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STUDY PROTOCOL

Design and Rationale of the Global Phase 3 NEURO-TTRansform Study of Antisense Oligonucleotide AKCEA-TTR-L_{Rx} (ION-682884-CS3) in Hereditary Transthyretin-Mediated Amyloid Polyneuropathy

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ABSTRACT

Introduction: AKCEA-TTR-L_{Rx} is a ligand-conjugated antisense (LICA) drug in development for the treatment of hereditary transthyretin amyloidosis (hATTR), a fatal disease caused by mutations in the transthyretin (*TTR*) gene. AKCEA-TTR-L_{Rx} shares the same nucleotide sequence as inotersen, an antisense medicine approved for use in hATTR polyneuropathy (hATTR-PN). Unlike inotersen, AKCEA-TTR-L_{Rx} is conjugated to a triantennary *N*-acetylgalactosamine moiety that supports receptor-mediated uptake by hepatocytes, the primary source of circulating TTR. This advanced design increases drug potency to allow for lower and less frequent dosing. The NEURO-TTRansform study will investigate whether AKCEA-TTR-L_{Rx} is safe and efficacious, with the aim of improv-

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C. Monteiro and L.-J. Tai were employees of Ionis Pharmaceuticals Inc. at the time of this work. L. St. L. O'Dea was an employee of Akcea Therapeutics at the time of this work.

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ing neurologic function and quality of life in hATTR-PN patients.

Methods/Design: Approximately 140 adults with stage 1 (independent ambulation) or 2 (requires ambulatory support) hATTR-PN are anticipated to enroll in this multicenter, open-label, randomized, phase 3 study. Patients will be assigned 6:1 to AKCEA-TTR-L_{Rx} 45 mg subcutaneously every 4 weeks or inotersen 300 mg once weekly until the prespecified week 35 interim efficacy analysis, after which patients receiving inotersen will receive AKCEA-TTR-L_{Rx} 45 mg subcutaneously every 4 weeks. All patients will then receive AKCEA-TTR-L_{Rx} through the remainder of the study treatment period. The final efficacy analysis at week 66 will compare the AKCEA-TTR-L_{Rx} arm with the historical placebo arm from the phase 3 trial of inotersen (NEURO-TTR). The primary outcome measures are between-group differences in the change from baseline in serum TTR, modified Neuropathy Impairment Score + 7, and Norfolk Quality of Life—Diabetic Neuropathy questionnaire.

Conclusion: NEURO-TTRansform is designed to determine whether targeted delivery of AKCEA-TTR-L_{Rx} to hepatocytes with lower and less frequent doses will translate into clinical and quality-of-life benefits for patients with hATTR-PN.

Trial Registration: The study is registered at ClinicalTrials.gov (NCT04136184) and EudraCT (2019-001698-10).

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PLAIN LANGUAGE SUMMARY

- Hereditary transthyretin amyloidosis with peripheral neuropathy (hATTR-PN for short) is a rare inherited condition.
  - In hATTR-PN, a protein called transthyretin (TTR for short) builds up and damages nerves throughout the body.
  - This neuropathy causes symptoms such as weakness, loss of sensation, and pain.
- Currently available medicines can slow disease progression, but researchers are looking for more effective treatments with fewer side effects.
- AKCEA-TTR-L_{Rx} is an investigational treatment for hATTR-PN.
  - AKCEA-TTR-L_{Rx} prevents the liver from making TTR, reducing the amount that causes disease progression.
  - It is similar to an existing treatment called inotersen, but designed for better delivery to the liver and is more potent.
- This article describes the NEURO-TTRansform study that will evaluate how effective AKCEA-TTR-L_{Rx} is for treating hATTR-PN.
  - Around 140 adults with hATTR-PN from the USA, Canada, and Europe will be able to take part in this study.
  - The study treatment period will be 85 weeks long. People will receive injections underneath the skin of either:

    AKCEA-TTR-L_{Rx} every 4 weeks, or
    Inotersen once a week for 35 weeks, followed by a switch to AKCEA-TTR-L_{Rx} every 4 weeks.
  - People may continue to receive AKCEA-TTR-L_{Rx} after the study treatment period ends.
- In this study, researchers will compare results from people who received AKCEA-TTR-L_{Rx} to results from people who received no active ingredients (called placebo) in a similar study (called NEURO-TTR).
- Researchers will measure the differences in peoples':

- Neuropathy symptoms.
- Quality of life.
- TTR protein levels in the blood.

Keywords: AKCEA-TTR-L_{rx}; Antisense oligonucleotide; Clinical trial design; Hereditary transthyretin-mediated amyloid polyneuropathy; Phase 3 clinical trial

Key Summary Points

Why carry out this study?

Hereditary transthyretin amyloidosis (hATTR) is a fatal disease caused by mutations in the transthyretin (*TTR*) gene that result in the synthesis of unstable TTR tetramers and consequent systemic deposition of insoluble amyloid fibrils, which most often manifest as cardiomyopathy or polyneuropathy (hATTR-PN).

Although existing treatments can slow or halt neurologic impairment associated with hATTR-PN, there remains a need for other disease-modifying agents that offer improved efficacy, safety, and convenience of use.

AKCEA-TTR-L_{Rx} is a novel antisense oligonucleotide (ASO) designed for enhanced delivery to hepatocytes, the primary site of TTR protein production, for increased potency and convenience of use to potentially translate into clinical and quality-of-life benefits for patients with hATTR-PN.

This paper describes the design and rationale of the NEURO-TTRansform trial, a multicenter, open-label, randomized, phase 3 study that aims to assess the efficacy and safety of AKCEA-TTR-L_{Rx} in approximately 140 adult patients with stage 1 (independent ambulation) or 2 (requiring ambulatory support) hATTR-PN.

DIGITAL FEATURES

This article is published with digital features, including a summary slide and a plain language summary, to facilitate understanding of the article. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.13677805.

INTRODUCTION

Hereditary transthyretin amyloidosis (hATTR) is a progressive, irreversible, and fatal autosomal dominant disorder that manifests most commonly as peripheral sensorimotor and autonomic axonal neuropathy or infiltrative cardiomyopathy [1–4]. hATTR is caused by transthyretin (*TTR*) gene mutations that result in the synthesis of unstable TTR tetramers [5, 6]. TTR tetramers consisting of mutant TTR monomers are more susceptible to tetramer dissociation and consequent misfolding of the monomer, resulting in aggregation into soluble oligomeric structures and insoluble TTR-derived amyloid deposits [7, 8]. The accumulation of TTR-derived amyloid fibrils in multiple organ systems results in a wide spectrum of clinical manifestations [1, 2]. TTR genotype–phenotype correlations have been reported for a predominant clinical phenotype; for example, pV142I mutation carriers manifest preferentially with hATTR cardiomyopathy, whereas pV50M mutation carriers manifest preferentially with hATTR polyneuropathy (hATTR-PN) [9–12]. Additionally, factors that contribute to the rate of progression, such as geographic location, sex of transmitting parent, genetic anticipation, and genetic modifiers, are associated with the variability of the clinical presentation [11–13]. Estimates suggest that the global prevalence of hATTR-PN is approximately 10,000 patients, although many individuals may be undiagnosed [14]. The median age of onset of hATTR-PN varies geographically (from 33 years in Japan and Portugal to 56 years in Sweden), and patients progress to death 6–12 years after symptom onset [1, 2, 15, 16].

Table 1 Treatments for polyneuropathy of hATTR

	Inotersen [24, 25]	**Patisiran** [22, 23]	**Tafamidis** [18, 29]	**Diflunisal** [20, 36]
Agent type	ASO	siRNA	Stabilizer	Nonsteroidal anti-inflammatory drug
Indication	Approved for ATTR-PN in the United States and for stage 1 or 2 hATTR-PN in the European Union, Canada, Brazil, and other countries[a]	Approved for ATTR-PN in the United States, Canada, and Japan and for stage 1 or 2 hATTR-PN in the European Union, Brazil, and Switzerland	Approved in the European Union (stage 1) and in over 40 countries[b]	Off-label
Dose	284 mg subcutaneously once weekly	0.3 mg/kg intravenously every 3 weeks	20 mg orally once daily	250–1000 mg daily
Premedication	None	Intravenous corticosteroid Oral acetaminophen Intravenous H1 blocker Intravenous H2 blocker	None	None
Precautions	Vitamin A supplementation	Vitamin A supplementation	None	None
Safety monitoring	Thrombocytopenia Glomerulonephritis Liver function	Infusion-related reactions	None	Serious cardiovascular thrombotic events, myocardial infarction, stroke, and serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines

ASO antisense oligonucleotide, *ATTR* transthyretin amyloidosis, *ATTR-PN* transthyretin-mediated amyloid polyneuropathy, *hATTR* hereditary transthyretin amyloidosis, *hATTR-PN* hereditary transthyretin-mediated amyloid polyneuropathy, *siRNA* small interfering ribonucleic acid

[a] Iceland, Liechtenstein, Norway, United Kingdom (license issued based on European Medicines Agency)

[b] Approved for ATTR-PN in over 40 countries, including Japan, European countries, Brazil, Mexico, Argentina, Israel, Russia, and South Korea

Clinical staging of hATTR-PN is often based on ambulatory status, which assesses the progression of sensorimotor axonal peripheral neuropathy severity. Disease metrics include the Familial Amyloid Polyneuropathy or Coutinho stage scoring system, and the Polyneuropathy Disability (PND) Score [1]. For the Coutinho scoring system, stage 1 is characterized by independent ambulation, stage 2 is characterized by the need for unilateral or bilateral support for ambulation, and stage 3 is characterized by being wheelchair-bound or bedridden [1].

Since the TTR protein is primarily produced by the liver, liver transplant has been the historical standard of care, acting to slow or halt disease progression by replacing a liver that produces mutant TTR protein with one that expresses only wild-type TTR [1]. Owing to donor shortages and progressive end-organ amyloid disease following liver transplantation, medical therapies have been pursued [1]. Over the past 13 years, several pharmacologic therapies have been developed, including TTR-stabilizing agents that preserve the tetrameric structure and ribonucleic acid (RNA)-targeting therapeutics that suppress *TTR* messenger RNA (mRNA) expression (Table 1). Oral tafamidis meglumine is a TTR-stabilizing agent that was approved for the treatment of familial amyloid polyneuropathy (FAP) stage 1 ATTR-PN in 2011 by the European Union, with subsequent approvals in Japan and other countries [17–19]. Diflunisal, a nonsteroidal anti-inflammatory drug, has been shown to significantly reduce the progression of neurological impairment among patients with hATTR-PN but is not approved for the treatment of hATTR-PN [20, 21]. With regard to RNA-targeting therapeutics, two treatments have recently been approved in the United States, the European Union, and other countries for patients with hATTR-PN: inotersen and patisiran [22–25]. Inotersen is an antisense oligonucleotide (ASO) inhibitor of TTR production, which is administered at a dosage of 300 mg by subcutaneous (SC) injection once weekly [24, 25]. Patisiran is a small interfering RNA (siRNA) inhibitor of TTR production, which is formulated in a lipid nanoparticle for administration at a dosage of 0.3 mg/kg by intravenous infusion every 3 weeks and requires premedication with a corticosteroid [22, 23].

Inotersen is approved for the treatment of adult patients with hATTR-PN [24, 25]. Inotersen binds to and induces ribonuclease H1-mediated degradation of *TTR* mRNA (wild-type and all known mutations), thereby lowering TTR protein production [21]. NEURO-TTR was a pivotal randomized, double-blind, placebo-controlled phase 2/3 study of inotersen 300 mg/week over 15 months in adult patients with stage 1 or stage 2 hATTR-PN [26]. Compared with placebo, inotersen significantly improved outcomes on the modified Neuropathy Impairment Score + 7 (mNIS + 7) and patient-reported Norfolk Quality of Life—Diabetic Neuropathy (Norfolk QOL-DN) questionnaire [26]. The maximum mean reduction from baseline in serum TTR was 74% [26]. The safety profile of inotersen was considered acceptable with regular safety laboratory assessments for thrombocytopenia and glomerulonephritis [24–26]. Although the approved treatments can slow or halt the neurologic impairment associated with hATTR-PN, there remains a need for more efficacious disease-modifying agents that offer a higher degree of TTR silencing, greater convenience of use, and a more favorable safety profile [26].

AKCEA-TTR-L_{Rx} (ION-682884) is a ligand-conjugated antisense (LICA) drug designed for preferential delivery to hepatocytes, the primary source of systemically circulating TTR (Fig. 1). This advanced antisense design utilizes a triantennary *N*-acetylgalactosamine (GalNAc) moiety to support productive receptor-mediated uptake by the high-capacity asialoglycoprotein receptors (ASGPR) expressed by hepatocytes [27, 28]. An integrated analysis of eight GalNAc-conjugated ASOs tested in phase 1 randomized placebo-controlled studies of healthy human volunteers showed a 20–30-fold increase in potency compared with the parent unconjugated ASOs [27]. Notably, these GalNAc-conjugated ASOs were well tolerated, with no discontinuations due to adverse events (AEs) [27].

AKCEA-TTR-L_{Rx} is a GalNAc-conjugated ASO with a nucleotide sequence identical to inotersen, but with fewer phosphorothioate-modified internucleotide linkages, which is expected to improve its safety profile. Results from a first-in-human phase 1 study of AKCEA-TTR-L_{Rx} in healthy volunteers demonstrated that AKCEA-TTR-L_{Rx} 45 mg SC once every 4 weeks produced sustained decreases in serum TTR levels within 30 days of treatment initiation, achieving a mean reduction from baseline of 86% after four doses of treatment [29]. No treatment-related safety or tolerability issues were identified under this dosing regimen. Consequently, AKCEA-TTR-L_{Rx} requires a lower dose and less frequent

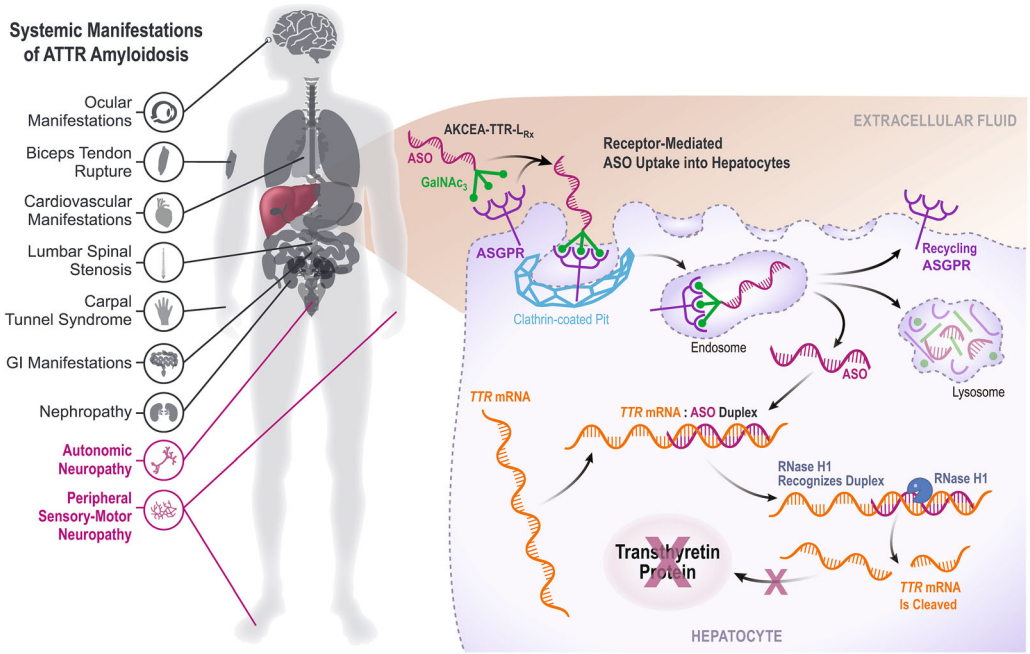

Fig. 1 ASGPR mediates the uptake of the $GalNAc_3$-conjugated ASO AKCEA-TTR-L_{Rx} by hepatocytes, where it binds to the *TTR* mRNA through Watson–Crick hybridization and prevents production of TTR protein via RNase H1-mediated degradation of the target *TTR* mRNA. *ASGPR* asialoglycoprotein receptor, *ASO* antisense oligonucleotide, *DNA* deoxyribonucleic acid, *mRNA* messenger ribonucleic acid, *RNase* ribonuclease, *TTR* transthyretin

administration (45 mg SC every 4 weeks, a 27-fold reduction in monthly exposure) than the unconjugated ASO, inotersen, to achieve a similar (if not better) pharmacological effect [26, 29, 30].

NEURO-TTRansform is a global, open-label, randomized phase 3 study that aims to evaluate the efficacy and safety of AKCEA-TTR-L_{Rx} in patients with hATTR-PN. This paper describes the rationale and the design of the trial.

METHODS

Study Design

NEURO-TTRansform (EudraCT: 2019-001698-10; ClinicalTrials.gov: NCT04136184; ION-682884-CS3) is a phase 3 multicenter, open-label, randomized study of patients with stage 1 or stage 2 hATTR-PN assigned to receive AKCEA-TTR-L_{Rx} or an active reference arm (inotersen; Fig. 2). The primary aim is to evaluate the efficacy of AKCEA-TTR-L_{Rx} in patients with hATTR-PN relative to the historical placebo control arm of the NEURO-TTR trial [26]. The primary endpoints include the percentage change from baseline in serum TTR, change from baseline in mNIS + 7, and Norfolk QOL-DN score (Fig. 3). Key efficacy endpoints will be assessed at week 35 (interim analysis), week 66 (final efficacy analysis), and week 85. The secondary aims are to evaluate the efficacy of AKCEA-TTR-L_{Rx} relative to the historical placebo group with respect to the Neuropathy Symptom and Change (NSC) score, Physical Component Summary (PCS) score of the 36-Item Short-Form Health Survey (SF-36), PND score, and modified body mass index (mBMI; Fig. 3). AE monitoring and safety laboratory assessment will be performed throughout the trial. Discontinuation of study drug will occur immediately if the patient becomes pregnant, receives a major organ transplantation, or presents with predefined laboratory test abnormalities.

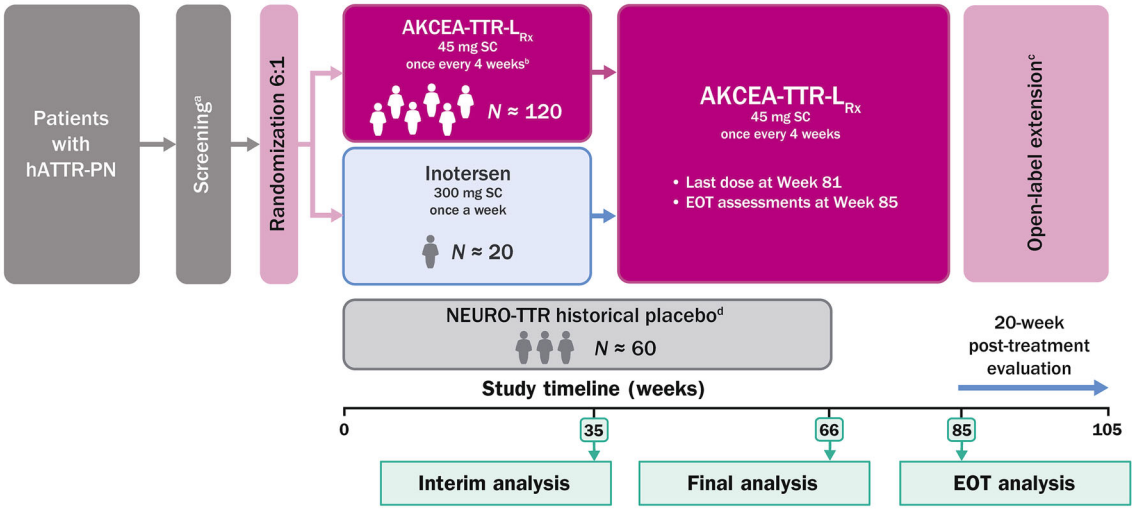

Fig. 2 NEURO-TTRansform study design. *EOT* end of treatment, *hATTR-PN* hereditary transthyretin-mediated amyloid polyneuropathy, *SC* subcutaneously. [a]The screening period is ≤ 6 weeks (or ≤ 10 weeks if genetic testing is required). [b]Concomitant therapy with tafamidis or off-label use of diflunisal is not allowed. Doxycycline use for the indication of infection (< 15 days) is allowed. [c]Patients not participating in the open-label extension will enter a 20-week post-treatment evaluation after completing EOT assessments. [d]Placebo arm of the NEURO-TTR study (NCT01737398)

Patients will be randomized 6:1 to receive SC injections of either AKCEA-TTR-L$_{Rx}$ ($n \approx 120$) 45 mg every 4 weeks or inotersen ($n \approx 20$) 300 mg once per week. As the analyses will utilize comparisons to the external placebo control arm of the NEURO-TTR trial, the inotersen reference arm is intended to ensure that no gross differences in patient population and response exist between the NEURO-TTR and the current study. Patients in the reference inotersen arm will be switched to receive AKCEA-TTR-L$_{Rx}$ at week 37. All patients will then continue receiving treatment with AKCEA-TTR-L$_{Rx}$ through week 81, with end-of-treatment (EOT) assessments at week 85, 4 weeks after the last dose. Following treatment and EOT assessments, patients will be eligible to enter an open-label extension study to continue receiving AKCEA-TTR-L$_{Rx}$ once every 4 weeks or enter a 20-week post-treatment evaluation period.

The distribution of patients according to the disease stage will mirror the NEURO-TTR trial. Patients will also take supplemental doses of the recommended daily allowance of vitamin A to ensure adequate delivery of vitamin A to tissues in a setting of low serum TTR.

The study protocol and amendments have been approved by the relevant local institutional review boards (IRBs) or ethics committees (ECs) for currently activated clinical study sites. Activation of additional clinical study sites is ongoing, and the study protocol and amendments will be approved by the relevant local IRBs/ECs before participants are allowed to enroll in the study. The full list of IRB/EC names and approval numbers is available upon request. The study is being conducted according to the International Conference on Harmonisation guidelines and relevant country-specific laws. All participants must provide written informed consent prior to study inclusion. A data and safety monitoring board will periodically review safety, tolerability, and efficacy data relating to AKCEA-TTR-L$_{Rx}$ and inotersen, including the results of the prespecified interim analysis at week 35.

Study Population

NEURO-TTRansform will enroll approximately 140 patients from ~ 70 clinical research sites in 15 countries. Adults aged 18–82 years are

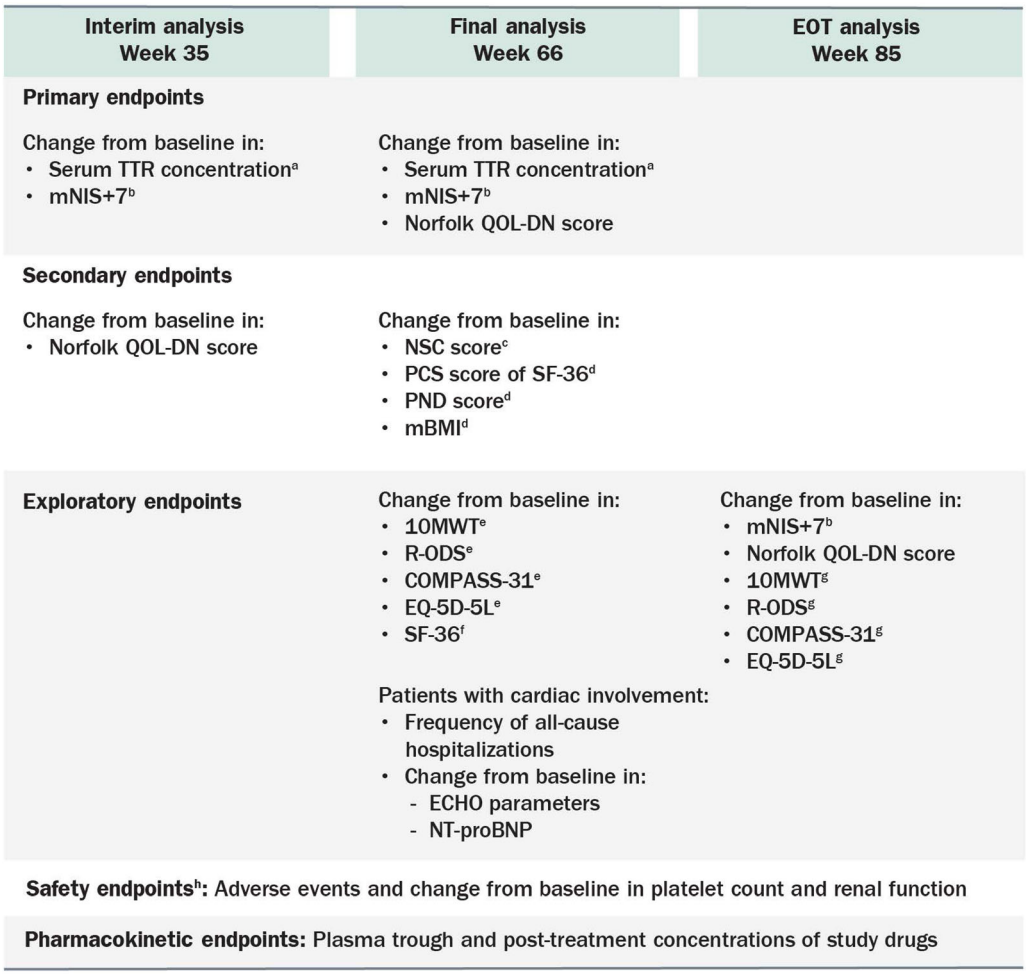

Fig. 3 NEURO-TTRansform study endpoints. *10MWT* 10-min walk test, *COMPASS-31* Composite Autonomic Symptom Score-31, *ECHO* echocardiogram, *EQ-5D-5L* 5-level EuroQol 5-dimension, *EOT* end of treatment, *mBMI* modified body mass index, *mNIS + 7* modified Neuropathy Impairment Score + 7, *Norfolk QOL-DN* Norfolk Quality of Life Questionnaire—Diabetic Neuropathy, *NSC* Neuropathy Symptom and Change, *NT-proBNP* N-terminal pro-brain natriuretic peptide, *PCS* Physical Component Summary, *PND* Polyneuropathy Disability Score, *R-ODS* Rasch-built Overall Disability Score, *SC* subcutaneously, *SF-36* 36-Item Short-Form Health Survey, *TTR* transthyretin. [a]Percentage change. [b]The mNIS + 7 assessment procedure includes: Neuropathy Impairment Score, quantitative sensory testing, heart rate response to deep breathing, and predetermined sensory and nerve conduction testing. Additional clinical evaluations done during mNIS + 7 assessment include: lower limbs function test and NSC. [c]weeks 35 and 66. [d]week 65. [e]week 37. [f]week 35. [g]week 81. [h]Vital signs, body weight, physical examination, clinical laboratory tests, electrocardiogram, use of concomitant medication, thyroid panel, inflammatory panel, coagulation, immunogenicity test

eligible for enrollment if they have stage 1 or stage 2 hATTR-PN, a documented *TTR* mutation, and signs and symptoms consistent with polyneuropathy (including an NIS $\geq$ 10 and $\leq$ 130). Key inclusion and exclusion criteria are shown in Fig. 4, and a complete list can be found in Table S1 in the supplementary material. Patients who have had a liver or heart transplant are ineligible. Any medications deemed necessary by the investigator are allowed, although concomitant tafamidis, inotersen, patisiran, or off-label use of diflunisal or doxycycline are not permitted. Patients who have received prior treatment with inotersen or

patisiran are not eligible; patients previously treated with tafamidis, diflunisal, or doxycycline are eligible as long as these medications were discontinued at least 2 weeks prior to initiation of study treatment (day 1).

Planned Outcomes

Co-primary endpoints at the week 35 interim analysis include percentage change from baseline to week 35 in serum TTR levels and change from baseline in mNIS + 7 (Fig. 3). Co-primary endpoints at the week 66 final analysis are the same as those for the week 35 interim analysis, but also include change from baseline in Norfolk QOL-DN score.

Secondary efficacy, safety, and exploratory endpoints measured at weeks 35, 66, and 85 (EOT) are shown in Fig. 3. The week 35 interim analysis key secondary endpoint is change from baseline to week 35 in the Norfolk QOL-DN score. The final analysis secondary endpoints will include NSC score at weeks 35 and 66, and SF-36 PCS score, PND score, and mBMI at week 65. Non-compartmental pharmacokinetic analysis of AKCEA-TTR-L_{Rx} or inotersen will be carried out on each individual patient data set in the pharmacokinetic subgroup, where plasma samples are collected following drug administration on days 1, 225, and 449.

All treatment-emergent AEs, serious AEs, and reasons for any withdrawals will be summarized for each treatment group using the Medical Dictionary for Regulatory Activities coding

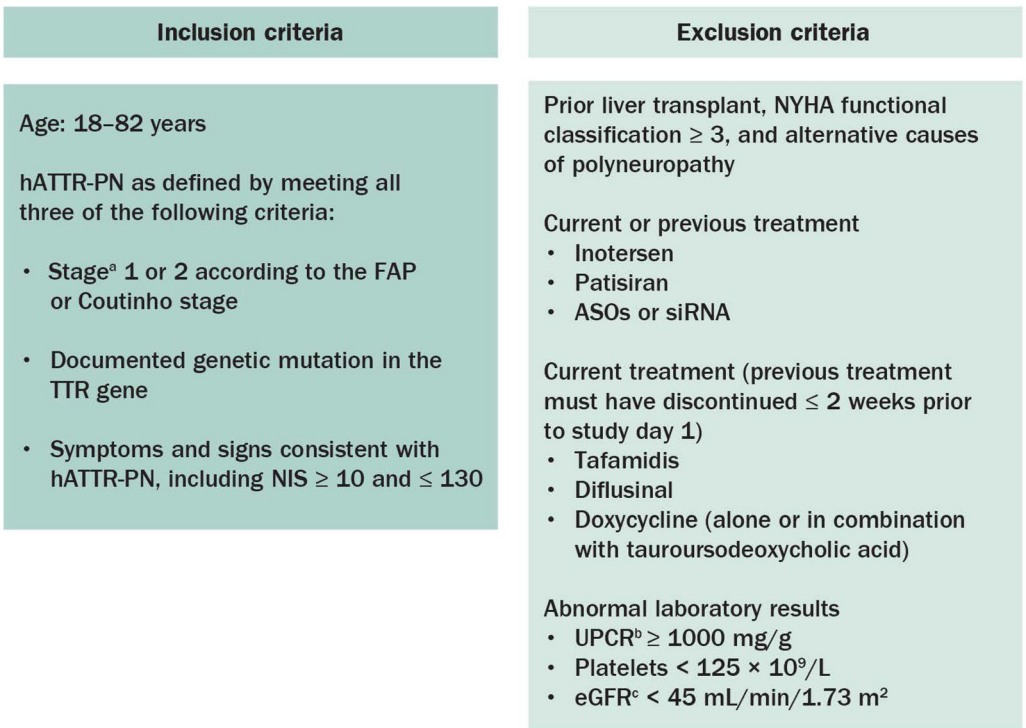

Fig. 4 Key inclusion and exclusion criteria for the NEURO-TTRansform study. *ASO* antisense oligonucleotide, *eGFR* estimated glomerular filtration rate, *FAP* familial amyloid polyneuropathy, *hATTR-PN* hereditary transthyretin-mediated amyloid polyneuropathy, *NIS* Neuropathy Impairment Score, *NYHA* New York Heart Association, *siRNA* small interfering ribonucleic acid, *TTR* transthyretin, *UPCR* urine protein/creatinine ratio. [a]Stage 1 (ambulatory without assistance) or stage 2 (ambulatory with assistance). [b]In the event of UPCR ≥ 1000 mg/g, eligibility may be confirmed by a repeat random urine test with UPCR < 1000 mg/g or a quantitative total urine protein measurement of < 1000 mg/24 h. [c]Chronic Kidney Disease Epidemiology Collaboration equation 1 formula

system, by system organ class, preferred term, relationship to AKCEA-TTR-L_{Rx} or inotersen, and severity. AEs of special interest (including serious or nonserious events) are severe reductions in platelet count ($< 50 \times 10^9$/L) accompanied by major bleeding or a clinically relevant non-major bleeding event, or a platelet count of $< 25 \times 10^9$/L independent of such bleeding events [31].

Statistical Analysis

The primary population for efficacy analyses will be the full analysis set, defined as all randomly assigned patients who receive ≥ 1 dose of AKCEA-TTR-L_{Rx} and who have one baseline and at least one post-baseline efficacy assessment for mNIS + 7 score or Norfolk QOL-DN questionnaire total score.

Power calculations for this study are based on the assumption that TTR reduction will be 80%. Approximately 140 patients (120 administered AKCEA-TTR-L_{Rx}) will be enrolled in NEURO-TTRansform to account for a 10% dropout rate. In the NEURO-TTR trial, there were 52 evaluable completers in the placebo arm [26]. A sample size of 108 evaluable patients in the AKCEA-TTR-L_{Rx} arm will provide ≥ 95% power to detect a 70.3% difference in the percentage change from baseline in serum TTR, ≥ 90% power to detect a 19.6-point difference in the change from baseline of mNIS + 7, and ≥ 80% power to detect a 10.7-point difference in the change from baseline of the Norfolk QOL-DN score between AKCEA-TTR-L_{Rx}–treated patients and the historical NEURO-TTR placebo arm (two-sided alpha level, 0.025).

A sequential multiple testing procedure will be used to control the overall type 1 error rate at 0.05. If both co-primary endpoints of the interim analysis (TTR and mNIS + 7) are significant at an alpha level of 0.025, then the secondary endpoint (Norfolk QOL-DN score) will be tested at the interim analysis at an alpha level of 0.025. Regardless of the interim analysis results, the study will proceed as planned and the data will be collected from week 66 onwards at all study endpoints. For endpoints that are statistically significant at the week 35 interim analysis, the corresponding tests at the week 66 final analysis will not be conducted. The non-significant endpoint(s) (TTR and/or mNIS + 7) and the co-primary endpoint Norfolk QOL-DN score will be tested at the final analysis. If all the co-primary endpoints are significant, the secondary endpoints will be tested with the multiplicity controlled by the ranking strategy. Testing of the statistical significance of subsequent endpoints is contingent upon the attainment of statistical significance by the previous endpoint analyzed.

The percentage change in TTR will be assessed with a mixed-effects model for repeated measures (MMRM) adjusted by propensity score weights. The MMRM assumes that missing data are at random and that patients who discontinue prematurely would have behaved similarly to other patients in the same treatment group. The MMRM will include the effects of treatment, time, disease stage, pV50M mutation, previous treatment, treatment-by-time interaction, baseline value of the endpoint, and baseline-by-time interaction. The propensity score will be calculated for each patient in the AKCEA-TTR-L_{Rx} or historical placebo arm using a logistic regression model with baseline value of the endpoint and covariates including disease stage, pV50M mutation, and previous treatment. For mNIS + 7 and Norfolk QOL-DN score, the treatment comparison at week 35 will be based on the analysis of covariance model adjusted by propensity score. In the week 66 final analysis, the aforementioned MMRM adjusted by propensity score weights will be used for the treatment comparison for the percentage change from baseline in serum TTR, change from baseline in mNIS + 7, and Norfolk QOL-DN score.

DISCUSSION

hATTR-PN is a rare, progressive, and fatal disease caused by mutations in the *TTR* gene that result in the synthesis of a destabilized TTR protein structure and lead to the formation of amyloid deposits in multiple organ systems [2, 7]. Although two *TTR* gene silencing treatments for hATTR-PN are available that reduce

TTR and slow or halt the progression of the disease, there is an unmet need for an effective treatment with a more favorable risk–benefit profile and greater convenience of use. Inotersen requires weekly SC administration and regular monitoring for thrombocytopenia and glomerulonephritis, while patisiran requires intravenous infusion every 3 weeks and premedication with a corticosteroid to prevent infusion-related reactions [26]. The ligand-conjugated antisense technology is a significant advancement in the selective delivery to hepatocytes, yielding an approximate 20–30-fold increase in potency that permits substantial reduction in systemic exposure and an improved safety and tolerability profile [27, 32–34]. Similar to other ASO conjugates in this class, preclinical and phase 1 data on AKCEA-TTR-L_{Rx} demonstrated an increase in pharmacological potency compared with inotersen, a currently approved antisense medicine for the treatment of hATTR-PN. On the basis of the pathogenesis of ATTR, it is likely that preventing TTR from being synthesized would eliminate the origin of misfolded protein and subsequent amyloid fibril deposition, thus leading to the improvement of symptoms of ATTR, including polyneuropathy and cardiomyopathy.

NEURO-TTRansform is a phase 3 multicenter, open-label study of patients with stage 1 or stage 2 hATTR-PN randomly assigned to the AKCEA-TTR-L_{Rx} arm or an inotersen reference arm. Efficacy and safety data from participants enrolled in the AKCEA-TTR-L_{Rx} treatment arm will be compared with results from the historical placebo group in the NEURO-TTR trial [26]. The open-label design was selected because of the availability of approved treatments for hATTR-PN in some countries, rendering inclusion of a placebo control arm unethical [1, 17, 22–25]. This was not the case while the NEURO-TTR study was conducted, when the only standard of care was liver transplantation and tafamidis (which was only available at the time for stage 1 ATTR-PN in some European countries [26]). Patient eligibility criteria and the distribution of stage 1 and 2 hATTR-PN in the NEURO-TTRansform study are similar to those in the NEURO-TTR study, with identical endpoints and assessment times [26]. This provides an opportunity to adequately compare outcomes between patients who received AKCEA-TTR-L_{Rx} in NEURO-TTRansform and those who received placebo in NEURO-TTR. The lack of blinding in NEURO-TTRansform means that many sources of conscious and unconscious bias could be introduced. However, these limitations can be assessed to an extent by comparing the performance of inotersen up until week 35 in NEURO-TTRansform and the NEURO-TTR study, as inotersen represents a common denominator between the two studies.

One of the primary endpoints assessed in this study, change from baseline in mNIS + 7, was also assessed as a primary endpoint in the NEURO-TTR study [26]. The mNIS + 7 scoring procedure utilizes highly standardized, quantitative, and referenced assessments that are specifically designed to assess hATTR-PN impairment [35]. Assessments include the NIS, sensory and motor nerve conduction testing, quantitative sensory testing, and measurement of heart rate variation with deep breathing. The nerve conduction test is an objective and quantitative assessment, thereby providing a highly validated measure of neuropathy [35]. In the NEURO-TTR study, the mNIS + 7 scoring approach showed highly significant improvement in global scores and essentially all subscores with inotersen treatment, including quantitative measures and potentially objective measures in scored neuropathy signs [26, 35]. The favorable outcome is a result of utilizing previously validated scores, using the same specially trained mNIS + 7 evaluator throughout the study for each individual participant, and surveillance by a central expert reading center [35].

The AKCEA-TTR-L_{Rx} dosage regimen under investigation in phase 3 was selected on the basis of the pharmacodynamics and safety analyses of the phase 1 randomized placebo-controlled study in 47 healthy volunteers. AKCEA-TTR-L_{Rx} dosed at 45 mg every 4 weeks produced a mean reduction from baseline in serum TTR of 86% after four doses and an absence of potential tolerability or safety issues, such as the increases in liver transaminase levels observed in the upper dose range of this study

[29]. The GalNAc-mediated delivery of AKCEA-TTR-L_{Rx} to hepatocytes supports low-dose therapy and a more convenient dosage regimen (i.e., monthly administration) relative to the unconjugated ASO therapy used for the treatment of hATTR-PN [22–25]. Since no placebo group will be utilized in this trial, enrolled participants will receive either an approved drug, inotersen, or AKCEA-TTR-L_{Rx} at a much lower dose to achieve a similar pharmacological effect. Eligible participants will have the option to enroll into a long-term extension trial after the completion of this study. As a targeted therapy, AKCEA-TTR-L_{Rx} has the potential to reduce the disease burden of hATTR-PN with lower and less frequent dose than the parent compound. The NEURO-TTRansform trial will determine whether a more effective and efficient reduction of TTR by AKCEA-TTR-L_{Rx} yields both an improved safety and tolerability profile and clinical benefit in terms of disease stability or regression.

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Compliance with Ethics Guidelines. The study protocol and amendments have been approved by the relevant local IRBs or ECs for currently activated clinical study sites. Activation of additional clinical study sites is ongoing, and the study protocol and amendments will be approved by the relevant local IRBs/ECs before participants are allowed to enroll in the study. The full list of IRB/EC names and approval numbers is available upon request. The study is being conducted according to the International Conference on Harmonisation guidelines and relevant country-specific laws. All participants must provide written informed consent prior to study inclusion. A data and safety monitoring board will periodically review safety, tolerability, and efficacy data relating to AKCEA-TTR-L_{Rx} and inotersen, including the results of the predetermined interim analysis at week 35.

Data Availability. The study is registered at ClinicalTrials.gov (NCT04136184) and EudraCT (2019-001698-10).

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