ORIGINAL RESEARCH ARTICLE

Ligand conjugated antisense oligonucleotide for the treatment of transthyretin amyloidosis: preclinical and phase 1 data

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Abstract

Aims Amyloidogenic transthyretin (ATTR) amyloidosis is a fatal disease characterized by progressive cardiomyopathy and/or polyneuropathy. AKCEA-TTR-L_{Rx} (ION-682884) is a ligand-conjugated antisense drug designed for receptor-mediated uptake by hepatocytes, the primary source of circulating transthyretin (TTR). Enhanced delivery of the antisense pharmacophore is expected to increase drug potency and support lower, less frequent dosing in treatment.

Methods and results AKCEA-TTR-L_{Rx} demonstrated an approximate 50-fold and 30-fold increase in potency compared with the unconjugated antisense drug, inotersen, in human hepatocyte cell culture and mice expressing a mutated human genomic *TTR* sequence, respectively. This increase in potency was supported by a preferential distribution of AKCEA-TTR-L_{Rx} to liver hepatocytes in the transgenic *hTTR* mouse model. A randomized, placebo-controlled, phase 1 study was conducted to evaluate AKCEA-TTR-L_{Rx} in healthy volunteers (ClinicalTrials.gov: NCT03728634). Eligible participants were assigned to one of three multiple-dose cohorts (45, 60, and 90 mg) or a single-dose cohort (120 mg), and then randomized 10:2 (active : placebo) to receive a total of 4 SC doses (Day 1, 29, 57, and 85) in the multiple-dose cohorts or 1 SC dose in the single-dose cohort. The primary endpoint was safety and tolerability; pharmacokinetics and pharmacodynamics were secondary endpoints. All randomized participants completed treatment. No serious adverse events were reported. In the multiple-dose cohorts, AKCEA-TTR-L_{Rx} reduced TTR levels from baseline to 2 weeks after the last dose of 45, 60, or 90 mg by a mean (SD) of −85.7% (8.0), −90.5% (7.4), and −93.8% (3.4), compared with −5.9% (14.0) for pooled placebo ($P < 0.001$). A maximum mean (SD) reduction in TTR levels of −86.3% (6.5) from baseline was achieved after a single dose of 120 mg AKCEA-TTR-L_{Rx}.

Conclusions These findings suggest an improved safety and tolerability profile with the increase in potency achieved by productive receptor-mediated uptake of AKCEA-TTR-L_{Rx} by hepatocytes and supports further development of AKCEA-TTR-L_{Rx} for the treatment of ATTR polyneuropathy and cardiomyopathy.

Introduction

Amyloidogenic transthyretin (ATTR) amyloidosis is a progressive and fatal disease manifested by the buildup of misfolded transthyretin (TTR) protein in major organ systems. There are two types of ATTR amyloidosis: one determined by genetic variation and the other by age.[1,2] The genetic form, hereditary ATTR (hATTR), is caused by pathogenic variants in the *TTR* gene that destabilize the normal tetrameric structure of the TTR oligomers, leading to the formation of insoluble, extracellular amyloid deposits in multiple organ systems.[3] Wild-type TTR can also misfold and deposit as amyloid, causing wild-type ATTR (wtATTR) amyloidosis in the elderly, which typically manifests primarily as a restrictive cardiomyopathy.[4]

ATTR amyloidosis is a complex disease of mixed phenotypes. The most common phenotypes are polyneuropathy (PN) and cardiomyopathy (CM), but other tissues and organs can be affected, including the kidneys, gastrointestinal tract, and eyes, causing symptoms in multiple organs.[5–7]

RNase H and RNA interference (RNAi) are two antisense RNA-degradation mechanisms broadly recognized for development of RNA-targeted drugs today.[8,9] Both mechanisms have recently been validated as an approach for treatment of patients with hATTR-PN in the pivotal phase 3 studies of inotersen[10] (NEURO-TTR) and patisiran[11] (APOLLO). Both drugs act through sequence-specific binding to the target *TTR* mRNA through Watson-Crick base pairing, to form an antisense : sense duplex. Once formed, the duplex is a substrate for an endoribonuclease (RNase H1 or argonaute 2 [Ago2] for RNAi), which degrades the mRNA at the site where the antisense strand is bound.[8,9] Degradation of the target mRNA reduces the amount of transcript (mRNA) available for translation, leading to subsequent reduction in synthesis of the protein product. In each respective pivotal phase 3 study, reduction of serum TTR protein levels by either antisense mechanism led to both clinical benefit in composite neurologic assessment and improved quality of life in patients with hATTR-PN.

Inotersen is a single-strand 2′-*O*-methoxyethyl (2′-MOE) modified full phosphorothioate (PS) antisense oligonucleotide (ASO) that supports RNase H1 activity with a central 'gap' of ten 2′-deoxynucleotides.[12] Inotersen includes five 2′-MOE modified ribonucleotides at each terminus to increase the affinity for the target *TTR* mRNA as well as to enhance drug stability.[13] The PS substitutions throughout the ASO backbone provide resistance to nuclease activity and increase plasma protein binding to facilitate broad tissue distribution and cell uptake.[14] Patisiran, on the other hand, is a double-stranded small interfering RNA (siRNA) that supports Ago2 activity and requires formulation in a lipid nanoparticle to provide sufficient stability, effective tissue distribution, and productive cellular uptake upon systemic intravenous delivery.[15]

AKCEA-TTR-L$_{Rx}$ (ION-682884) is an ASO of similar design and identical sequence as inotersen that is conjugated to a triantennary N-acetyl galactosamine (GalNAc$_3$) moiety. This moiety acts as a ligand for productive receptor-mediated uptake by the high-capacity asialoglycoprotein receptors (ASGR) expressed by hepatocytes,[16,17] which are the principal source of systemically circulating TTR. After internalization into cells by the ASGR1-mediated endocytic pathway, the GalNAc$_3$ cluster is metabolized to release 'free ASO' inside the hepatocyte for binding to the target mRNA.[18] The ligand-conjugated antisense (LICA) technology has provided a 20-fold to 30-fold increase in potency for targets expressed by liver hepatocytes.[19,20] Additionally, targeted receptor-mediated delivery eliminates the need for a full PS-modified backbone to facilitate tissue distribution and cell uptake. Based on prior

clinical experience with GalNAc$_3$-conjugated ASOs, AKCEA-TTR-L$_{Rx}$ is expected to support lower, less frequent dosing and achieve an improved safety and tolerability profile compared with inotersen.[19,21–24] Here, we present the preclinical comparison of inotersen and AKCEA-TTR-L$_{Rx}$ pharmacologic activity in human hepatocyte cell culture and a human *TTR* transgenic mouse model, and the results from first-in-human dosing with AKCEA-TTR-L$_{Rx}$ in a phase 1 study in healthy volunteers.

Methods

AKCEA-TTR-L$_{Rx}$ and inotersen are 2′-MOE-modified ASOs that target the 3′ untranslated region (3′UTR) of the human *TTR* pre-messenger RNA (NM_000371.3; base pairs 618 to 637; 5′-TCTTGGTTACATGAAATCCC-3′). The target sequence has no known hATTR pathogenic mutations and was identified through an extensive ASO screening process described previously.[25] Both ASOs are 20 nucleotides in length with five 2′-MOE-modified ribonucleotides at each terminus and a central region of ten 2′deoxynucleotide residues. AKCEA-TTR-L$_{Rx}$ utilizes a LICA technology for targeted delivery to hepatocytes through conjugation of a triantennary cluster of GalNAc sugars on the 5′ end.[26] The conjugated form of the ASO has six phosphodiester linkages in the 2′MOE modified ends, as compared with the full number of PS linkages contained in inotersen. Decreasing the PS content of single-stranded oligonucleotides has been found to attenuate sequence-dependent proinflammatory effects[27] and, in the conjugated form, shows a similar or slightly enhanced increase in potency.[26]

Detailed methods for the preclinical model studies are provided in the Supporting Information, *Data S1*.

Phase 1 study

AKCEA-TTR-L$_{Rx}$ was evaluated in a randomized, double-blind, placebo-controlled, dose-ascending phase 1 study at a single site in Canada from December 2018 to February 2020. The study was approved by Institutional Review Board Services (Ontario, Canada) and conducted in compliance with the World Medical Association Declaration of Helsinki (October 2002), Good Clinical Practice (GCP) guidelines, and all national, state, and local laws of the appropriate regulatory authorities. All participants provided written informed consent prior to participation in the study. Participants, monitors, study centre personnel, and the sponsor were blinded to treatment throughout the study. Only the pharmacist who allocated the drug was unblinded to treatment.

Healthy male and non-pregnant, non-lactating female participants between the ages of 18 and 65 years with a body mass index <32 kg/m^2 and a willingness to take vitamin A

supplements were eligible for the study. Women of child-bearing potential were excluded. Participants with clinically significant abnormalities in medical history, physical examinations, vital signs, electrocardiogram (ECG), or laboratory tests were also excluded.

Eligible participants were randomized 10:2, active to placebo, in one single-dose cohort of 120 mg and three multiple-dose cohorts of 45, 60, or 90 mg. Study drug was administered by SC injection. Participants in the multiple-dose cohorts received one dose of the study drug once every 4 weeks on Days 1, 29, 57, and 85 (Supporting Information, *Figure S1*).

Phase 1 endpoints

Safety assessments included adverse events (AEs), physical examination findings, ECG results, vital signs, and routine laboratory tests for blood chemistry, coagulation, complement, haematology, and urinalysis.

Pharmacokinetic (PK) parameters were determined based on nominal times from intensive PK sampling following SC injection in the multiple-dose (Days 1 and 85) and single-dose (Day 1) cohorts. Concentrations of total full-length ASO in human plasma were measured using a validated hybridization-based assay with electrochemiluminescence (ECL) detection, a variation on the method reported previously.[28] Total full-length ASO included full and partial (one-, two-, and three-sugar deletions) conjugated forms and the unconjugated form of AKCEA-TTR-L_{Rx}. The assay was validated for precision, accuracy, selectivity, sensitivity, dilution linearity, anti-drug antibody interference, and stability of AKCEA-TTR-L_{Rx} quantitation prior to analysis of the plasma samples. Equivalent mass concentrations (μg eq./mL) were determined using AKCEA-TTR-L_{Rx} standard curves. Plasma sample analyses were conducted at PPD Inc. and were performed based on the principles and requirements described in 21 CFR Part 58. The assay conducted with synthesized putative shortened oligonucleotide metabolite standards showed no measurable cross-reactivity, confirming the assay's specificity for the parent full-length oligonucleotides. The quantitation range was 0.129–86.1 ng/mL, with the low and high ends of the range defining the lower and upper limits of quantitation, respectively. Samples anticipated to be above the upper limits of quantitation were diluted prior to the hybridization-ECL analysis.

Pharmacodynamic (PD) assessments evaluated the per cent change from baseline in serum TTR and retinol binding protein 4 (RBP4) concentration. Laboratory tests were performed by a central laboratory (Medpace Reference Laboratories). Serum TTR and RBP4 concentrations were determined using ELISA.

Phase 1 analysis

The sample size was based on previous experience with ASOs in the same chemical class to adequately assess the safety, tolerability, PK, and pharmacodynamics of AKCEA-TTR-L_{Rx} while minimizing unnecessary participant exposure.

Safety and pharmacodynamic populations included all participants who were randomized and received at least one dose of study drug. The baseline for pharmacodynamic analysis was defined as the average of the pre-dose measurement closest to Day 1 and the pre-dose measurement on Day 1, where Day 1 was the time of the first dose of study drug. Participants assigned to placebo treatment in the multiple-dose cohorts were pooled for analysis.

Noncompartmental PK analysis of AKCEA-TTR-L_{Rx} was carried out on each individual subject data set (Phoenix WinNonlin version 8.1). Plasma PK parameters for total full-length ASOs (ION-682884 equivalent) included peak plasma concentration (C_{max}) and time to C_{max} (t_{max}), area under the concentration-time curve from 0 to 24 h (AUC_{0-24h}), AUC_{τ} after multiple doses, $AUC_{0-\infty}$ and clearance (CL/F) for the single-dose cohort only, clearance at steady-state following multiple doses (CL_{ss}/F), and apparent terminal elimination half-life ($t_{1/2\lambda z}$).

Per cent change from baseline in serum TTR and RBP4 levels was compared between each AKCEA-TTR-L_{Rx} treatment group and pooled placebo at each post-baseline visit using one-way ANOVA. If data departed substantially from normality, the Wilcoxon rank sum test was applied. Statistical tests were conducted using two-sided tests with a 5% type 1 error rate (SAS version 9.4).

Results
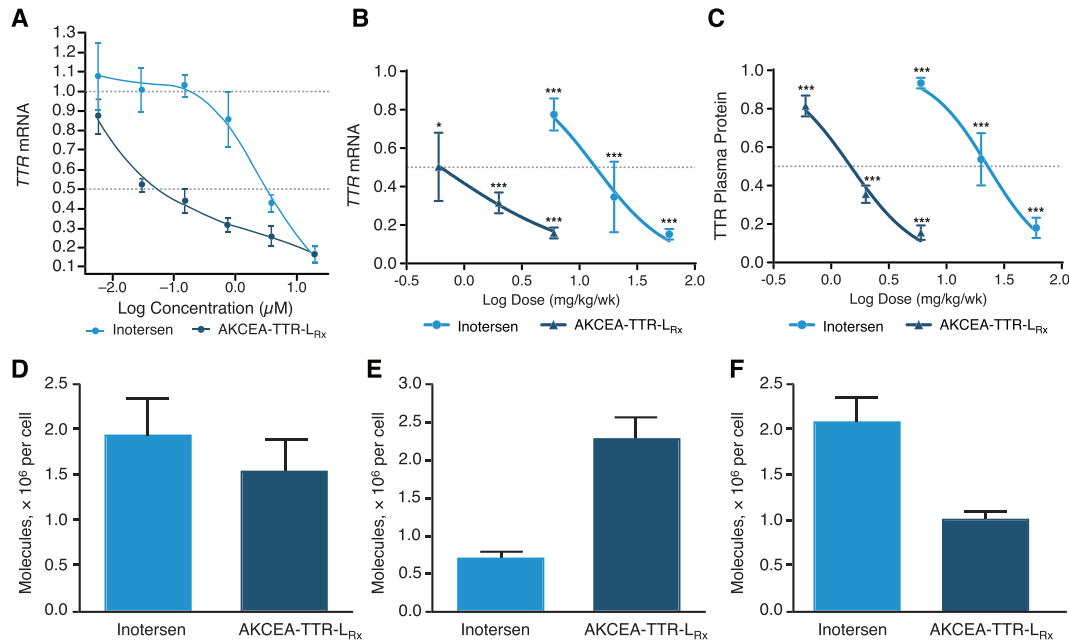
Preclinical studies

In vitro pharmacodynamics of AKCEA-TTR-L_{Rx}

In HepatoPac cell culture, inotersen and AKCEA-TTR-L_{Rx} both exhibited dose-dependent reductions in wild-type *TTR* mRNA expression. AKCEA-TTR-L_{Rx} had a median EC_{50} of 0.059 μM compared with 3.01 μM for inotersen (*Figure 1A*). AKCEA-TTR-L_{Rx} showed greater reductions in *TTR* mRNA at the same drug concentrations and was approximately 50-fold more potent than inotersen.

In vivo pharmacodynamics of AKCEA-TTR-L_{Rx} in hTTR-Tg mice

The in vivo pharmacologic activity of inotersen and AKCEA-TTR-L_{Rx} was compared in transgenic mice expressing the human genomic *TTR* transgene containing the Ile84Ser pathogenic mutation. Three once-weekly doses of inotersen or AKCEA-TTR-L_{Rx} resulted in dose-dependent reductions of hepatic *hTTR* mRNA levels with a maximum target reduction of 85% for both drugs at the highest doses (60 and 6 mg/kg/week, respectively) compared with PBS-treated controls (*Figure 1B*). The ED_{50} was 0.5 mg/kg/week for AKCEA-TTR- L_{Rx} and 13.9 mg/kg/week for inotersen,

Figure 1 AKCEA-TTR-L$_{Rx}$ produced dose-dependent reductions in *TTR* mRNA in human hepatocytes and human *TTR* mRNA and protein levels in hTTR-Tg mice, with preferential distribution to liver hepatocytes in hTTR-Tg mice. (A) Mean percentage *TTR* mRNA levels relative to untreated primary human hepatocytes after culture with AKCEA-TTR-L$_{Rx}$ at concentrations ranging from 0.0064 to 40 μM. (B) Mean liver human *TTR* mRNA levels relative to that of phosphate buffered saline-treated hTTR-Tg mice. (C) Mean plasma hTTR protein levels relative to baseline pre-treatment levels in hTTR-Tg mice. (D–F) Number of ASO molecules per cell in whole liver (D), hepatocytes (E), and non-parenchymal cells (F) in hTTR-Tg mice treated with equi-molar amounts of inotersen or AKCEA-TTR-L$_{Rx}$. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

representing a 28-fold improvement in the potency for liver *hTTR* mRNA knockdown. Reductions in liver *hTTR* mRNA correlated with reductions in hTTR plasma protein (*Figure 1C*). AKCEA-TTR-L$_{Rx}$ had an ED$_{50}$ of 1.5 mg/kg/week compared with 22.9 mg/kg/week for inotersen, representing a 15-fold improvement in TTR plasma protein reduction.

Following administration of a single dose of equal molar concentration of ASO (1.4 mmol/kg) to hTTR-Tg mice, inotersen distributed preferentially to nonparenchymal cells by a mean (SD) of 2.09 (0.52) molecules per nonparenchymal cell, and 0.7 (0.19) molecules per hepatocyte cell, see *Figure 1D–F*. Conversely, AKCEA-TTR-L$_{Rx}$ preferentially accumulated in hepatocytes by a mean (SD) of 2.27 (0.60) molecules per hepatocyte cell and 1.18 (0.12) molecules per nonparenchymal cell, leading to an approximate three-fold increase of drug delivered to hepatocytes.
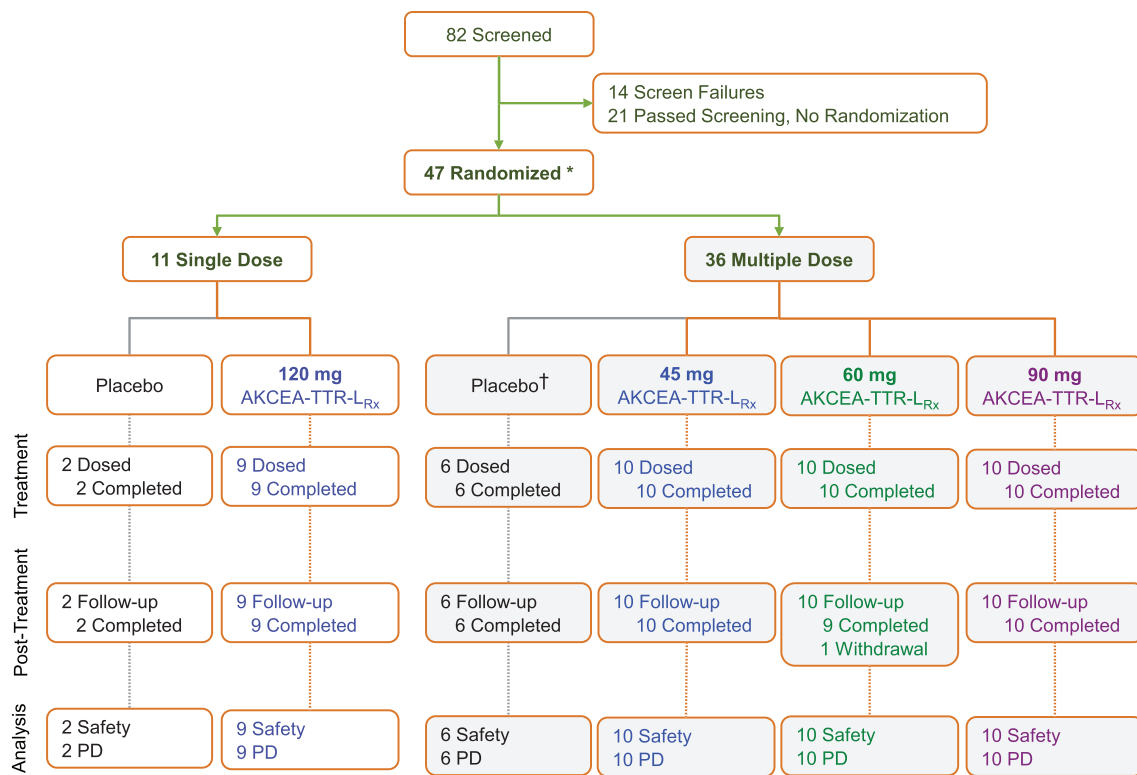
Phase 1 trial

Forty-seven eligible adults (20 women and 27 men) were assigned to one of three multiple-dose cohorts (*N* = 36) or a single-dose cohort (*N* = 11). Subjects were then randomized within each cohort to receive a total of four doses of either 45 mg (*n* = 10), 60 mg (*n* = 10), or 90 mg (*n* = 10) AKCEA-TTR-L$_{Rx}$, or placebo (*n* = 6, pooled), SC once every 4 weeks

in the multiple dose cohorts; or one dose of 120 mg AKCEA-TTR-L$_{Rx}$ (*n* = 9), or placebo (*n* = 2), in the single-dose cohort (*Figure 2*). Across treatment groups, mean age ranged from 43.4 to 51.6 years and mean BMI ranged from 24.7 to 30.4 kg/m^2 (*Table 1*). All participants completed dosing and were included in the pharmacodynamic and safety analyses.

Pharmacokinetics

Following SC administration, AKCEA-TTR-L$_{Rx}$ was absorbed rapidly into the systemic circulation with median t$_{max}$ values of one to 6 h (*Table S1*). Similar t$_{max}$ values were observed at all dose levels. Mean C$_{max}$ and total exposure (AUC) were dose-dependent and near dose-proportional over the studied SC dose range of 45 to 120 mg. After reaching C$_{max}$, mean plasma concentrations of AKCEA-TTR-L$_{Rx}$ declined over time in a biphasic fashion, with an initial rapid distribution phase that dominated the plasma clearance, followed by a slower elimination phase with an apparent terminal half-life (t$_{1/2λz}$) of 2 to 4 weeks. This result was consistent with the slow elimination of AKCEA-TTR-L$_{Rx}$ observed in monkeys and the comparatively long half-lives observed for this chemical class.[20] There was little or no increase in mean plasma C$_{max}$ or AUC after four repeated SC injections once every 4 weeks as compared with the first dose, consistent with the expected result of little or no plasma accumulation and time-invariant plasma kinetics.

Figure 2 Flow of study participants in phase 1 trial of AKCEA-TTR-L$_{Rx}$.

PD, pharmacodynamics. * Randomized by dose cohort. † Pooled placebo.

Table 1 Demographics and baseline characteristics of phase 1 study participants by treatment groups

	Single-dose cohort		Multiple-dose cohorts			
		AKCEA-TTR-L$_{Rx}$			AKCEA-TTR-L$_{Rx}$	
Characteristic	Placebo	120 mg	Placebo[a]	45 mg	60 mg	90 mg
n	2	9	6	10	10	10
Age, years	57.0 (53, 61)	43.4 (26, 60)	49.2 (39, 57)	51.6 (28, 61)	51.6 (23, 65)	51.1 (28, 62)
Gender (M : F)	0:2	8:1	2:4	7:3	4:6	6:4
Race, n (%)						
White	2 (100.0%)	3 (33.3%)	4 (66.7%)	6 (60.0%)	4 (40.0%)	6 (60.0%)
Black	0 (0.0%)	3 (33.3%)	1 (16.7%)	3 (30.0%)	3 (30.0%)	1 (10.0%)
Asian	0 (0.0%)	3 (33.3%)	1 (16.7%)	1 (10.0%)	3 (30.0%)	3 (30.0%)
BMI, kg/m^2	30.4 (0.2)	25.2 (2.4)	24.7 (2.5)	26.3 (2.6)	26.2 (3.7)	25.4 (2.8)
TTR, mg/L	237.4 (10.7)	317.8 (70.7)	324.3 (84.5)	316.0 (99.3)	303.5 (53.5)	304.6 (79.2)
RBP4, mg/L	29.59 (3.09)	32.14 (9.67)	34.045 (6.73)	37.95 (10.08)	25.35 (4.39)	29.93 (7.42)

BMI, body mass index; RBP4, retinol binding protein 4; SD, standard deviation; TTR, transthyretin.
Values shown for BMI, TTR, and RBP4 are the mean (standard deviation) and for age are the mean (minimum, maximum).
[a]Pooled placebo.

Pharmacodynamics

Four SC doses of AKCEA-TTR-L$_{Rx}$ once every 4 weeks produced dose-dependent and prolonged decreases in TTR levels over time compared to placebo (*Figure 3*). Mean per cent changes from baseline at 2 weeks after the fourth dose were statistically significant compared with pooled placebo

(−5.9%) in each AKCEA-TTR-L$_{Rx}$ multiple-dose group of 45 mg (−85.7%, $P < 0.001$), 60 mg (−90.5%, $P < 0.001$), and 90 mg (−93.8%, $P < 0.001$) (*Table 2*) and at all observations from 1 week after the first dose (Day 8) to 3 months after the last dose (Day 176) (*Table S2*). Dose-dependent and prolonged reductions in RBP4 levels also occurred, with a

Figure 3 Mean per cent change from baseline in TTR over time in the multiple-ascending dose cohorts. Subjects were administered four SC doses of 45, 60, or 90 mg AKCEA-TTR-L$_{Rx}$ or placebo, once every 4 weeks. Shaded region represents treatment period and solid red triangles indicate dosing days.
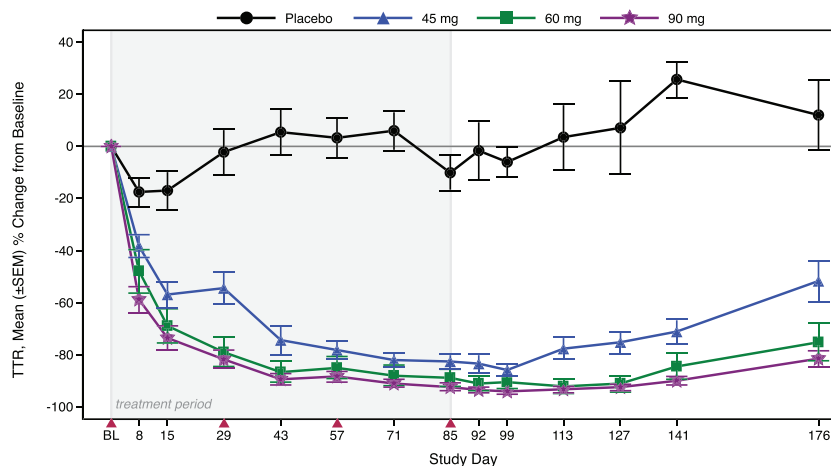

Table 2 Mean per cent change in serum TTR and RBP4 levels from baseline to 2 weeks after the last dose in the multiple dose groups

% change from baseline	Placebo	AKCEA-TTR-L$_{Rx}$		
		45 mg	60 mg	90 mg
TTR				
n	6	10	10	10
Mean (SD)	−5.9 (14.0)	−85.7 (8.0)	−90.5 (7.4)	−93.8 (3.4)
Min, Max	−28.5, 10.0	−93.5, −71.7	−97.2, −71.8	−98.4, −87.0
P-value [A]		<0.001	<0.001	<0.001
RBP4				
n	6	10	10	10
Mean (SD)	−0.2 (13.3)	−77.3 (12.5)	−79.2 (5.5)	−84.0 (4.6)
Min, Max	−15.5, 21.4	−89.7, −51.1	−89.0, −70.2	−90.2, −74.6
P-value [T]		0.006	0.006	0.006

[A] ANOVA test P-value, [T] Wilcoxon rank sum t approximation test two-sided P-value. Max, maximum; Min, minimum; RBP4, retinol binding protein 4; SD, standard deviation; TTR, transthyretin.

mean per cent change from baseline to 2 weeks after last dose of −77.3%, −79.2%, and −84.0% in the 45, 60, and 90 mg AKCEA-TTR-L$_{Rx}$ multiple-dose groups, respectively (*Figure S2, Table 2*).

A single dose of 120 mg AKCEA-TTR-L$_{Rx}$ produced a rapid mean (SD) reduction in TTR levels of −60.4% (14.0) 1 week after dosing, with a maximum mean (SD) reduction from baseline of −86.3% (6.5) on Day 29 (*Figure S3*). RBP4 showed a similar profile over time, with a mean (SD) per cent change from baseline of −53.6% (12.1) 1 week after dosing and maximum mean (SD) reduction of −77.4% (6.2) on Day 29 in the single-dose group.

Safety and tolerability

No serious AEs occurred in this study, and no dose discontinuations occurred due to an AE. AEs that occurred in two or more participants included headache in two subjects of the 120 mg single-dose group, alanine aminotransferase (ALT) increase in four subjects in the 90 mg multiple-dose group, and blood creatine phosphokinase (CK) increase in one placebo-treated subject and four subjects in the 90 mg multiple-dose group (*Table 3*). The increase in ALT levels of two of the four subjects was three-fold above the upper limit of normal (ULN) and reversed after the last dose without sequelae. There were no abnormalities in laboratories values of bilirubin, international normalized ratio (INR), or alkaline phosphatase in these subjects. CK increases were transient, and subjects were asymptomatic. Two of the four subjects in the 90 mg group who had a mild transient increase in CK levels on treatment also had a transient increase prior to dosing.

No local cutaneous injection site reactions, defined as injection site erythema, pain, swelling, or pruritis that persisted for two or more days post-injection, were reported in this study. Influenza-like illness was experienced by one subject

Table 3 Treatment-emergent adverse events reported in two or more participants: (A) single-dose cohort and (B) multiple-dose cohorts

(A)

Preferred term[a], *n*	Placebo (2)	AKCEA-TTR-L$_{Rx}$ 120 mg (9)		
Headache	0	2		

(B)

Preferred term[b], *n*	Placebo (6)	AKCEA-TTR-L$_{Rx}$ 45 mg (10)	60 mg (10)	90 mg (10)	Total (30)
Alanine aminotransferase increased	0	0	0	4	4
Blood creatine phosphokinase increased	1	0	0	4	4

[a]One subject had an event considered mild in severity; a second subject had an event of moderate severity.
[b]All events were considered mild in severity.

in the 120 mg single-dose group. No significant abnormalities were observed in either renal or haematologic parameters, such as increased serum creatinine levels or reduced platelet counts, respectively (*Table S3*).

Discussion

AKCEA-TTR-L$_{Rx}$ is under development for treatment of both hATTR and wtATTR. In healthy volunteers, AKCEA-TTR-L$_{Rx}$ produced an overall reduction of approximately 90% in TTR levels after four SC doses of 45, 60, or 90 mg once every 4 weeks. The mean reduction in serum TTR at the lowest dose tested was 86% from baseline under the pre-steady state conditions of this dose regimen. As predicted by the PK properties of this class of LICA drugs, serum TTR reductions were maintained between doses and up to 3 months after the last dose in the multi-dose treatment groups, supporting infrequent dosing. Except for one moderate AE of headache at the highest dose tested, all other reported AEs were mild in severity and resolved spontaneously without sequelae. AKCEA-TTR-L$_{Rx}$ was well tolerated at all doses tested in phase 1, with all participants receiving the full number of planned doses.

The pharmacodynamics of AKCEA-TTR-L$_{Rx}$ observed in healthy volunteers was supported by an approximately 50-fold reduction in EC$_{50}$ in human hepatocyte cell culture and a 28-fold reduction in ED$_{50}$ (<1 mg/kg) in the Ile84Ser hTTR-Tg mouse model, upon head-to-head comparison with inotersen at the target *TTR* mRNA level. Preferential uptake of the ligand-conjugated drug to hepatocytes was also demonstrated in hTTR-Tg mice, further confirming the basis of the increase in potency across species. These results are consistent with other drugs in this LICA class, showing an improved pharmacodynamic profile compared with the unconjugated parent ASO across species[16,19–23,29,30] and support the potential for an increase in safety margin upon longer-term exposure in the clinic.[24]

GalNAc$_3$-conjugated siRNAs have also been investigated in the clinic. The first that advanced to late-stage clinical development in patients with hATTR-CM was revusiran in the pivotal phase 3 study ENDEAVOUR.[31,32] This trial was halted early, however, due to an unfavourable imbalance in mortality rate between the revusiran and placebo arms.[33] Further to this outcome, treatment-emergent peripheral neuropathy and severe hepatic events were only reported in the revusiran arm, and four revusiran-treated patients had elevations in liver transaminase accompanied by increases in total bilirubin.[34] Consequently, the revusiran development program was terminated.

Vutrisiran, a next-generation siRNA of identical sequence as revusiran, is now in phase 3 investigations in patients with ATTR amyloidosis. This siRNA and its predecessor, revusiran, target the same site of the 3'-UTR of the *TTR* mRNA as AKCEA-TTR-L$_{Rx}$ and inotersen but differ in structure, chemistry, and the enzyme-mediated mechanism of target mRNA reduction. Vutrisiran differs from revusiran in its chemical modifications,[35] through incorporation of two PS modifications at the 5' terminus of each of the two strands for increased stability[36] and reduction in the number of 2'-fluoro-modified sugar residues (from 22 to 9 residues) to potentially minimize hybridization-dependent off-target effects[37] and lessen exposure to 2'-fluoro nucleoside metabolites.[38] Outcomes from phase 3 assessments will provide further insight on the effect of these modifications.

An alternative approach to treating ATTR amyloidosis is to stabilize the functional TTR tetramer to prevent its dissociation to the monomeric form, a catalyst for formation of amyloidogenic fibrils.[39] Tafamidis is a TTR stabilizer and the first medicine to be approved for the treatment of patients with ATTR-CM. Though tafamidis was found to attenuate disease progression in patients with ATTR-CM, including morbidity and mortality rate, consistent worsening in patients' functional capacity and quality of life were observed over time despite continued treatment.[40] Thus, there remains an unmet medical need for more effective treatments.

The increased potency of AKCEA-TTR-L$_{Rx}$ demonstrated first preclinically in human hepatocytes and hTTR-Tg mice supports and predicts an equivalent pharmacologic effect to inotersen despite lower and less frequent dosing in humans. Based on the PK and PD findings for AKCEA-TTR-L$_{Rx}$ and the absence of potential tolerability or safety issues, such as the increases in liver transaminase levels observed in the upper dose range of the current phase 1 trial, the 45 mg dose SC once every 4 weeks was chosen for the phase 3 studies.

The lower, less frequent SC dose is expected to improve the drug safety profile by reducing total systemic exposure and to provide a more convenient and tolerable dosing regimen for patients compared with inotersen.[10,41,42] Additionally, based on the absorption, distribution, metabolism, and excretion of this class of drugs,[20,43] combination with tafamidis or medications such as statins, antihypertensives, or diuretics is not expected to be contraindicated due to drug–drug interactions.[44] Finally, because AKCEA-TTR-L$_{Rx}$ is designed to reduce both wild-type and mutant *TTR* mRNA, treatment of both forms of ATTR is possible.

AKCEA-TTR-L$_{Rx}$ is currently under investigation in two phase 3 trials, NEURO-TTRansform in patients with hATTR-PN (ClinicalTrials.gov, NCT04136184) and CARDIO-TTRansform in patients with either hATTR-CM or wtATTR-CM (NCT04136171). Prior experience with dosing in the inotersen trials and the predictable antisense platform effect, as well as modelling, provide confidence that the reduction in circulating TTR levels will be replicated in patients with either hATTR or wtATTR amyloidosis. Notably though, TTR amyloidosis affects persons who are older in age than the participants in the current phase 1 trial and particularly those with the wild-type form. Thus, a broader characterization of the safety profile of this investigational antisense drug awaits further evaluation in the phase 3 trials, which allow patients up to 82 and 90 years of age, respectively, as well as those with mild to moderate renal impairment and elevated hepatic transaminase levels.

The efficacy and safety of 45 mg AKCEA-TTR-L$_{Rx}$ SC once every 4 weeks are being investigated in both pivotal, phase 3 trial. An approximate 30-fold increase in potency was observed under pre-steady state conditions at this dose of AKCEA-TTR-L$_{Rx}$ in healthy volunteers, relative to 300 mg weekly SC dose of the unconjugated 2'-MOE-modified ASO inotersen.[10,25] Based on the profile of AKCEA-TTR-L$_{Rx}$ and the LICA platform overall, AKCEA-TTR-L$_{Rx}$ is expected to produce greater efficacy compared with existing RNA-targeted medicines with an attractive safety profile and the convenience of a once monthly injection.

Acknowledgements

Conflict of interest

Funding

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Methods - Preclinical Models
Figure S1. Phase 1 study design in healthy volunteers
Figure S2. Mean percent change from baseline in RBP4 over time with multiple doses
Figure S3. Mean percent change from baseline in TTR and RBP4 after a single dose
Table S1. Plasma pharmacokinetics of AKCEA-TTR-LRx in healthy subjects
Table S2. Summary of percent change from baseline in plasma TTR overtime
Table S3. Clinical laboratory measurements

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