Efficient and Durable Ocular Gene Silencing of TTR after Single Intravitreal Administration of siRNA Conjugates

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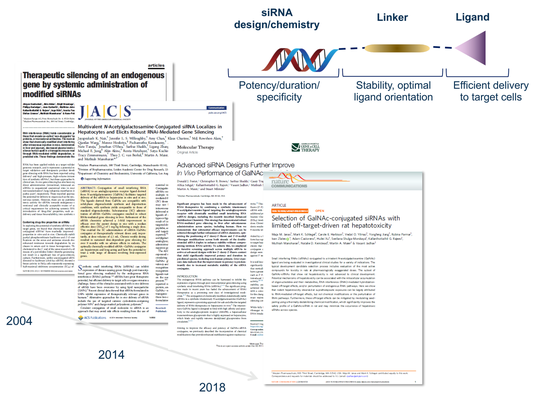
Abstract

- Ocular transthyretin produced locally in retinal pigment epithelium (RPE) and ciliary epithelia (CE) can cause amyloid deposits, resulting in significant visual impairment, including blindness, in approximately 10% of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) patients.
- Liver transplantation does not resolve ocular amyloidosis and liver-directed therapies are not expected to be efficacious against ocular manifestations.
- Silencing the expression of TTR in the eye using RNAi Therapeutics would represent a novel treatment approach for development.
- Here we show that siRNA conjugates targeting TTR can be delivered to the relevant cell types in the eye and produce efficient and durable gene silencing after single intravitreal administration.
- Preclinical efficacy and safety of siRNA conjugates in rodents and nonhuman primates will be presented.

Figure 1. Alnylam Advancements in Conjugate-Based siRNA Delivery



Evolution of conjugate potency (mouse, SD ED_{50})

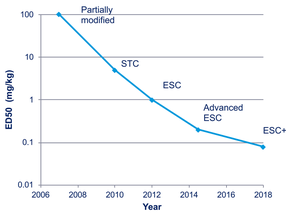
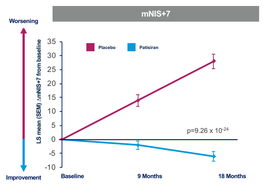


Figure 2. RNAi Therapeutics for hATTR Amyloidosis

APOLLO Phase 3 Study Results

Met Primary and all Secondary Endpoints ONPATTRO™ Now Approved in the U.S. and EU

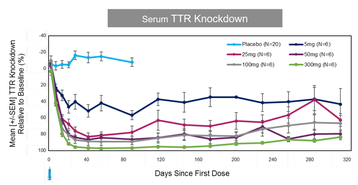


Safety

- No deaths considered related to study drug
- Majority of AEs mild or moderate in severity
- Encouraging safety & tolerability in cardiac subpopulation
- No safety signals related to steroid pre-medication regimen or TTR knockdown
- No safety signals regarding liver function tests, hematology including thrombocytopenia, or renal dysfunction related to patisiran

ALN_TTRsc02 Phase 1 Study Results

Mean max TTR KD of 97.1%; ~80% TTR KD at nearly 1 year after single 50 mg dose



Safety (N= 80)

- No SAEs and no discontinuations due to AEs
- All AEs mild or moderate in severity

ALN_TTRsc02: Expect to initiate Phase 3 study in Late 2018

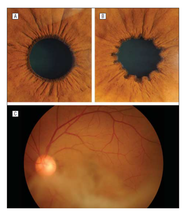
Figure 3. TTR is Produced in the Eye as Well as the Liver

Ocular manifestations of hATTR in patients

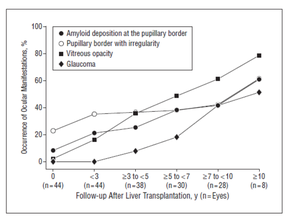
Intraocular (% of patients with ocular symptoms)

- Glaucoma (~12%-23%)
- Vitreous opacities (Wide range)
- Retinal abnormalities (~4% - 15%)
  - retinal amyloid deposit
  - retinal angiopathy
- Iris abnormalities (~14% - 38%)
  - iris amyloid deposit
  - scalloped iris
- Amyloid deposits on lens (~33%)

I



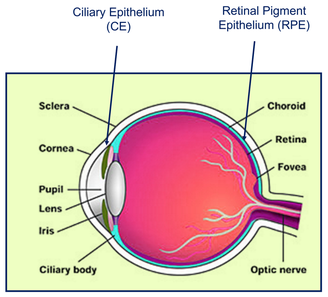
II



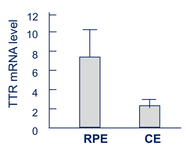
I. Pupillary deposits and abnormality predict onset of glaucoma
Ocular manifestations in transthyretin-related familial amyloid polyneuropathy. A, Amyloid deposition at the pupillary border. B, Pupillary border with irregularity. C, Vitreous opacities

II. Study of Japanese liver transplant patients with V30M continue to have ocular symptoms
Changes in the occurrence of ocular manifestations in transthyretin-related familial amyloid polyneuropathy. Occurrence of all the ocular manifestations increased with time after liver transplantation

Figure 4. Two Sites of TTR Production in the Eye



A



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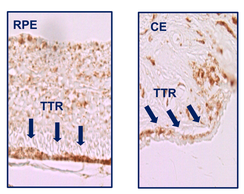


A. Expression of TTR mRNA in RPE cells and CPE cells with paraffin embedded tissue samples. The relative levels of mRNA expression were quantified and normalized to levels of G6PD mRNA expression. For each group in paraffin-embedded tissue samples, n=4.

B. In situ hybridization studies of rabbit eye sections via a tyramide signal amplification system with a fluorescein-labeled RNA probe for rabbit TTR. The tissues were analyzed under light microscopy.

Figure 5. Ocular TTR Silencing by Differentially Modified siRNA Conjugates in Rat After Single Intravitreal Injection

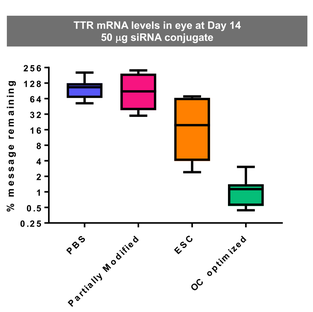
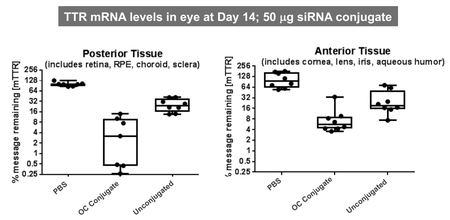


Figure 6. Robust TTR Silencing in Both CE and RPE in Rat



- TTR KD observed in anterior and posterior tissue suggesting activity across eye
- No treatment-related clinical observation and histopathology analysis did not identify any treatment related findings at dose levels tested

Relative mRNA knockdown versus a saline control in rat eye at Day 14 by qPCR. TTR mRNA expression was quantified by RT-qPCR and normalized to GAPDH expression. The data are represented as mean ± SD (n=3).

Figure 7. TTR Ocular Activity in hTTR Transgenic Mice

Specificity of Target Knockdown

Ocular silencing of hTTR in transgenic mice with optimized conjugate design

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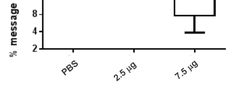
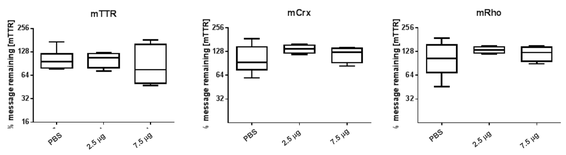


Specificity: No impact on expression of mouse TTR, Cone-Rod Homeobox or Rhodopsin

II

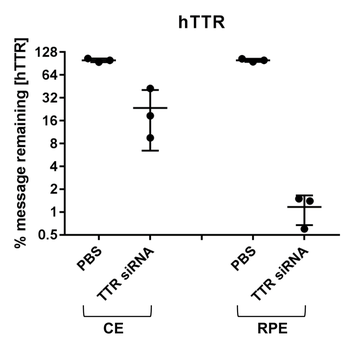


I. Relative mRNA knockdown versus a saline control in hTTR transgenic mice eye at Day 14 by qPCR. hTTR mRNA expression was quantified by RT-qPCR and normalized to GAPDH expression. The data are represented as mean ± SD (n=3).

II. mTTR, mCrx and mRho expressions were quantified by RT-qPCR and normalized to GAPDH expression. The data are represented as mean ± SD (n=3).

Figure 8. Ocular TTR Silencing by siRNA Conjugates in Non-Human Primates (NHP)

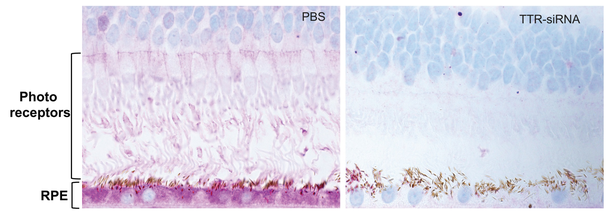
TTR mRNA levels in retinal pigment epithelium (RPE) and ciliary epithelia (CE) at day 31 in NHP 3 mgs siRNA conjugate



Relative mRNA knockdown versus a saline control in NHP eye at Day 31 was calculated by qPCR. TTR mRNA expression was quantified by RT-qPCR and normalized to GAPDH expression. The data are represented as mean ± SD (n=3).

Figure 9. Ocular TTR Silencing by OC Optimized Conjugates in NHP

TTR protein analysis by IHC
Single intravitreal injection of 3 mgs in 50 µL



Near Complete reduction of TTR Protein (magenta in each image) at Day 31 in retinal pigment epithelium (RPE)

Safety of Ocular siRNA Conjugates in NHP

Ophthalmoscopic Examination Summary (Days -7, 3, 8, 30)

PBS (Individual Animals, N=3)	TTR siRNA (Individual Animals, N=3)
Normal	Normal
Blepharitis Eye/Right	Normal
Normal	Normal

Histopathology Summary (Day 31)

Eye (right)	PBS (N=3)	TTR siRNA (N=3)
Cornea/Conjunctiva/Sclera	Normal	Normal
Anterior Chamber/Lens	Normal	Normal
Posterior Chamber/Vitreous body	Normal	Normal
Choroid/Retina/Optic nerve	Normal	Normal

Ocular Opportunity for RNAi Therapeutics

- The siRNA conjugates specifically designed for ocular delivery show robust and durable RNAi activity
  - Silencing demonstrated at both sites of ocular TTR expression (RPE and CE)
  - Encouraging initial safety results
- Successful translation to higher species
- RNAi therapeutics directed to disease-causing, intraocular gene targets represents a significant opportunity for further development

References

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