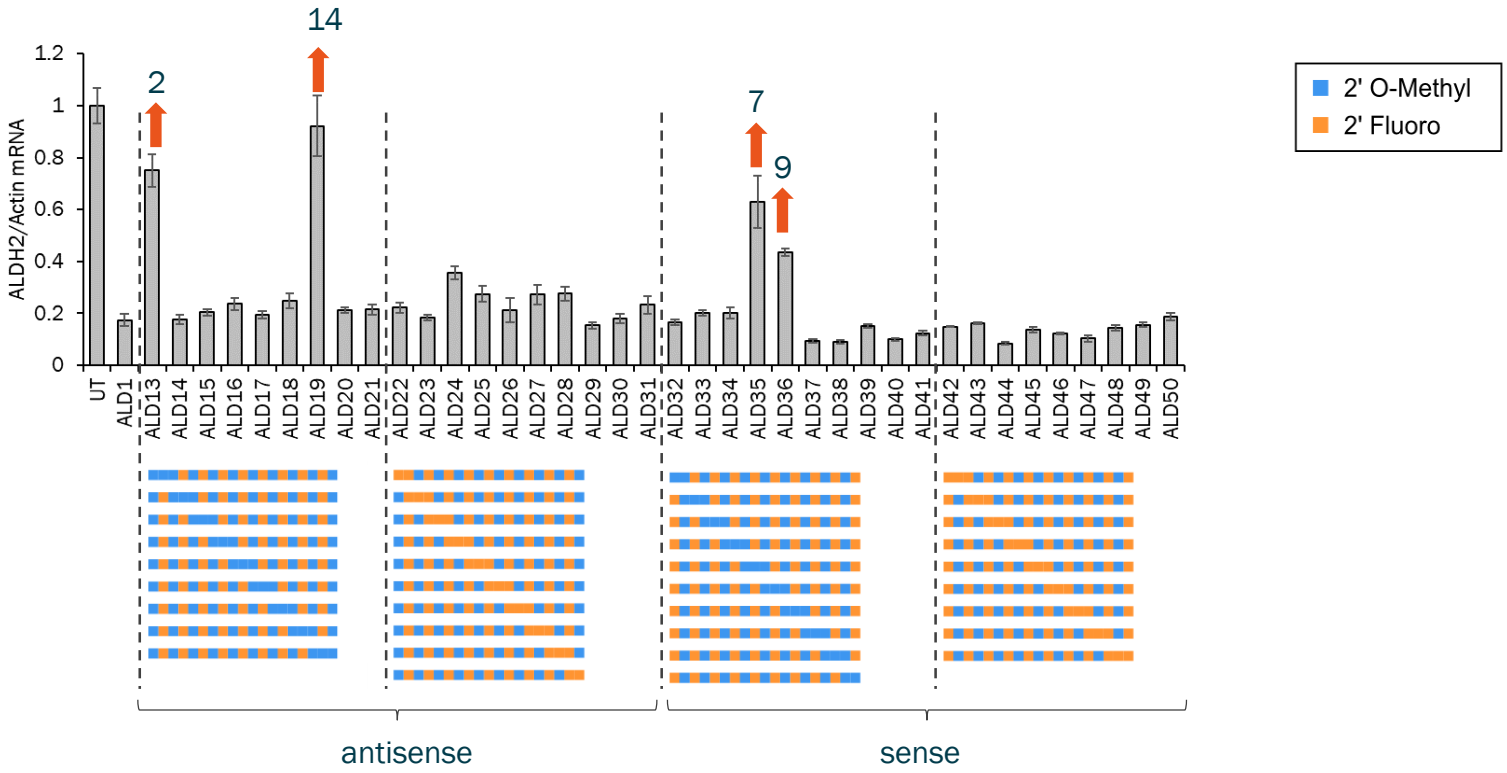
Primary hepatocyte activity: with GalNAc

Animal activity: with GalNAc

> Variations were tested in a systematic manner to identify patterns with reduced non-natural nucleotide content from ~50% to less than 15%

© Silence Therapeutics 2018

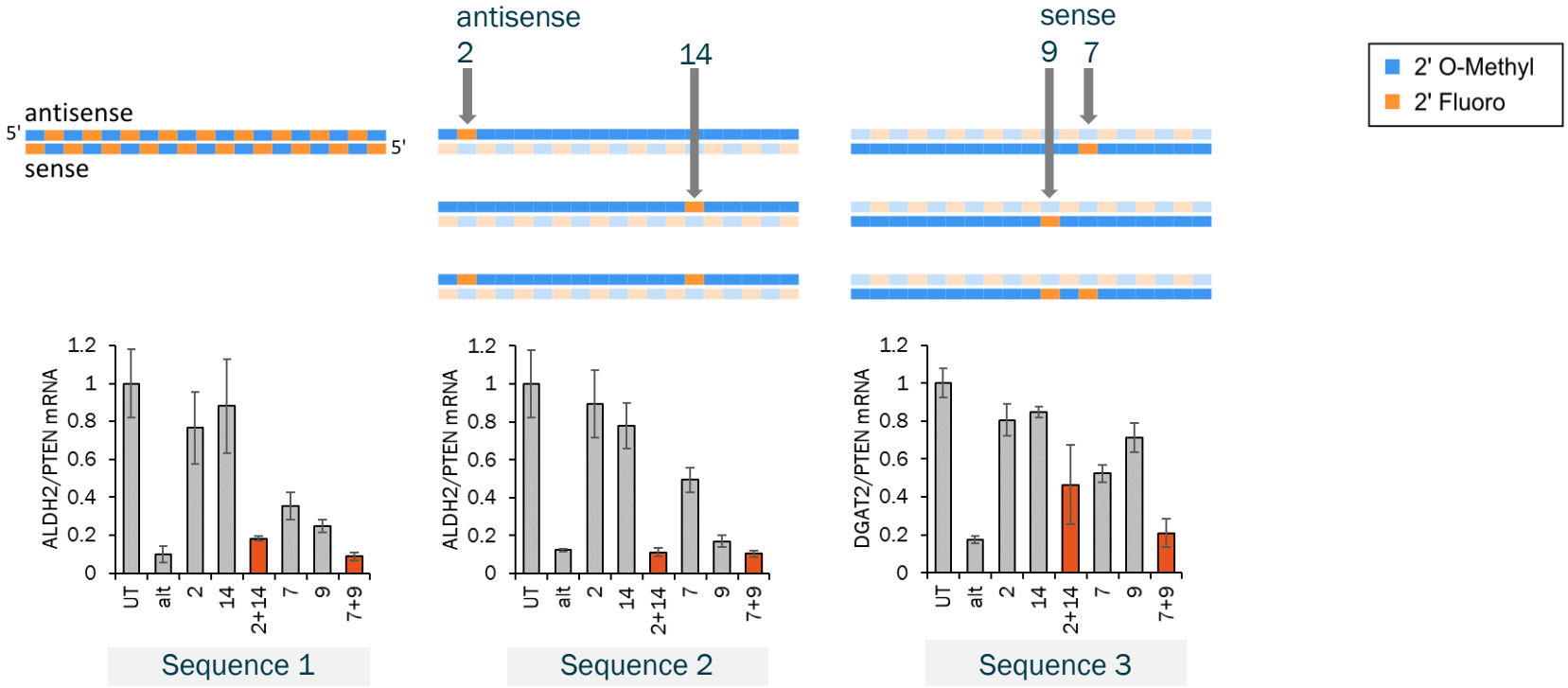
Systematic mapping of 2‘-OMe/2‘-F modifications



Hep3B, 150 000 cells/6-well, 0.1 nM siRNA, 1 µg/ml Atufect transfection reagent, lysis 2 dpt

© Silence Therapeutics 2018

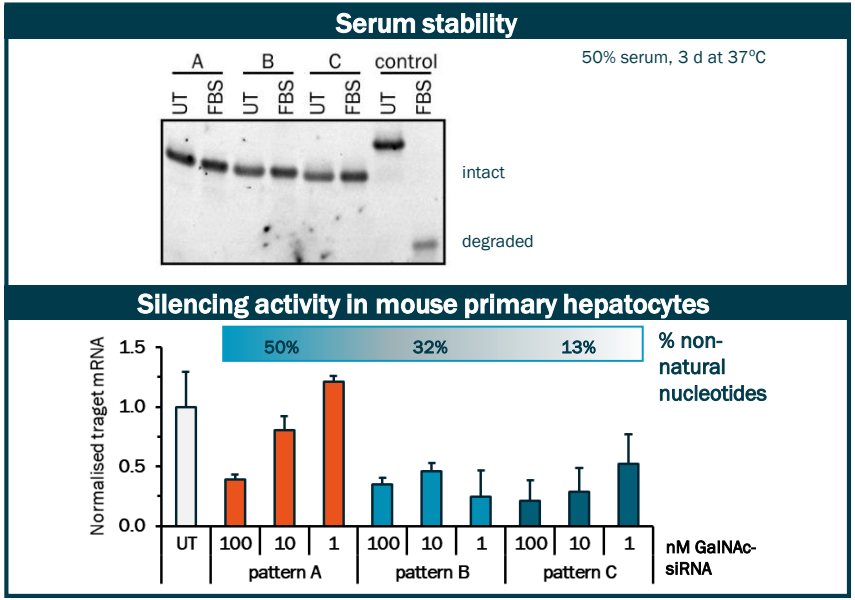
Validation of identified key positions in three different sequences

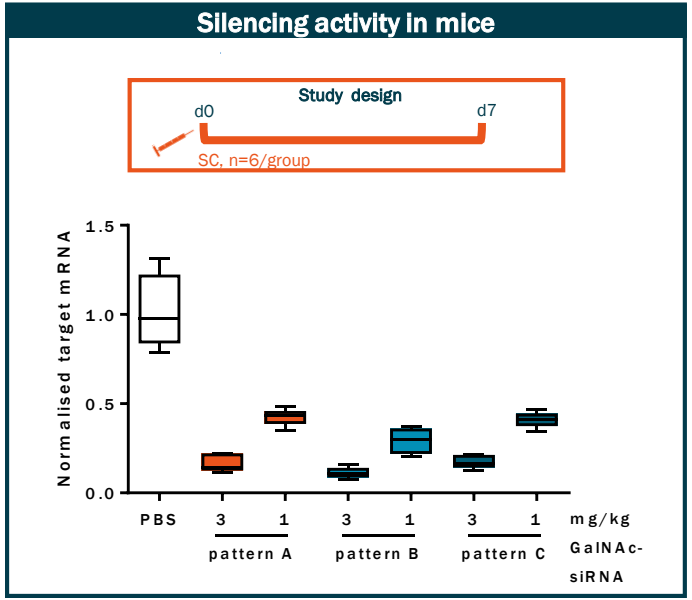


Hep3B, 150 000 cells/6-well, 0.1 nM siRNA, 1 µg/ml Atufect transfection reagent, lysis 2 dpt (ALDH2)
Huh-7, 80 000 cells/6-well, 1 nM siRNA, 1 µg/ml Atufect transfection reagent, lysis 2 dpt (DGAT2)

© Silence Therapeutics 2018

GalNAc-conjugated siRNA with reduced 2'F-modified nucleobase content





- Non-natural siRNA modifications can be dramatically reduced with retained serum stability, and activity in primary hepatocytes, compared to molecules with 50% non-natural modifications
- GalNAc-conjugated siRNAs with reduced non-natural nucleotide content demonstrate similar or improved dose-dependent reduction of target transcript in livers of treated mice (compared to 50% non-natural modifications)

© Silence Therapeutics 2018

Optimizing number and position of GalNAc units

© Silence Therapeutics 2018

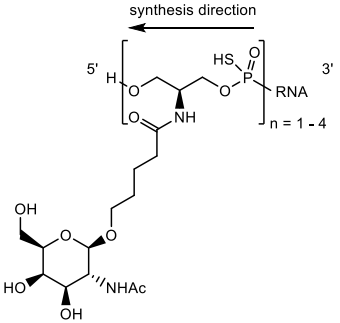
Stepwise path to new design

> **Step 1:** investigating optimal number and location for “serial clusters”

- 1-4 serinol-linked GalNAc units were conjugated to 3’ or 5’ sense or antisense strand
- All conjugates were screened for stability (tritosomes) and *in vitro* activity (primary mouse hepatocytes), several conjugates were also tested *in vivo* (mice)

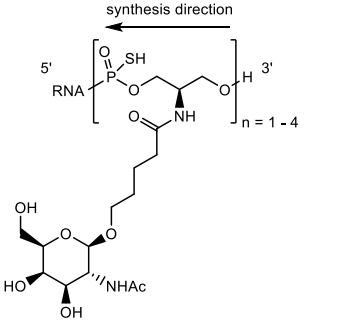
> Result from step 1:

- 5’ antisense conjugates are stable but inactive *in vitro*
- All other positions gave stable conjugates with only minor differences between 2-4 GalNAc units and a triantennary control compound



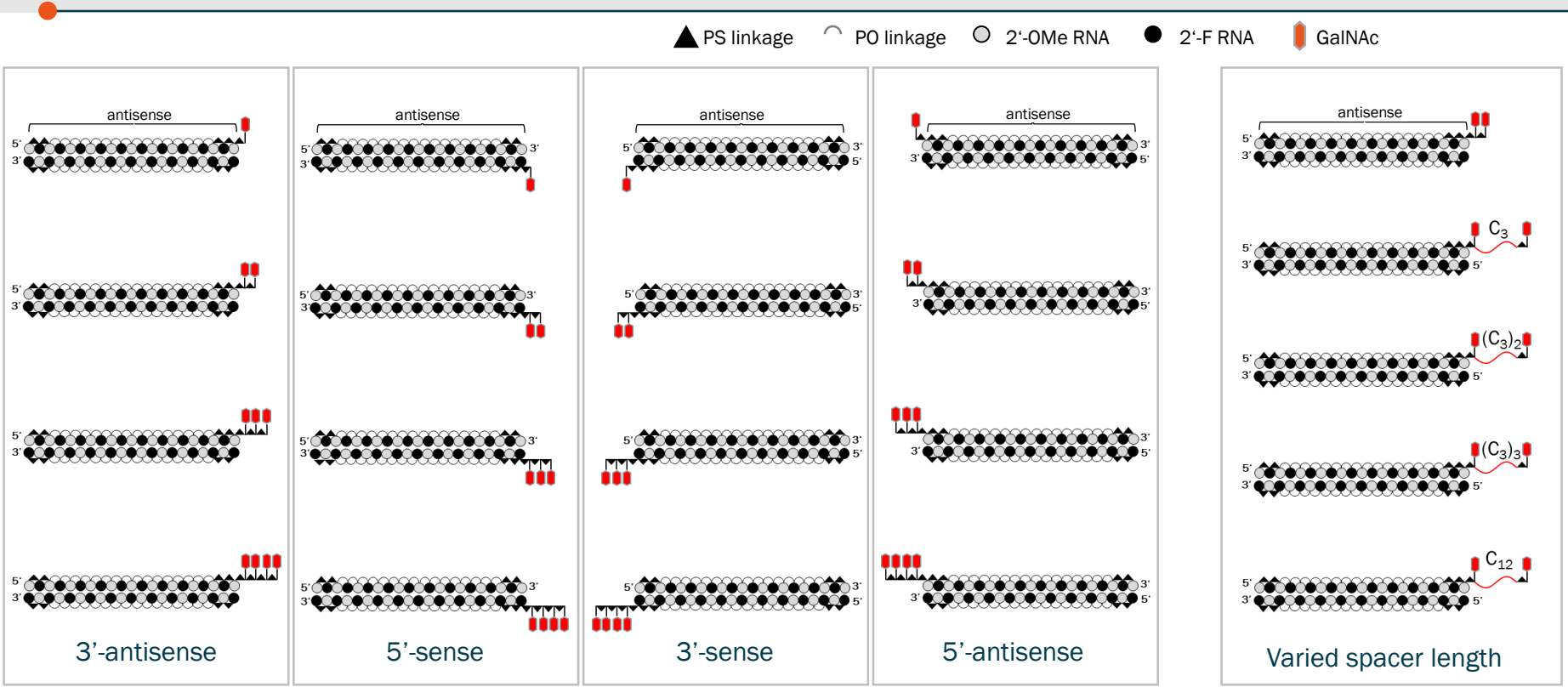
> **Step 2:** investigating optimal distance between two 3’ antisense positioned GalNAc units

- Similar *in vitro* activity (primary mouse hepatocytes) for two serinol linked GalNAc units comparing short or long spacing (longest spacer a C12 hydrocarbon chain)



© Silence Therapeutics 2018

Conjugate designs with serial, serinol-linked GalNAcs



© Silence Therapeutics 2018

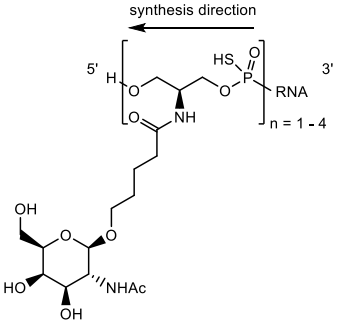
Stepwise path to new design

> **Step 1:** investigating optimal number and location for “serial clusters”

- 1-4 serinol-linked GalNAc units were conjugated to 3’ or 5’ sense or antisense strand
- All conjugates were screened for stability (tritosomes) and *in vitro* activity (primary mouse hepatocytes), several conjugates were also tested *in vivo* (mice)

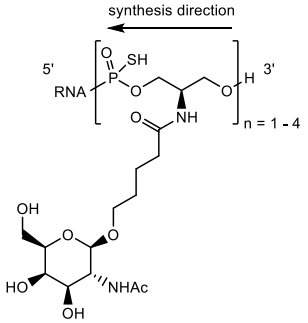
> Result from step 1:

- 5’ antisense conjugates are stable but inactive *in vitro*
- All other positions gave stable conjugates with only minor differences between 2-4 GalNAc units and a triantennary control compound



> **Step 2:** investigating optimal distance between two 3’ antisense positioned GalNAc units

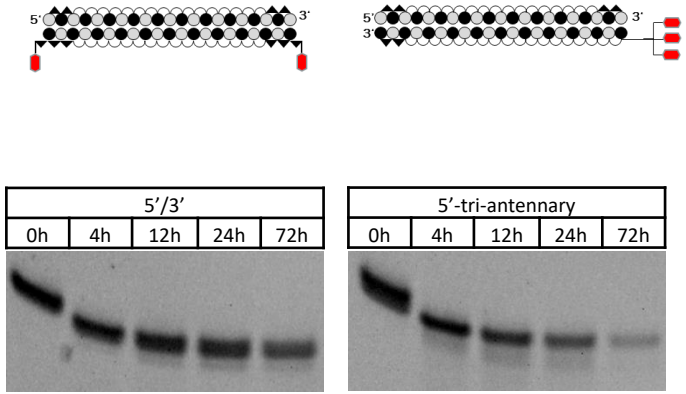
- Similar *in vitro* activity (primary mouse hepatocytes) for two serinol linked GalNAc units comparing short or long spacing (longest spacer a C12 carbohydrate chain)



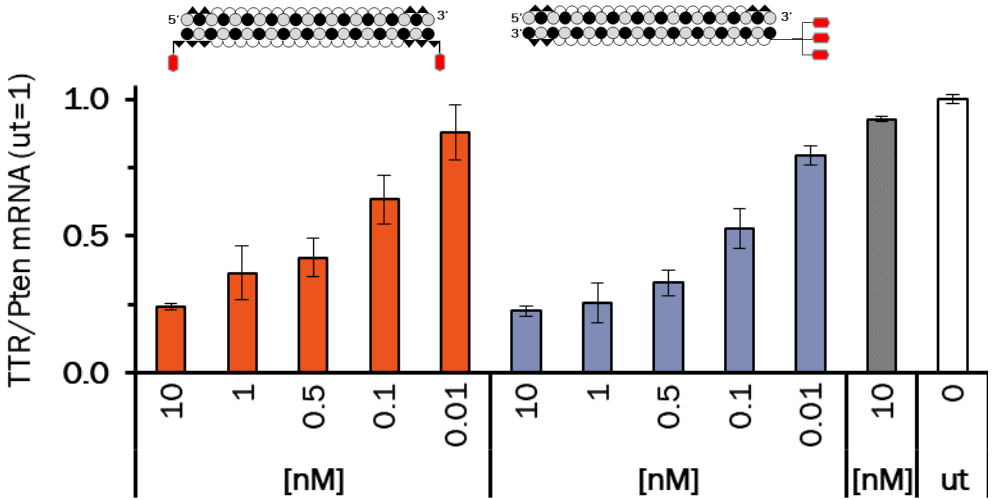
> **Step 3:** Asking the question “If two GalNAc units, close or spaced far between, are sufficient for *in vitro* activity, what will happen if we position single GalNAc units at different end locations in the siRNA?”

© Silence Therapeutics 2018

Step 3: GalNAc conjugated siRNA with single GalNAc units at both ends of the sense strand



Rat liver tritosome stability at 37 °C and pH=5

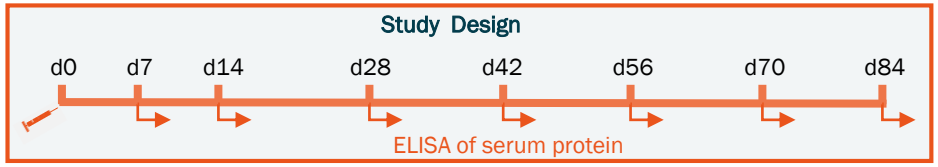


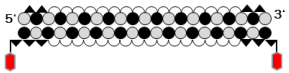
Mouse hepatocytes incubated for 24h
Grey bar: non-targeting control, white bar: un-transfected cells.

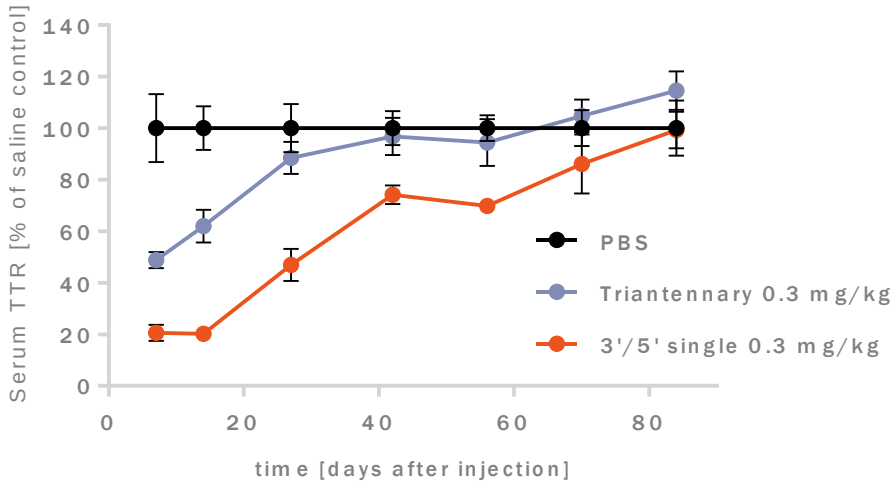
> Single GalNAc unit with 3’ and 5’ sense strand location are remarkably stable and demonstrate equal, or slightly reduced, *in vitro* activity compared to a tri-antennary GalNAc cluster conjugate of the same siRNA

© Silence Therapeutics 2018

Single GalNAc units at both ends of the sense strand increase *in vivo* activity





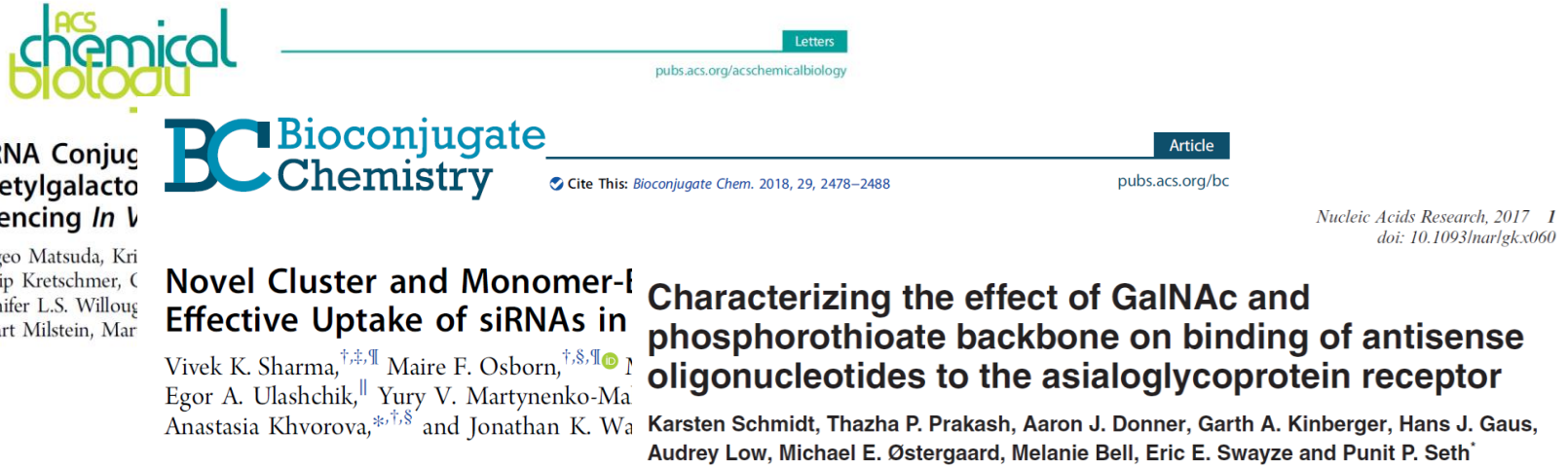


- The new conjugate design is 2-3 times more active *in vivo* compared to a tri-antennary GalNAc cluster conjugate of the same siRNA
- Similar increase in *in vivo* activity has been demonstrated for three other test sequences
- Evaluation is in progress for combination with other new design features, i.e. reduced 2'F nucleobase content

C57Bl/6J mice injected s.c. d0, n=4/group. Data represents mean±SD.
Data presented as percent of saline control at respective time point.

© Silence Therapeutics 2018

Is this really a new idea?



ACS Chemical Biology

Letters

pubs.acs.org/acschemicalbiology

siRNA Conjug
Acetylgalacto
Silencing *In V*

Shigeo Matsuda, Kri
Philip Kretschmer, (
Jennifer L.S. Willoug
Stuart Milstein, Mar

Bioconjugate Chemistry

Article

Cite This: *Bioconjugate Chem.* 2018, 29, 2478−2488

pubs.acs.org/bc

Nucleic Acids Research, 2017 **1**
doi: 10.1093/nar/gkx060

**Novel Cluster and Monomer-l
Effective Uptake of siRNAs in**

Vivek K. Sharma,[†,‡,¶] Maire F. Osborn,[†,§,¶] M
Egor A. Ulashchik,[‖] Yury V. Martynenko-Ma
Anastasia Khvorova,[*,†,§] and Jonathan K. Wa

Characterizing the effect of GalNAc and phosphorothioate backbone on binding of antisense oligonucleotides to the asialoglycoprotein receptor

Karsten Schmidt, Thazha P. Prakash, Aaron J. Donner, Garth A. Kinberger, Hans J. Gaus, Audrey Low, Michael E. Østergaard, Melanie Bell, Eric E. Swayze and Punit P. Seth*

In summary:

> Linear arrangements of 2-4 GalNAc units has previously been shown to give good uptake via ASGPR clusters, but *in vivo* activity is usually not as good as for equimolar dose of triantennary GalNAc conjugates

> To conjugate GalNAc units in both ends of an oligonucleotide is not new (Schmidt *et al*) – the novelty here is that the combination with Silence Therapeutics siRNA design, two single GalNAc units in respective ends of the sense strand improve activity compared to triantennary clusters

© Silence Therapeutics 2018

Summary – novel GalNAc-conjugated siRNA design

- Reduced non-natural nucleotide content to minimum 15% with retained or increased activity
- Surprisingly, two single GalNAc units with 3’ and 5’ sense strand location resulted in increased stability and *in vivo* activity
- Ongoing work includes evaluation of combination of design elements, most of which demonstrates that “less is more”
- 10 siRNA design patent applications filed 2017-18

© Silence Therapeutics 2018

Acknowledgements

Silence Preclinical Drug Discovery and Drug Development Teams:
Ute Schaeper, Steffen Schubert, Manuela Aleku, Christian Frauendorf & Jens-Uwe Künstler

Silence Technology Innovation Team:
Adrien Weingärtner, Judith Hauptmann, Lucas Bethge

© Silence Therapeutics 2018