Activity of Enhanced Stabilization Chemistry (ESC)-GalNAc-siRNA Conjugates via Lung Delivery Using Microsprayer®

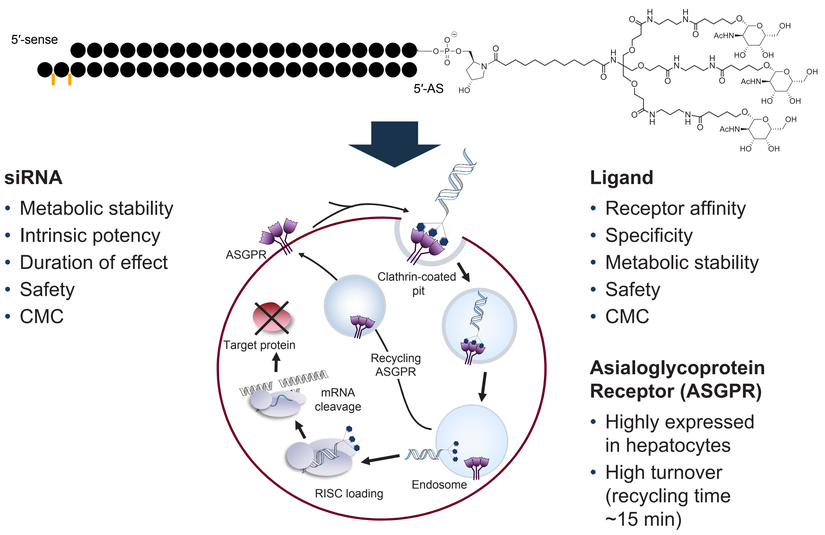
Vasant Jadhav, Jennifer LS Willoughby, Svetlana Morskaya, Stuart Milestein, Martin Maier, Muthiah Manoharan, Laura Sepp-Lorenzino and Rachel Meyers
Alnylam Pharmaceuticals, Inc., 300 Third Street, Cambridge, MA, USA

Abstract

We have recently developed the ESC-GalNAc-siRNA conjugate platform for subcutaneous (SC) delivery of potent, specific and durable RNAi therapeutics. Initial clinical results with an ESC conjugate targeting antithrombin (ALN-AT3) show ~25% target KD at a dose of 0.03 mg/kg confirming the potency of this compound in healthy volunteers. The SC route of administration provides sustained release of conjugates into systemic circulation resulting in improved activity compared to intravenous injection. Although SC administration is preferred for ease of use over IV injection, needle-free administration of conjugates could further expand their utility. We have evaluated a Microsprayer® high pressure syringe to deliver ESC GalNAc-siRNA conjugates to mouse lungs, as a route into systemic circulation. The results indicate that Microsprayer® mediated delivery via lung achieves comparable efficacy, duration and plasma exposure to that of SC delivery. This opens up possibility for needle-free injection of conjugates in clinic as an alternative strategy to achieve systemic exposure to the liver. Further studies would include translation of inhalation delivery in higher species.

Figure 1. siRNA-GalNAc conjugates

SC-administered platform for targeted delivery to hepatocytes



siRNA
- Metabolic stability
- Intrinsic potency
- Duration of effect
- Safety
- CMC

Ligand
- Receptor affinity
- Specificity
- Metabolic stability
- Safety
- CMC

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

Adapted from Essentials of Glycobiology *(2009)*

Figure 2. GalNAc-siRNA conjugate lead optimization process

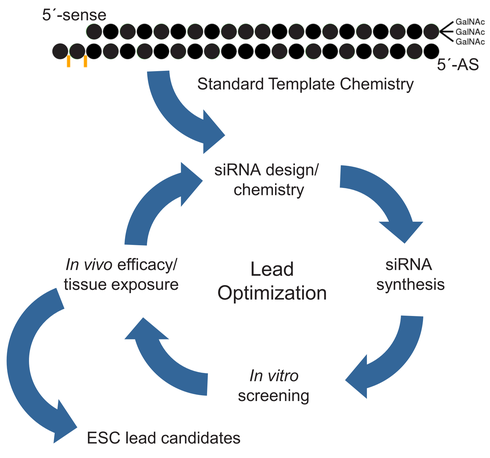
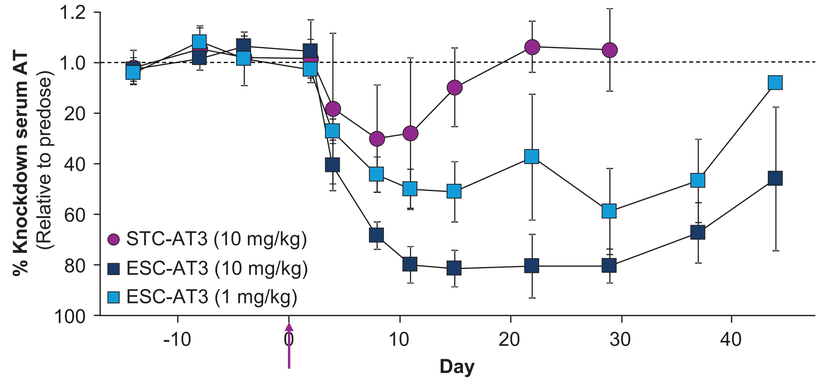


Figure 3. ESC significantly enhances efficacy and duration

Potent and durable silencing achieved after single SC dose in NHP

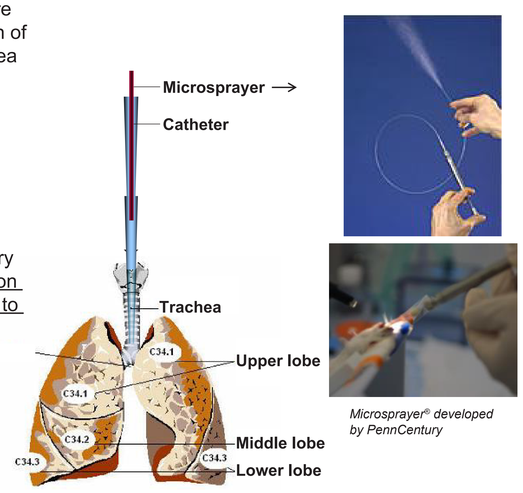
- >10-fold improvement in efficacy over standard template chemistry



Early clinical data with ALN-AT3
- ESC potency improvements translated favorably in healthy volunteers
- Statistically significant KD of antithrombin after single 0.03 mg/kg dose

Microsprayer® mediated intra-tracheal delivery of GalNAc-conjugates- alternative strategy for systemic delivery

- Microsprayer® – A high pressure syringe for direct administration of aerosol at the junction of trachea for delivery in lung
- Avoids loss of material from mouth/nose to trachea
- Systemic exposure of siRNA and LNA-antisense oligos by intra tracheal dosing has been reported.[1] We utilized Microsprayer® mediated delivery as proof of concept for inhalation delivery of GalNAc-conjugates to liver



Microsprayer® developed by PennCentury

[1]Molecular Therapy *2011 19 (12), 2163–2168*

Study design to compare SC vs. intra-tracheal delivery using Microsprayer®

ESC GalNAc-siRNA conjugates targeting TTR and FVII
- These targets provide serum biomarker to monitor duration and efficacy
- ESC conjugates of TTR and FVII have potent *in vitro* activity when transfected with lipofectamine

Study Design

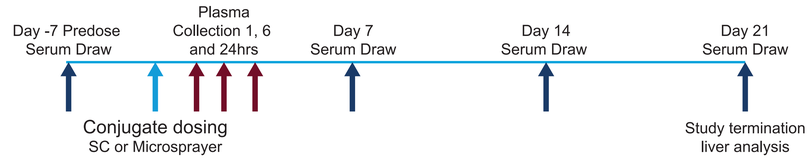


Figure 4. Microsprayer® mediated dosing achieves comparable potency to SC delivered GalNAc-conjugates in mouse liver

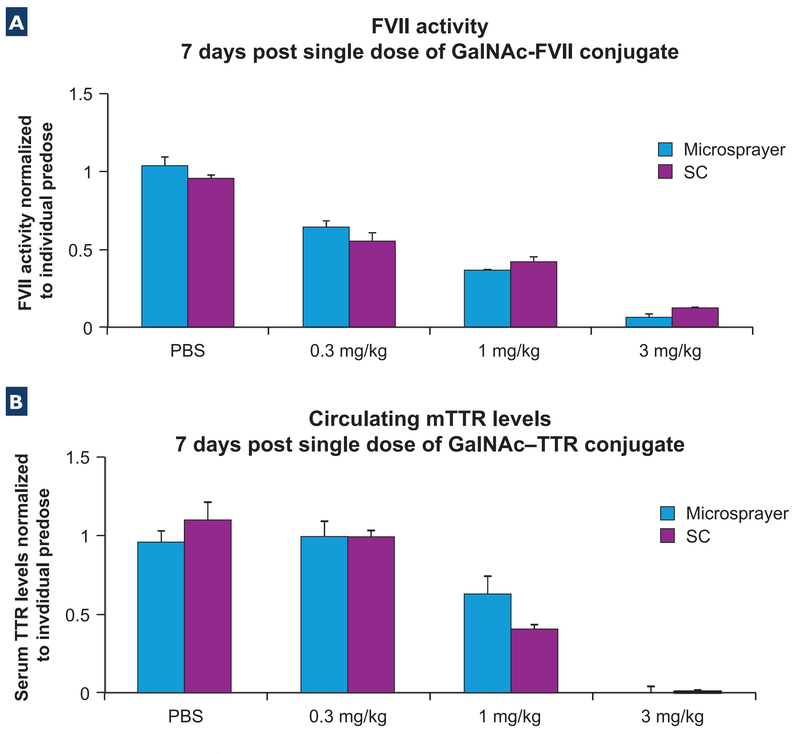


Figure 4. Microsprayer® dosing of GalNAc-FVII or GalNAc-TTR results in dose dependent reduction of target. (A) Serum FVII levels determined by FVII Activity Assay analyzed 7 days after each dose (n = 4 per group) **(B)** Serum mTTR levels measured by ELISA analyzed 7 days after each dose (n = 4 per group)

Figure 5. Microsprayer® mediated dosing achieves comparable duration of activity to SC delivered ESC conjugates in mouse liver

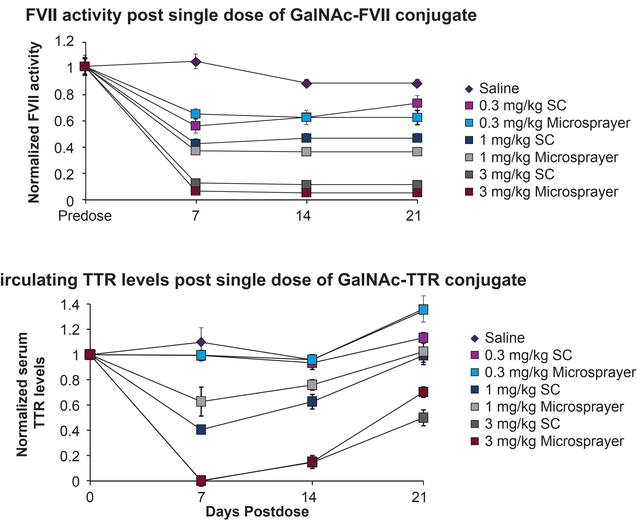


Figure 5. FVII activity or serum TTR levels reveal dose dependent and durable knockdown following conjugate delivery by microsprayer or subcutaneous delivery. Efficacy profile in wild type C57BL/6 mice following a single Microsprayer or SC dose of 3, 1, or 0.3 mg/kg FVII-GalNAc or TTR-GalNAc (N = 4 per group). Serum collected pre-dose, 7, 14 and 21 days post dose for analysis. **(A)** FVII levels normalized to the individual animal pre-dose. Reduction of FVII activity reaches maximum suppression at approximately 7 days post-dose. Duration of FVII silencing is observed out to Day 21. **(B)** TTR levels normalized to the individual animal pre-dose. Reduction of TTR reaches maximum suppression at approximately 7 days post-dose. Microsprayer dosing leads to comparable silencing observed with SC administration at the dose levels examined.

Figure 6. Comparable systemic exposure of ESC conjugates observed with Microsprayer® and SC delivery

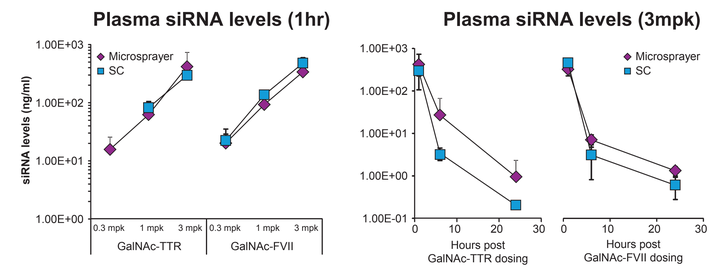
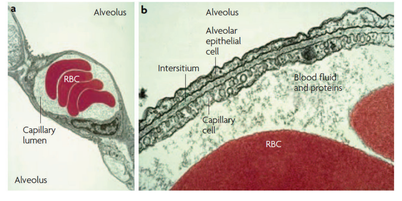


Figure 6. Plasma siRNA levels are comparable following conjugate delivery by microsprayer or SC dosing. siRNA levels assessed by stem-loop PCR method in plasma from wild type C57BL/6 mice following a single Microsprayer or SC dose of 3, 1, or 0.3 mg/kg mTTR-GalNAc (N = 4 per group). Plasma collected at 1, 6 and 24 hours postdose for analysis.

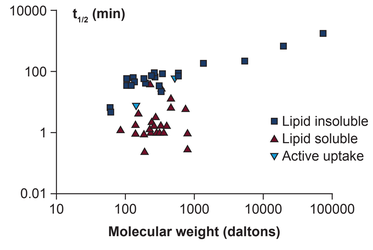
GalNAc-conjugate absorption in systemic circulation via lung

- Absorption via lung is reported for number of compounds including oligos[1] though the mechanism not fully understood
- MannKind's inhaled insulin drug 'Afrezza' approved in 2014

Massive network of lung capillaries in close proximity to alveolar epithelial cells–huge surface area for absorption[2]



Absorption appears to depend on MW for hydrophilic compounds–slower for >50 kDa size compounds[3]



[1]Molecular Therapy *2011* ***19*** *(12), 2163–2168*
[2]Nature Reviews Drug Discovery *2007,* ***6*** *(1), 67-74*
[3]Biochemical Pharmacology *1983,* ***32*** *(17) 2599-2601*

Summary

- ESC GalNAc-siRNA conjugates show comparable efficacy and duration in mouse liver when administered by Microsprayer®-mediated intra-tracheal delivery via lung to that observed with SC administration
- The data support that efficient systemic exposure of GalNAc-siRNA conjugates and delivery to liver can be achieved via lung
- This opens up the possibility for development of needle-free, non-invasive administration of conjugates by inhalation delivery
  - Specific formulations and devices would need to be applied
- Future studies would involve translating these results for inhalation delivery in NHP

Acknowledgements

We would like to thank Biomedical Research Models, INC. Worcester, MA for conducting in-life portion of studies to evaluate SC vs. Microsprayer® mediated delivery.