Transthyretin (TTR)-mediated amyloidosis (ATTR) is an inherited, progressively debilitating, and fatal disease caused by mutations in the TTR gene. TTR is a serum transport protein predominantly expressed in the liver. Destabilizing mutations in TTR result in protein misfolding and accumulation of pathogenic protein (including both mutant and wild type) deposits in peripheral tissues including nerves and heart, resulting in neuropathy and/or cardiomyopathy. Although the clinical manifestation of ATTR depends on the nature and location of deposit accumulation, the underlying cause is siTTRsc 25.0, 2.5, or 1.0 mg/kg, qwx12

RNAi therapeutic hypothesis for treatment of ATTR

• Subcutaneous administration, QW

Unstable circulating TTR

Tafamidis stabilization of TTR tetramer

• LNP-siTTR or control formulation LNP-siCTRL

Overview

• In direct comparison with an ASO targeting TTR, administration of ALN-TTRsc achieved more rapid and potent knockdown at 2 log lower drug levels in liver.

RNAi mediated suppression of TTR resulted in robust and significant regression of mutant TTR deposition in peripheral tissues, consistent with previous work.

• The extent of regression of mutant TTR deposits correlates with the absolute circulating TTR protein levels at steady state knockdown.

Study Termination

<table>
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Figure 2. RNAi-mediated suppression leads to superior TTR tissue deposit regression relative to tetramer stabilization

Figure 3. (A) Study design. (B) hTTR V30M/hsf1-/+ transgenic mice received subcutaneous administration of siTTRsc once weekly for 12 weeks at dose levels of 1, 2.5, and 25 mg/kg. Plasma TTR protein levels were measured by ELISA. Data points represent...
Abstract

Translthyretin (TTR)-mediated amyloidosis (ATTR) is an inherited, progressively debilitating, and fatal disease caused by mutations in the TTR gene. TTR is a serum transport protein predominantly expressed in the liver. Destabilizing mutations in TTR result in protein misfolding and accumulation of pathogenic protein (including both mutant and wild type) deposits in peripheral tissues including nerves and heart, resulting in neuropathy and/or cardiomyopathy. Although the clinical manifestation of ATTR depends on the nature and location of deposit accumulation, the underlying cause is amyloidogenic TTR protein expression. As such, any successful therapeutic intervention must impact the deleterious effects of mutant protein expression. RNA interference (RNAi) is a clinically validated technology that offers great promise for the treatment of genetic diseases. RNAi is mediated by small interfering RNAs (siRNA) that function by facilitating the degradation of mRNA, the biological precursor essential for protein production. In the context of ATTR, the therapeutic objective of RNAi-based drugs is to eliminate both wild type and mutant TTR mRNA, thereby preventing the expression and resultant negative effects of amyloidogenic TTR protein. To that end, two investigational RNAi-based therapeutics for the treatment of ATTR are currently in clinical development, including patisiran for familial amyloidotic polyneuropathy (FAP) and ALN-TTRsc for TTR cardiac amyloidosis. In preclinical studies presented here, we demonstrate that both investigational drugs result in robust and durable suppression of serum TTR protein. These investigational therapeutics successfully target all amyloidogenic TTR mutations tested to date, demonstrating the broad applicability of this platform toward all forms of ATTR. Finally, in a mouse model of V30MATTR, we demonstrate significant reductions of TTR protein deposition in peripheral tissues typically associated with disease.

RNAi therapeutic hypothesis for treatment of ATTR

Mutant TTR is genetic cause of ATTR

- Autosomal dominant with >100 defined mutations
- Misfolds and forms amyloid deposits in nerves, heart, other tissues
- Wild-type TTR also accumulates in amyloid plaques
  - Limits benefits of liver transplantation

Summary

Context

Previously, we have demonstrated that RNAi-mediated suppression of TTR in a transgenic mouse model expressing amyloidogenic mutant human V30M TTR leads to inhibition and regression of TTR deposition in peripheral tissues. These data support RNAi as a promising therapeutic approach for the treatment of ATTR. Here we present new data from experiments in the human V30M TTR transgenic mouse model designed to address two key questions regarding the robustness of this approach. Specifically:

1. How does RNAi compare to other therapeutic approaches?
2. What is the extent of TTR suppression needed to impact TTR deposition?

Summary of key findings

- In direct comparison with an ASO targeting TTR, administration of ALN-TTRscs achieved more rapid and potent knockdown at 2 log lower drug levels in liver.
- RNAi-mediated suppression of TTR resulted in robust and significant regression of mutant TTR deposition in peripheral tissues, consistent with previous work.
  - In contrast, the TTR tetramer stabilizer Tafamidis resulted in minimal regression of TTR deposits at levels of tetramer stabilization that equal or surpass that observed in the clinic.
- The extent of regression of mutant TTR deposits correlates with the absolute circulating TTR protein levels at steady state knockdown.
  - This finding supports the therapeutic hypothesis and suggests that clinical benefit will directly correlate with the extent of RNAi-mediated TTR protein suppression.

Figure 1. RNAi-mediated suppression of TTR with lower drug exposure in target tissue than ASO

A) Study design

<table>
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- hTTR V30M/hsf1−/+ transgenic mice age 8-10 months
- Subcutaneous administration, BIW

B) Direct comparison of TTR ASO to ALN-TTRsc

- LNP-siTTR suppression of TTR protein

C) 2 to 3-log higher liver and kidney levels for TTR ASO compared to ALN-TTRsc at same dose level

Figure 1. (A) Study design. (B) RNAi achieves more rapid and potent knockdown than TTR ASO. ALN-TTRsc or TTR ASO were administered subcutaneously twice a week for 4 weeks at 2.5 or 10 mg/kg in hTTR V30M/hsf1−/+ transgenic mice. Plasma TTR protein concentration was measured by ELISA. Data points represent the mean relative plasma TTR protein concentration; error bars are the standard deviation. (C) 2 to 3-log higher liver and kidney levels for TTR ASO compared to ALN-TTRsc at same dose level. ALN-TTRsc and TTR ASO liver and kidney tissue levels were measured using hybridization based HPLC assays. LLOQ for liver was 100 ng/g and 150 ng/g and for kidney was 400 ng/g and 300 ng/g for ALN-TTRsc and TTR ASO, respectively. Liver and kidney drug levels were measured 24h after the 1st and 8th doses and 4 weeks after the last dose. The relative kd at each time point is indicated on the bar graphs.

Figure 2. Relative TTR deposition in peripheral tissues (A) at 2.5 mg/kg and (B) at 10 mg/kg of ALN-TTRsc or TTR ASO administered subcutaneously twice a week for 4 weeks in hTTR V30M/hsf1−/+ transgenic mice. Data fit to a linear regression (p-value <0.01).

Figure 3. TTR regression correlates with reduction in exposure to circulating TTR...
Summary of key findings

1. How does RNAi compare to other therapeutic approaches?

Previously, we have demonstrated that RNAi-mediated suppression of TTR in a transgenic mouse model expressing amyloidogenic mutant human V30M TTR leads to inhibition and regression of disease.

Context

including patisiran for familial amyloidotic polyneuropathy (FAP) and ALN-TTRsc for TTR cardiac amyloidosis. In preclinical studies presented here, we demonstrate that both investigational drugs have the potential to act as precursors for protein production. In the context of ATTR, the therapeutic objective of RNAi-based drugs is to eliminate both wild type and mutant TTR mRNA, thereby preventing the expression and resultant negative effects of amyloidogenic TTR protein. To that end, two investigational RNAi-based therapeutics for the treatment of ATTR are currently in clinical development, which act to knock down both mutant and wild-type TTR production.

Preclinical Evaluation of RNAi Therapeutics for the Treatment of ATTR: An Update

ALN-TTRsc and TTR ASO liver and kidney tissue levels

KD

Regression of TTR deposition in peripheral tissues

Relative TTR deposition

Days

R² = 0.4125

Comparison of TTR tissue deposition regression

Figure 2. RNAi-mediated suppression leads to superior TTR tissue deposit regression relative to tetramer stabilization

Study design

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Overview

- V30M hTTR, HSF +/- HM Tg mice
  - Animal age at study start = 15 months
- LNP-siTTR or control formulation LNP-siCTRL
  - 1 mg/kg, i.v. administration every 2 weeks for a total of 4 doses (q2w x4)
- Tafamidis or Meglumine Control Formulation
  - 2 mg/mL x 0.4 mL (0.8 mg) administered subcutaneously three times weekly (M,W,F) for a total of 17 doses
  - Tafamidis formulated as a Tafamidis/meglumine complex; control formulation is meglumine only

Figure 2. (A) Study design. (B) Administration of LNP-siTTR results in robust and durable suppression of serum TTR protein. Serum TTR protein concentration measured by ELISA. Data points represent the mean relative serum TTR protein concentration at each respective time point; error bars represent standard error of the mean (SEM). Statistical significance evaluated using a repeated-measures ANOVA significant at p<0.05; asterisks indicate significance of pairwise comparisons to respective time point according to Holm-Sidak multiple comparisons tests (***, p<0.001). (C) Administration of Tafamidis results in robust TTR Tetramer stabilization. % TTR Tetramer stabilization measured as previously described. Data represent mean value at each point; error bars represent SEM. Statistical significance evaluated using a repeated-measures ANOVA significant at p<0.05; asterisks indicate significance of pairwise comparisons to respective time point according to Holm-Sidak multiple comparisons tests (**, p<0.01). (D) RNAi mediated suppression leads to superior TTR tissue deposit regression. Relative V30M TTR tissue deposition determined by IHC as previously described. Statistical significance evaluated using 1-way ANOVA significant at p<0.05; asterisks indicate significance of pairwise comparisons of LNP-siTTR treated animals relative to Tafamidis treated animals (*, p<0.05; **, p<0.01; ***, p<0.001).

Figure 1. (A) Study design. (B) hTTR V30M/hsf1-/+ transgenic mice age 8-10 months. (C) Bars are the standard deviation.

Figure 3. TTR regression correlates with reduction in exposure to circulating TTR
The extent of regression of mutant TTR deposits correlates with the absolute circulating TTR protein levels at steady state knockdown. LLOQ for liver was 100 ng/g and 150 ng/g and for kidney was 400 ng/g and 300 ng/g for ALN-TTRsc and TTR ASO, respectively. Liver and kidney levels for TTR ASO compared to ALN-TTRsc at same dose level.

2. What is the extent of TTR suppression needed to impact TTR deposition?

Mutant TTR is genetic cause of ATTR, biological precursor essential for protein production. In the context of ATTR, the therapeutic objective of RNAi-based drugs is to eliminate both wild type and mutant TTR mRNA, thereby preventing nerves and heart, resulting in neuropathy and/or cardiomyopathy. Although the clinical manifestation of ATTR depends on the nature and location of deposit accumulation, the underlying cause is.

Preclinical Evaluation of RNAi Therapeutics for the T reatment of ATTR: An Update

Abstract

Study design

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</table>

Study Termination

Concentration in Liver

~70%

Figure 2. RNAi-mediated suppression leads to superior TTR tissue deposit regression relative to tetramer stabilization

Concentration (µg/g)

0 10 20 30 40 50 60 70 80

Days

0 10 20 30 40 50 60 70 80

Figure 3. TTR regression correlates with reduction in exposure to circulating TTR

Study design

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<th>Dose</th>
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• HTR V30M/hsf1−− transgenic mice; 14-16 months old
• Subcutaneous administration, QW

Regression of TTR deposition in peripheral tissues

Figure 3. (A) Study design. (B) Subcutaneous administration of siTTRsc results in robust, dose dependent and sustained plasma TTR protein suppression. hTTR V30M/hsf1−− transgenic mice received subcutaneous administration of siTTRsc once weekly for 12 weeks at dose levels of 1, 2.5, and 25 mg/kg. Plasma TTR protein levels were measured by ELISA. Data points represent the mean plasma TTR protein concentration; error bars represent standard error of the mean (SEM). Statistical significance of steady knockdown evaluated using a repeated-measures ANOVA. Pairwise comparisons with control group p<0.01, p<0.001 and p<0.0001 at 1, 2.5 and 25 mg/kg siTTRsc, respectively. (C) siTTRsc suppression of TTR leads to dose dependent regression of TTR deposition in peripheral tissues. TTR tissue deposition (brown stain) was detected by immunohistochemical staining with an anti-TTR antibody. Brown stained regions represent positive TTR antibody staining. (D) Reduction in steady state TTR exposure correlates with extent of TTR regression. Data points for each individual animal are the relative V30M TTR tissue deposition determined by IHC as previously described plotted relative to the total circulating plasma TTR exposure at steady state knockdown for each animal. Data fit to a linear regression (p-value <0.01).

References