

ORIGINAL ARTICLE

Safety and Efficacy of RNAi Therapy for Transthyretin Amyloidosis

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ABSTRACT

BACKGROUND

Transthyretin amyloidosis is caused by the deposition of hepatocyte-derived transthyretin amyloid in peripheral nerves and the heart. A therapeutic approach mediated by RNA interference (RNAi) could reduce the production of transthyretin.

METHODS

We identified a potent antitranssthyretin small interfering RNA, which was encapsulated in two distinct first- and second-generation formulations of lipid nanoparticles, generating ALN-TTR01 and ALN-TTR02, respectively. Each formulation was studied in a single-dose, placebo-controlled phase 1 trial to assess safety and effect on transthyretin levels. We first evaluated ALN-TTR01 (at doses of 0.01 to 1.0 mg per kilogram of body weight) in 32 patients with transthyretin amyloidosis and then evaluated ALN-TTR02 (at doses of 0.01 to 0.5 mg per kilogram) in 17 healthy volunteers.

RESULTS

Rapid, dose-dependent, and durable lowering of transthyretin levels was observed in the two trials. At a dose of 1.0 mg per kilogram, ALN-TTR01 suppressed transthyretin, with a mean reduction at day 7 of 38%, as compared with placebo ($P=0.01$); levels of mutant and nonmutant forms of transthyretin were lowered to a similar extent. For ALN-TTR02, the mean reductions in transthyretin levels at doses of 0.15 to 0.3 mg per kilogram ranged from 82.3 to 86.8%, with reductions of 56.6 to 67.1% at 28 days ($P<0.001$ for all comparisons). These reductions were shown to be RNAi-mediated. Mild-to-moderate infusion-related reactions occurred in 20.8% and 7.7% of participants receiving ALN-TTR01 and ALN-TTR02, respectively.

CONCLUSIONS

ALN-TTR01 and ALN-TTR02 suppressed the production of both mutant and nonmutant forms of transthyretin, establishing proof of concept for RNAi therapy targeting messenger RNA transcribed from a disease-causing gene. (Funded by Alnylam Pharmaceuticals; ClinicalTrials.gov numbers, NCT01148953 and NCT01559077.)

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TRANSTHYRETIN AMYLOIDOSIS IS A LIFE-threatening disorder caused by the deposition of hepatocyte-derived transthyretin amyloid in various tissues and organs.^{1,2} Circulating transthyretin is derived from the liver³ and can form amyloid deposits in peripheral nerves and in the gastrointestinal tract, heart, and kidneys. Transthyretin is also synthesized by the retina and choroid plexus,^{4,5} which can lead to vitreal and leptomeningeal deposits. More than 100 genetic variants of the gene encoding transthyretin (TTR) are associated with autosomal dominant forms of the disease, known as familial amyloidotic polyneuropathy^{6–8} and familial amyloidotic cardiomyopathy.^{9–11}

The most common mutation associated with familial amyloidotic polyneuropathy is V30M; in addition to polyneuropathy, cardiac involvement can be manifested early as conduction disturbances in patients with the V30M mutation and as cardiomyopathy in some patients with the V30M mutation who have advanced disease and in patients with familial amyloidotic polyneuropathy with other mutations.^{12,13} The predominant mutation associated with familial amyloidotic cardiomyopathy is V122I; patients with this mutation are susceptible to isolated cardiac involvement and usually do not have polyneuropathy.⁹ Most patients are heterozygous for the TTR mutations, and the amyloid deposits consist of mutant and nonmutant transthyretin.^{14,15}

Liver transplantation is performed in patients with familial amyloidotic polyneuropathy but has substantial limitations, including transplant availability and substantial morbidity and mortality.^{16–20} Also, transplantation eliminates the production of mutant but not nonmutant transthyretin, so further deposition of nonmutant transthyretin occurs after transplantation, leading to cardiomyopathy and worsening of neuropathy. Furthermore, since liver transplantation does not affect the production of transthyretin in the eye and central nervous system, ocular or leptomeningeal disease can develop after transplantation.²¹ Tafamidis, a small-molecule stabilizer of the transthyretin tetramer, was recently approved in the European Union on the basis of its ability to slow the progression of neuropathy in early-stage familial amyloidotic polyneuropathy.²²

RNA interference (RNAi) is an endogenous cellular mechanism for controlling gene expression in which small interfering RNAs (siRNAs) that are bound to the RNA-induced silencing com-

plex (RISC) mediate the cleavage of target messenger RNA (mRNA).^{23,24} The ability to turn siRNAs into drugs has depended on chemical processes that confer druglike properties and facilitate safe and effective delivery to target organs.^{25,26} Formulations of lipid nanoparticles have emerged as agents to deliver siRNAs to hepatocytes and have resulted in a robust and durable reduction in genetic expression (called knockdown) of a variety of hepatocyte targets across multiple species.^{27–29} Biodistribution of these parenterally administered lipid nanoparticles is predominantly to the liver, with a small fraction distributing to other organs that have a fenestrated endothelium (e.g., spleen).^{30,31} Penetration into the central nervous system or eye does not occur after systemic administration of such formulations.

ALN-TTR01 and ALN-TTR02 are first- and second-generation formulations of lipid nanoparticles as agents to deliver siRNAs. These formulations have similar physicochemical properties but different ionizable lipid components, which are key determinants of potency.³² Each formulation encapsulates an identical siRNA that targets a conserved sequence in the 3′ untranslated region of nonmutant and mutant mRNA in TTR, thereby affecting both forms of TTR (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). To determine the activity and relative potencies of these two formulations in lowering transthyretin levels, and thereby select the best formulation to take forward, we performed studies in nonhuman primates and two sequential phase 1 trials.

METHODS

STUDY PATIENTS

Patients who were 18 years of age or older were eligible for the ALN-TTR01 phase 1 trial if they had a biopsy-proven diagnosis of transthyretin amyloidosis with mild-to-moderate neuropathy, a Karnofsky performance-status score of at least 60, a body-mass index (BMI; the weight in kilograms divided by the square of the height in meters) of 18.5 to 33.0, a New York Heart Association class of II or less, and adequate liver, renal, and thyroid function. Men and women between the ages of 18 and 45 years were eligible for the ALN-TTR02 phase 1 trial if they were healthy (as determined on the basis of a medical history, physical examination, and 12-lead electrocardiography), had a BMI of 18.0 to 31.5, had adequate liver function

and blood counts, and did not have childbearing potential.

STUDY DESIGN

The trial to test ALN-TTR01 enrolled participants from July 2010 through September 2011, and the trial to test ALN-TTR02 (which was developed after the ALN-TTR01 trial) enrolled participants from March through May 2012. Both phase 1 trials were multicenter, randomized, single-blind, placebo-controlled, dose-ranging studies to evaluate the safety and efficacy of a single dose of ALN-TTR01 or ALN-TTR02 in patients with transthyretin amyloidosis or in healthy adult volunteers, respectively.

Series of participants, consisting of four each, were randomly assigned to receive ALN-TTR01 (at doses of 0.01 to 1.0 mg per kilogram of body weight) or ALN-TTR02 (at doses of 0.01 to 0.5 mg per kilogram) or placebo (normal saline) in a 3:1 ratio. ALN-TTR01 and ALN-TTR02 were administered intravenously during a period of 15 minutes and 60 minutes, respectively. In the two trials, participants received similar premedication the evening before and the day of infusion to reduce the risk of infusion-related reactions.^{33,34} These medications included dexamethasone, acetaminophen, diphenhydramine or cetirizine, and ranitidine. In the event of a mild-to-moderate infusion-related reaction, the infusion could be temporarily interrupted and resumed at a slower rate.

STUDY OVERSIGHT

The study protocols were approved by the ethics committee at each site. All participants provided written informed consent. The studies were designed jointly by the principal investigators at the study sites and representatives of the sponsor, Alnylam Pharmaceuticals. Data were collected by the investigators, gathered by Medpace, and analyzed by Veristat and the sponsor. All the authors interpreted the data and collaborated in the preparation of the manuscript. All the authors made the decision to submit the manuscript for publication, and all vouch for the completeness and accuracy of this report and its fidelity to the protocol, available at NEJM.org.

CLINICAL ACTIVITY, SAFETY, AND PHARMACOKINETIC EVALUATIONS

We measured the pharmacodynamic activity of ALN-TTR01 and ALN-TTR02, as reflected by serum transthyretin levels, using a validated enzyme-linked immunosorbent assay (ELISA) for total

transthyretin (Charles River Laboratories). For patients with the V30M mutation in the ALN-TTR01 study, measurement of mutant and nonmutant transthyretin was performed at Tandem Laboratories with the use of liquid chromatography–tandem mass spectrometry (see the Methods section in the Supplementary Appendix for more details). Measurements of serum retinol-binding protein and vitamin A were performed at Biomnis. Baseline levels of transthyretin, retinol-binding protein, and vitamin A for each patient were defined as the mean of two to three measurements before the administration of ALN-TTR01 or four measurements before the administration of ALN-TTR02.

Adverse events were monitored from the start of drug administration through day 28. Safety monitoring also included hematologic evaluations, blood chemical analyses, and thyroid-function tests.

We evaluated the plasma pharmacokinetics of the TTR siRNA contained in both ALN-TTR01 and ALN-TTR02 by means of a validated ELISA-based hybridization assay. For detection and quantification of siRNA, we used an oligonucleotide probe (lower limit of quantification, 0.5 ng per milliliter) (Charles River Laboratories) in the ALN-TTR01 trial and the ATTO-Probe-HPLC assay (lower limit of quantification, 1.0 ng per milliliter) (Tandem Laboratories) in the ALN-TTR02 trial. We did not obtain data that addressed the question of whether plasma levels of TTR siRNA were proportionate to levels of transthyretin-lowering siRNA entering the hepatocyte. We used WinNonlin (Pharsight) to determine the pharmacokinetic estimates.

STATISTICAL ANALYSIS

We measured the knockdown of transthyretin, vitamin A, and retinol-binding protein as compared with baseline levels. Geometric means and confidence intervals were calculated by means of natural-log transformation, back-transformed for presentation (Fig. 1A, 2A, and 2C). Repeated-measures analysis of variance examined log knockdown ratios. Main effects were dose, time, and interaction. Covariance was unstructured, and the day after drug administration was a nominal variable. Dose-by-time interaction was significant in both ALN-TTR01 ($P=0.0054$) and ALN-TTR02 ($P<0.001$) trials. We used *t*-tests to perform comparisons between the ALN-TTR01 and ALN-TTR02 groups and the placebo groups (Fig. 1A and 2A). We used linear regression to test correlations between either nonmutant or mutant (log) concen-

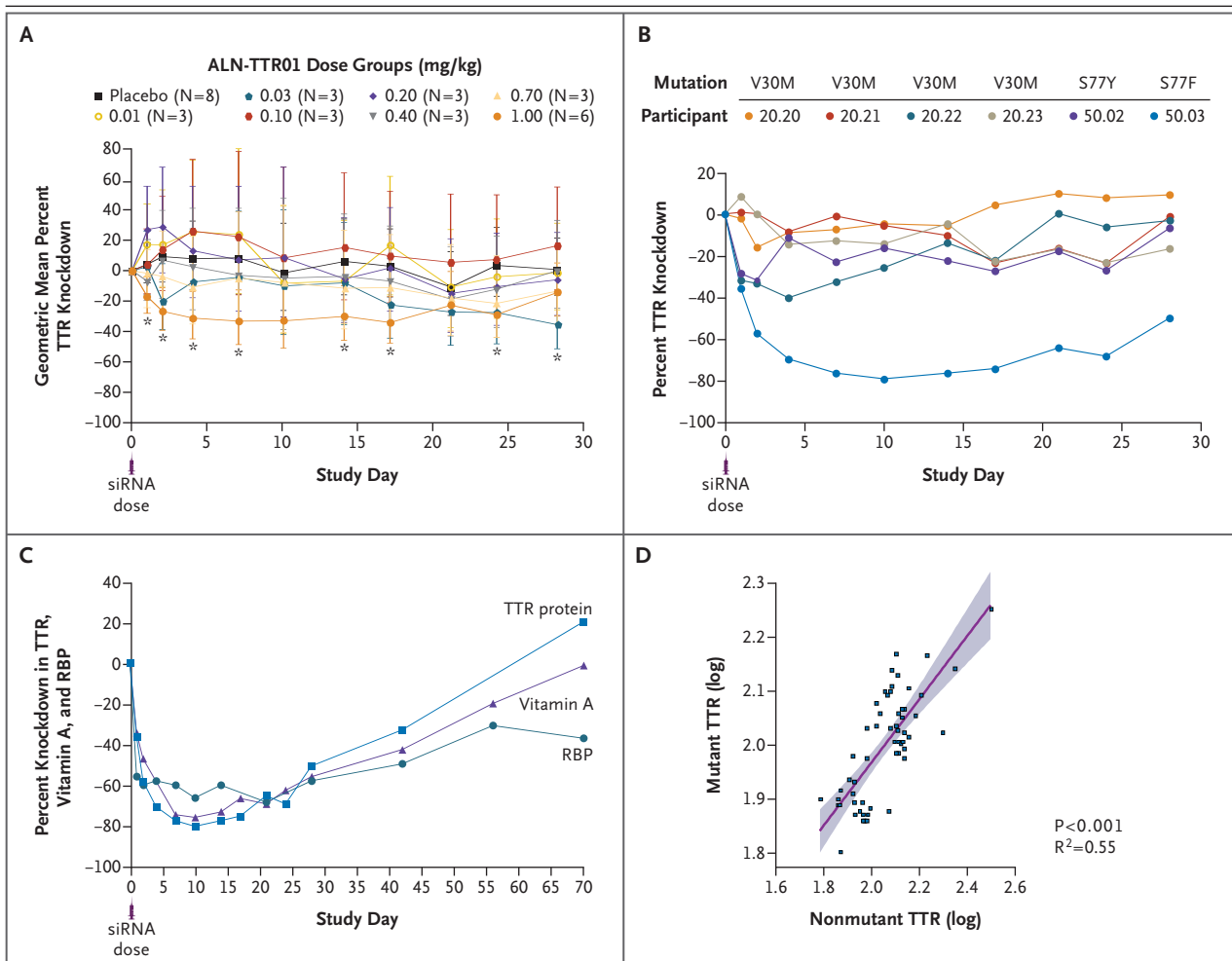


Figure 1. Efficacy of ALN-TTR01 in Patients with Transthyretin Amyloidosis.

Panel A shows the geometric mean percent serum transthyretin (TTR) knockdown (reduction in genetic expression), as compared with baseline, at the indicated time points over a 28-day period in groups of patients receiving either increasing doses of placebo or ALN-TTR01, a formulation of lipid nanoparticles containing a small interfering RNA (siRNA) targeting mutant and nonmutant transthyretin. Negative values indicate increased knockdown levels. Asterisks indicate $P < 0.05$ for the comparison between the group receiving 1.0 mg of the drug per kilogram and the placebo group, according to repeated-measures analysis of variance and post hoc tests. The I bars indicate 95% confidence intervals. Panel B shows the percent serum transthyretin knockdown for individual patients treated at a dose of 1.0 mg per kilogram, according to *TTR* mutational status. Panel C shows the time course for suppression of transthyretin, vitamin A, and retinol-binding protein (RBP) in Participant 50.03, who received a dose of 1.0 mg per kilogram. Panel D shows the correlation between mutant and nonmutant transthyretin at all time points before and after drug administration in four patients with the V30M mutation who received a dose of 1.0 mg per kilogram. The coefficient of determination (R^2) and P values were calculated according to simple linear regression. Shading indicates the 95% confidence interval.

trations (Fig. 1D) and the knockdown of transthyretin, vitamin A, and retinol-binding protein (Fig. S3 in the Supplementary Appendix). Nadir levels were defined as the minimum level per participant during the 28-day period after drug administration. We used analysis of variance or Tukey's tests to analyze nadir levels and (log) knockdown (Fig. 2C). We used the Cochran-Mantel-Haenszel test with continuity correction (adjustment for participant) to examine cleavage

products versus transthyretin-aligned clones before and after drug administration. All statistical analyses were performed with the use of SAS software, version 9.3, and R software, version 2.13.2.

RESULTS

NONHUMAN PRIMATE STUDIES

The region of the *TTR* 3' untranslated region that was targeted by the siRNA in ALN-TTR01 and

ALN-TTR02 is identical in cynomolgus monkeys and humans. In nonhuman primates that received ALN-TTR01 in a single dose of 1.0 mg per kilogram, the mean percent transthyretin knockdown at the nadir level (7 days after administration) was approximately 50%, with recovery to the baseline level by day 28 (Fig. S2A in the Supplementary Appendix). By contrast, ALN-TTR02 showed an improvement in potency by a factor of more than 10. Knockdown of approximately 75% was seen at day 7 at a dose of 0.1 mg per kilogram and 90% at day 14 at a dose of 0.3 mg per kilogram, with more than 70% suppression persisting at day 28 in the high-dose group.

To explore whether the effect of ALN-TTR02 was maintained with multiple doses, monkeys received 0.3 mg per kilogram every 4 weeks for five doses. The kinetics of transthyretin knockdown and recovery were essentially identical after each dose, though the degree of transthyretin suppression at nadir levels increased with each consecutive dose (85% and 94% suppression after doses 1 and 5, respectively), and the extent of recovery before drug administration decreased (Fig. S2B in the Supplementary Appendix).

STUDY PARTICIPANTS

Baseline characteristics of the patients with transthyretin amyloidosis and healthy volunteers in the two studies are shown in Table 1. All the patients with transthyretin amyloidosis had early-stage familial amyloidotic polyneuropathy,³⁵ with the majority enrolled in Portugal and Sweden, where the disease is endemic.⁶ The most common mutation in participants was V30M.^{6–8} Healthy volunteers had no known TTR mutation. None of the patients with the V30M mutation had received previous therapy with tafamidis. All participants in the ALN-TTR02 trial were male, since the protocol excluded women with childbearing potential in this early-phase study involving healthy volunteers. At study entry, serum transthyretin levels were modestly lower in patients with transthyretin amyloidosis than in healthy volunteers, a finding that was consistent with reports showing lower transthyretin levels in patients with transthyretin amyloidosis than in either asymptomatic disease carriers or healthy controls.³⁶

EFFICACY

ALN-TTR01

After a single dose of ALN-TTR01, no significant change from pretreatment baseline levels was ob-

served in serum transthyretin levels, as compared with the placebo group, at doses of 0.01 to 0.7 mg per kilogram. In contrast, significant lowering from baseline was seen in the group receiving 1.0 mg per kilogram as early as day 1, with a mean reduction of 38% in transthyretin levels on day 7 ($P=0.01$) (Fig. 1A). In the group receiving 1.0 mg per kilogram, which included two series of four participants each, there was considerable variability among participants in the observed degree of transthyretin knockdown.

Among four participants with the V30M mutation and one with the S77Y mutation, peak knockdown ranged from 16 to 41% and recovered close to predose baseline levels by day 28 (Fig. 1B). However, the one patient with the S77F mutation (Participant 50.03) had a robust response, with more than 50% transthyretin knockdown at day 2 and a nadir level of 81% by day 10 (Fig. 1B and 1C). By day 28, there was still more than 50% knockdown, and recovery to the baseline level did not occur until day 70. Since the main role of transthyretin is to bind retinol-binding protein, and lowering is therefore expected to reduce circulating levels of retinol-binding protein and vitamin A (retinol),^{37,38} the assays of serum retinol-binding protein and retinol provided additional measures of the pharmacodynamic activity of ALN-TTR01. As anticipated, the lowering of transthyretin in Participant 50.03 resulted in similar kinetics of lowering and recovery for retinol-binding protein and vitamin A (Fig. 1C).

Among the four patients with the V30M mutation in the group receiving 1.0 mg per kilogram, there was a strong correlation between mutant and nonmutant transthyretin (Fig. 1D), showing that both forms of the protein were reduced to the same extent, with the same kinetics of lowering and recovery (data not shown).

ALN-TTR02

We observed no significant changes in transthyretin levels, as compared with placebo, at the two lowest doses of ALN-TTR02. However, substantial transthyretin knockdown was observed in all participants receiving doses of 0.15 to 0.5 mg per kilogram (Fig. 2A). Transthyretin knockdown was rapid, potent, and durable across all three dose levels, with highly significant changes, as compared with placebo ($P<0.001$), through day 28. In light of the robust response seen at 0.15 and 0.3 mg per kilogram and modest incremental improvement in response at 0.5 mg per kilogram,

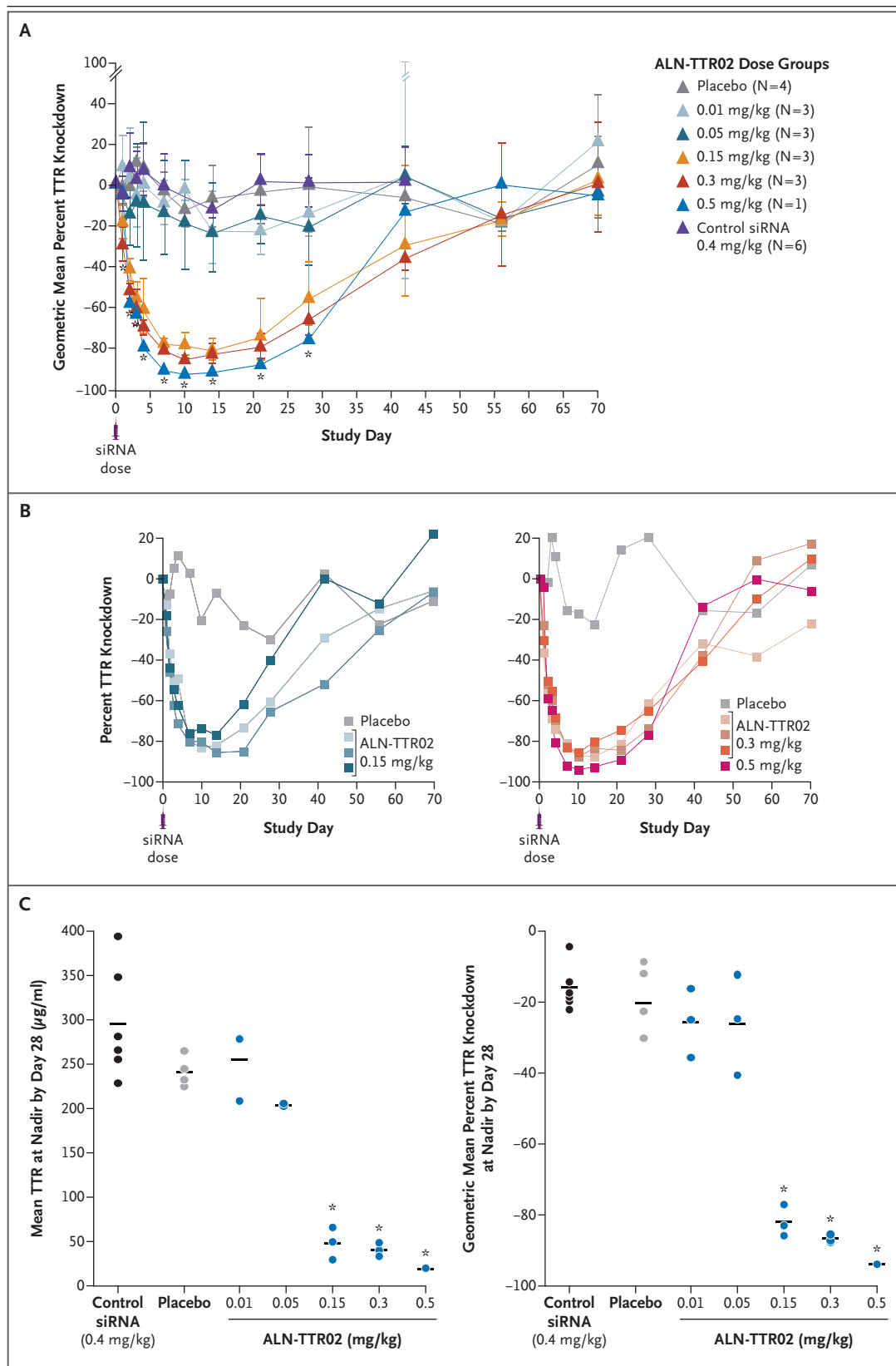


Figure 2 (facing page). Efficacy of ALN-TTR02 in Healthy Volunteers.

Panel A shows the geometric mean percent serum transthyretin knockdown, as compared with baseline, at the indicated time points over a 70-day period in groups of patients receiving placebo, control siRNA (siRNA targeting *PCSK9* formulated in the same type of lipid nanoparticles as were used in ALN-TTR02), or increasing doses of ALN-TTR02. Asterisks denote $P < 0.001$ for the comparison between the group receiving a dose of 0.3 mg per kilogram and the placebo group, according to repeated-measures analysis of variance and post hoc tests. The comparisons for groups receiving doses of 0.15 mg and 0.5 mg per kilogram maintained similar significance from days 2 through 28. The I bars indicate 95% confidence intervals. Panel B shows the percent serum transthyretin knockdown for one participant treated with placebo and three participants treated with doses of 0.15 mg of ALN-TTR02 per kilogram (left) and for one participant treated with placebo and three participants treated with doses of 0.3 mg of ALN-TTR02 per kilogram and one treated with a dose of 0.5 mg per kilogram (right). Panel C shows mean serum transthyretin levels at nadir levels (left) and the geometric mean percent serum transthyretin knockdown at nadir levels (right) for control siRNA, placebo, or increasing doses of ALN-TTR02. Circles show results for individual participants, and horizontal lines show means for each group. Asterisks indicate $P < 0.001$ for pairwise comparisons with placebo, according to analysis of variance and post hoc tests.

only one participant received the dose of 0.5 mg per kilogram.

There was little variability among participants in the kinetics of response (Fig. 2B), especially at doses of at least 0.3 mg per kilogram, with more than 50% lowering by day 3, a nadir level by approximately day 10, and continued suppression of more than 50% at day 28, with full recovery occurring by day 70. Maximum values for transthyretin knockdown for participants receiving 0.15 mg, 0.3 mg, and 0.5 mg per kilogram were 85.7%, 87.6%, and 93.8%, respectively. The average nadirs at doses of 0.15 mg and 0.3 mg per kilogram were 82.3% (95% confidence interval [CI], 67.7 to 90.3) and 86.8% (95% CI, 83.8 to 89.3), respectively; these nadirs showed little variability among participants when analyzed as either absolute transthyretin levels or percent transthyretin knockdown and were highly significant, as compared with placebo ($P < 0.001$) (Fig. 2C). The degree of knockdown appeared to determine the duration of suppression, with mean reductions at day 28 of 56.6% (95% CI, 11.6 to 78.7) and 67.1%

(95% CI, 45.5 to 80.1) for participants receiving 0.15 mg and 0.3 mg per kilogram, respectively, and a 76.8% reduction at day 28 for the single patient receiving 0.5 mg per kilogram. The transthyretin knockdown observed in humans at a dose of 0.3 mg per kilogram was virtually identical to that seen in nonhuman primates at the same dose level (Fig. S2C in the Supplementary Appendix). These reductions in transthyretin by ALN-TTR02 correlated with changes in levels of retinol-binding protein and vitamin A (Fig. S3 in the Supplementary Appendix).

To further demonstrate the specificity of the effect of ALN-TTR02, transthyretin was also measured in a group of healthy volunteers in a phase 1 trial of ALN-PCS, which contains an siRNA targeting *PCSK9*²⁹ (a target for cholesterol lowering) that is formulated in the same type of lipid nanoparticle used in ALN-TTR02. A single dose of 0.4 mg of ALN-PCS per kilogram (so-called control siRNA) had no effect on transthyretin (Fig. 2A and 2C), which showed that the effect of ALN-TTR02 on transthyretin was due to specific targeting by the siRNA and not a nonspecific effect of the formulation of lipid nanoparticles.

We obtained additional evidence in support of the specificity and mechanism of action of the pharmacodynamic effect of ALN-TTR02 using the 5' RACE (rapid amplification of complementary DNA ends) assay^{28,29} on blood samples obtained from participants receiving a dose of 0.3 mg per kilogram to detect the predicted TTR mRNA cleavage product in circulating extracellular RNA (for details, see the Methods section in the Supplementary Appendix). TTR mRNA was detected both in predose samples and in samples obtained 24 hours after drug administration. Consistent with the RNAi mechanism, the predicted mRNA cleavage product was absent in the predose samples and present in postdose samples in all three participants (Table 2).

SAFETY AND PHARMACOKINETICS

The use of ALN-TTR01 and ALN-TTR02 did not result in any significant changes in hematologic, liver, or renal measurements or in thyroid function, and there were no drug-related serious adverse events or any study-drug discontinuations because of adverse events (Tables 3 and 4, and Table S1 in the Supplementary Appendix). Mild-to-moderate infusion-related reactions occurred in 20.8% of participants receiving ALN-TTR01 at

Table 1. Baseline Characteristics of the Study Participants.

Characteristic	ALN-TTR01 Trial	ALN-TTR02 Trial
Disease status	Transthyretin amyloidosis	No disease
No. of participants (drug:placebo ratio)	32 (24:8)	17 (13:4)
Median age (range) — yr	37 (21–77)	27 (21–44)
Male sex — %	56	100
Country of enrollment — no. of participants		
Portugal	23	0
Sweden	5	0
France	3	0
United Kingdom	1	17
TTR genotype — no. of participants		
Val30Met (V30M)	29	0
Ser77Tyr (S77Y)	2	0
Ser77Phe (S77F)	1	0
Mean serum transthyretin (range) — $\mu\text{g/ml}$	253.3 (123.8–558.9)	290.8 (195.3–370.9)

doses of at least 0.4 mg per kilogram. A moderate infusion-related reaction occurred in the single participant who received ALN-TTR02 at a dose of 0.5 mg per kilogram (overall incidence, 7.7%). Mild reactions resolved spontaneously, whereas moderate reactions readily responded to temporary interruption of the infusion with or without the administration of additional glucocorticoids, followed by resumption of drug administration at a slower rate. The infusion-related reactions that were observed in the two trials were not associated with any inductions of proinflammatory cytokines. Levels of complement C3a were transiently elevated in the single participant who had

an infusion-related reaction in the ALN-TTR02 trial. Antibodies to the pegylated lipid component of ALN-TTR02 were not detected in any participants.

The plasma pharmacokinetic profiles of ALN-TTR01 and ALN-TTR02 showed that the values for the peak plasma concentration and for the area under the curve through the last day for the TTR siRNA increased in an approximately dose-proportional manner over the range of doses that were tested (Fig. S4A and S4B in the Supplementary Appendix).

DISCUSSION

Harnessing RNAi holds promise for the treatment of human disease and requires safe and effective delivery. Results with formulations of lipid nanoparticles as agents to deliver siRNAs in animals, including nonhuman primates,^{28,29} have shown activity toward silencing of disease-modifying genes expressed in the liver. Our data provide proof that siRNAs can be delivered to human liver and therein modulate gene expression through RNAi.

The findings for ALN-TTR01 in patients with transthyretin amyloidosis showed that a single dose could rapidly and potently suppress hepatic production of transthyretin and that both mutant and nonmutant proteins were similarly affected. In one patient with 81% knockdown, substantial

Table 2. Detection of Transthyretin Messenger RNA Cleavage Product in Three Participants Receiving 0.3 mg of ALN-TTR02 per Kilogram.*

Participant No.	Clones Aligned with Transthyretin†		Transthyretin-Aligned Clones with Predicted Cleavage	
	Before Therapy	After Therapy‡	Before Therapy	After Therapy§
		no.		no. (%)
014	46	34	0	34 (100)
015	65	49	0	48 (98)
016	8	35	0	14 (40)

* The listed results were obtained with the use of the 5' RACE (rapid amplification of complementary DNA ends) assay.

† A total of 96 clones were selected for sequencing.

‡ Analyses were performed 24 hours after drug administration.

§ P<0.001 by the Mantel–Haenszel chi-square test with continuity correction.

Table 3. Select Adverse Events in the ALN-TTR01 Trial.*

Adverse Event	ALN-TTR01 (N=24)							Placebo (N=8)	
	0.01 mg/kg (N=3)	0.03 mg/kg (N=3)	0.1 mg/kg (N=3)	0.2 mg/kg (N=3)	0.4 mg/kg (N=3)	0.7 mg/kg (N=3)	1.0 mg/kg (N=6)		
	All Doses								
	number (percent)								
Infusion reaction	0	0	0	0	1 (33.3)	1 (33.3)	3 (50.0)	5 (20.8)	0
Fatigue	1 (33.3)	1 (33.3)	0	0	0	0	0	2 (8.3)	0

* A complete list of adverse events is provided in Table S1 in the Supplementary Appendix.

Table 4. Select Adverse Events in the ALN-TTR02 Trial.*

Adverse Event	ALN-TTR02 (N = 13)						Placebo (N = 4)	
	0.01 mg/kg (N = 3)	0.05 mg/kg (N = 3)	0.15 mg/kg (N = 3)	0.3 mg/kg (N = 3)	0.5 mg/kg (N = 1)	All Doses		
	number (percent)							
Skin erythema†	1 (33.3)	2 (66.7)	0	2 (66.7)	1 (100.0)	6 (46.2)	2 (50.0)	
Infusion reaction	0	0	0	0	1 (100.0)	1 (7.7)	0	

* A complete list of adverse events is provided in Table S1 in the Supplementary Appendix.

† Skin erythema consisted of transient, asymptomatic flushing of the face, trunk, or both.

suppression of more than 50% was still maintained 28 days after drug administration, which underscored the finding from previous studies in animals that the duration of target suppression was in part a function of the degree of initial knockdown.^{28,29}

The results for ALN-TTR02 were remarkable for the exceptional potency, as compared with ALN-TTR01, a finding that was predicted in the studies of nonhuman primates. Within the active dose range of 0.15 to 0.5 mg per kilogram, there was little variability among participants within each dose group in nadir levels of transthyretin knockdown and in the kinetics of suppression and recovery. From the standpoint of nadir levels of transthyretin and the degree of suppression at 28 days, the response appeared to plateau at 0.3 mg per kilogram, with incremental additional suppression seen in the one participant who was treated at a dose of 0.5 mg per kilogram. The safety profile associated with this degree of activity was encouraging.

The one clear adverse event that was associated with RNAi therapy and not with placebo was a moderate infusion-related reaction in the participant who received ALN-TTR02 at a dose of 0.5 mg per kilogram. This reaction was managed by slowing the infusion rate. The reduced rate of moder-

ate infusion-related reactions in the ALN-TTR02 trial, as compared with the ALN-TTR01 trial, was probably due to the use of lower doses of the drug and an extended infusion time (60 minutes vs. 15 minutes).³⁹ There was a reversible decline in levels of retinol-binding protein and vitamin A associated with transthyretin knockdown, which was consistent with transthyretin binding of retinol-binding protein.^{37,38} Although transthyretin has a minor role in binding thyroxine in humans, we observed no significant change in thyroid function in participants with up to 94% suppression of transthyretin. The safety of long-term suppression of transthyretin in patients with transthyretin amyloidosis with respect to its effect on levels of retinol-binding protein and circulating vitamin A remains to be determined. However, the benign clinical phenotype and absence of findings of severe vitamin A deficiency in TTR-knockout mice or in humans deficient in retinol-binding protein who have very low circulating vitamin A levels^{40,41} suggest that there are mechanisms for vitamin A transport and tissue uptake that are independent of retinol-binding protein.⁴²

The ability of ALN-TTR01 and ALN-TTR02 to suppress hepatic transthyretin production and reduce circulating levels of both mutant and non-mutant transthyretin has implications for the

treatment of patients with transthyretin amyloidosis. Liver-derived transthyretin has been shown to be the disease-causing protein in studies of the effect of eliminating mutant transthyretin with liver transplantation in patients with early-stage familial amyloidotic polyneuropathy^{16,17} and of the development of amyloidosis in recipients of livers from patients with familial amyloidotic polyneuropathy in “domino” transplantations.⁴³ In addition, the beneficial effect of reducing circulating amyloidogenic precursors by more than 50% has been shown in both secondary (AA) amyloidosis and amyloid light-chain (AL) amyloidosis.^{44,45}

These findings suggest that the robust, sustained reduction in transthyretin levels that we have observed may ameliorate the course of dis-

ease in patients with transthyretin amyloidosis. The ability of ALN-TTR02 to lower both mutant and nonmutant proteins may also provide a major advantage over liver transplantation, which affects the production of only mutant protein. Further studies of ALN-TTR02 are needed to examine the clinical efficacy and safety of long-term transthyretin knockdown in patients with familial amyloidotic polyneuropathy.

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