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Patisiran Pharmacokinetics, Pharmacodynamics, and Exposure-Response Analyses in the Phase 3 APOLLO Trial in Patients With Hereditary Transthyretin-Mediated (hATTR) Amyloidosis

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Abstract

Hereditary transthyretin-mediated (hATTR) amyloidosis is an inherited, rapidly progressive, life-threatening disease caused by deposition of abnormal transthyretin protein. Patisiran is an RNA interference therapeutic comprising a novel, small interfering ribonucleic acid (ALN-18328) formulated in a lipid nanoparticle targeted to inhibit hepatic transthyretin protein synthesis. The lipid nanoparticle also contains 2 novel lipid excipients (DLin-MC3-DMA and PEG_{2000}-C-DMG). Here we report patisiran pharmacokinetics (PK), pharmacodynamics (PD), and exposure-response analyses from the phase 3 APOLLO trial, in which patients with hATTR amyloidosis with polyneuropathy were randomized 2:1 to receive patisiran 0.3 mg/kg or placebo intravenously every 3 weeks over 18 months. In patisiran-treated patients, mean maximum reduction in serum transthyretin level from baseline was 87.8%. Patisiran PK exposure was stable following chronic dosing. There were no meaningful differences in PK exposure, serum transthyretin reduction, and efficacy (change from baseline in modified Neuropathy Impairment Score+7) across all subgroups analyzed (age, sex, race, body weight, genotype status of valine-to-methionine mutation at position 30 [V30M] and non-V30M, prior use of tetramer stabilizers, mild/moderate renal impairment, and mild hepatic impairment). transthyretin reduction and efficacy were similar across the interpatient PK exposure range for ALN-18328. There was no trend in the incidence of adverse events or serious adverse events across the interpatient PK exposure range for all 3 analytes. Incidence of antidrug antibodies was low (3.4%) and transient, with no impact on PK, PD, efficacy, or safety. The patisiran dosing regimen of 0.3 mg/kg every 3 weeks is appropriate for all patients with hATTR amyloidosis.

Keywords

exposure-response, hereditary transthyretin-mediated amyloidosis (hATTR), patisiran, pharmacodynamics (PD), pharmacokinetics (PK), small interfering ribonucleic acid (siRNA)

Hereditary transthyretin-mediated (hATTR) amyloidosis is an inherited, multisystem, rapidly progressive, and often fatal disease caused by mutations in the transthyretin gene.[1,2] The transthyretin protein is primarily produced in the liver[3] and forms a tetramer that transports vitamin A (retinol) in association with retinol-binding protein (RBP) in the plasma and cerebrospinal fluid.[4,5] To date, more than 120 *transthyretin* mutations have been identified,[6] of which the most common is the valine-to-methionine mutation at position 30 (V30M).[7] These pathogenic transthyretin mutations result in misfolded transthyretin proteins that accumulate as amyloid deposits at multiple sites including peripheral nerves, heart, kidney, and gastrointestinal tract.[1,8] This gives rise to a heterogeneous clinical presentation including sensory, motor, and autonomic polyneuropathy and cardiomyopathy, as well as other disease manifestations.[1,2,9–11] The disease has a rapid progression, with a median survival of 4.7 years following diagnosis, reduced to 3.4 years for patients presenting with cardiomyopathy.[12–15]

Therapeutic treatment options for hATTR amyloidosis include transthyretin tetramer stabilizers (tafamidis and diflunisal), transthyretin-reduction pharmacotherapies (patisiran and inotersen), and surgical intervention (orthotopic liver transplant).[1,16,17] Patisiran is a first-in-class RNA interference (RNAi) therapeutic recently approved in the United States and Europe to treat the polyneuropathy caused

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by hATTR amyloidosis.[18–20] The recommended dose of patisiran is 0.3 mg/kg administered via intravenous infusion every 3 weeks, with a maximum dose of 30 mg for patients weighing ≥100 kg. The patisiran drug substance is a novel small interfering RNA (siRNA) formulated as a lipid nanoparticle.[21] lipid nanoparticle technology is used as a delivery system for RNAi therapeutics and gene therapy[22,23] to protect therapeutic oligonucleotides from degradation by endogenous enzymes in biological fluids[24] and to facilitate targeted siRNA delivery into hepatocytes.[21–23] The structure of an RNAi therapeutic lipid nanoparticle consists of a largely hydrophobic core with inverted micelles of lipid that encapsulate the siRNA drug substance, which is surrounded by a roughly homogeneous outer coating of polyethylene glycol (PEG) lipids.[23,25] In patisiran, the lipid nanoparticle is composed of the siRNA (ALN-18328) and 4 lipid excipients, 2 of which have been used in marketed drugs (DSPC [1,2 distearoyl-sn-glycero-3-phosphocholine] and cholesterol)[23] and 2 novel lipids, which are being used as excipients for the first time (DLin-MC3-DMA [(6Z, 9Z, 28Z, 31Z)-heptatriaconta-6, 9, 28, 31-tetraen-19-yl-4-(dimethylamino) butanoate] and PEG_{2000}-C-DMG (α-(3′-{[1,2-di(myristyloxy) proponoxy]carbonylamino}propyl)-ω-methoxy, polyoxyethylene). PEG_{2000}-C-DMG aids lipid nanoparticle stability in circulation after intravenous administration and provides optimum circulation time, thereby enabling uptake of patisiran into the liver, the primary site of transthyretin synthesis.[21–23] DLin-MC3-DMA is important for particle formation, fusogenicity, cellular uptake, and endosomal release of the siRNA into the cell's cytoplasm.[23,25] In addition, DSPC and cholesterol provide physicochemical stability to the lipid nanoparticle.[23] The lipid nanoparticle facilitates siRNA delivery into hepatocytes through apolipoprotein E uptake, mediated by low-density lipoprotein receptors, following which the lipid nanoparticle structure is perturbed, resulting in release of ALN-18328 into the cytoplasm.[21,23] There, ALN-18328 binds to the RNA-induced silencing complex, resulting in the targeted degradation of wild-type and mutant *transthyretin* messenger RNA and subsequent reduction of transthyretin synthesis.[26,27]

The clinical development program for patisiran comprised 6 clinical studies.[28] In the phase 1 and 2 studies, ALN-18328 plasma exposure increased in an approximately dose-proportional manner over the dose range of 0.01 to 0.5 mg/kg, resulting in rapid and dose-dependent reduction of serum transthyretin levels.[21,29] Based on phase 1 and 2 studies, patisiran 0.3 mg/kg once every 3 weeks was selected for evaluation in patients with hATTR amyloidosis with polyneuropathy in the subsequent pivotal APOLLO trial.

Efficacy and safety results from the APOLLO trial have been reported elsewhere.[17] Patisiran significantly improved polyneuropathy, quality of life, gait speed, activities of daily living, and nutritional status in patients with hATTR amyloidosis.[17]

Here we report pharmacokinetics (PK), pharmacodynamics (PD), and antidrug antibody (ADA) results from the APOLLO trial. The objectives of the present analyses were to evaluate (1) plasma PK of ALN-18328 and the 2 novel lipid excipients (DLin-MC3-DMA and PEG_{2000}-C-DMG); (2) PD effects on serum levels of transthyretin, vitamin A, and RBP; (3) the effect of intrinsic and extrinsic factors on PK, PD, and efficacy; (4) the effect of PK exposure on transthyretin reduction, efficacy, and safety; (5) the incidence and titer of ADAs; and (6) the effect of ADAs on PK exposure, transthyretin reduction, efficacy, and safety.

Methods

Study Design

The APOLLO study methods have been described in detail previously[17,30] and are briefly summarized here. APOLLO was a randomized, multicenter, double-blind, placebo-controlled phase 3 study in adult patients with hATTR amyloidosis with polyneuropathy (NCT01960348). The study was conducted at 44 sites worldwide,[17] and was performed in accordance with the principles associated with the World Health Organization Declaration of Helsinki and the Health Insurance Portability and Accountability Act of 1996. The study protocol was approved by the local or central institutional review boards and ethics committees for each study site. Written informed consent was obtained from all participating patients before any study-related procedures were performed.

Patients were randomized 2:1 to receive patisiran 0.3 mg/kg or placebo intravenously every 3 weeks for 18 months. The maximum dose of patisiran was 31.2 mg for patients who weighed 104 kg or more. All patients received premedications to minimize the risk of infusion-related reactions. In addition, patients received a daily oral vitamin A supplement containing the recommended daily allowance. The primary end point was change from baseline in modified Neuropathy Impairment Score+7 (mNIS+7)[31] at 18 months. Safety assessments included adverse-event (AE) monitoring and clinical laboratory safety tests throughout the study. AEs and serious AEs (SAEs) were coded according to the Medical Dictionary for Regulatory Activities (version 18.0).

Pharmacokinetic Assessments

In the APOLLO trial, a sparse PK sample strategy was implemented, as previous phase 1 and 2 trials had investigated PK properties with intensive PK

sampling.[21,29,32] Blood samples for plasma PK analysis were taken at the following times over the 18-month treatment period: predose in weeks 0, 3, 18, 36, 57, and 78 (C_{min} predose plasma concentration [C_{min}]); at the end of infusion in weeks 0, 18, and 57 (maximum postinfusion plasma concentration [C_{max}]); and 30 minutes after the end of infusion in weeks 3, 36, and 78 (concentration 30 minutes postinfusion [$C_{p(30min)}$]).

Plasma concentrations of ALN-18328 were measured with a validated ATTO probe assay using a high-performance liquid chromatography (HPLC)/fluorescence detection method with a lower limit of quantification (LLOQ) of 1 ng/mL.[21] Precision was ≤9.1% (% coefficient of variation [CV%]) and accuracy ranged from −2.7% to 2.5% (% difference between found and nominal concentration). Plasma concentrations of DLin-MC3-DMA and PEG_{2000}-C-DMG were measured using 2 validated liquid chromatography with tandem mass spectrometry methods with LLOQ of 0.5 and 5.0 ng/mL, respectively. For the DLin-MC3-DMA high-range assay (LLOQ, 5 ng/mL), precision was ≤13.4%, and accuracy ranged from 0.6% to 6.7%. For the DLin-MC3-DMA low-range assay (LLOQ, 0.5 ng/mL), precision was ≤9.7%, and accuracy ranged from −2.5% to 0.3%. For the PEG_{2000}-C-DMG assay, precision was ≤7.4%, and accuracy ranged from −1.3% to 1.4%.

Pharmacodynamic Assessments

Blood samples for PD analysis (serum transthyretin, vitamin A, and RBP levels) were collected: predose in weeks 0, 3, 15, 36, 39, 57, and 78, 2-7 days postdose in weeks 37-38 (month 9) and weeks 79-80 (month 18), and in week 81, 3 weeks after the last dose. Blood samples were obtained before vitamin A supplementation.

Total serum transthyretin was quantified using a custom-developed, validated, sandwich enzyme-linked immunosorbent assay (ELISA; LLOQ, 1.13 ng/mL).[33] Serum vitamin A and RBP were analyzed by commercial in vitro diagnostic methods: vitamin A using an HPLC method (Chromsystems Instruments and Chemicals GmBH, Munich, Germany) and RBP using immunonephelometry (Siemens, Erlangen, Germany).

ADA Assessments

Blood samples to evaluate ADA status in serum were collected at predose in weeks 0, 3, 18, 36, 57, and 78. A validated ELISA method that specifically detected antibodies to PEG_{2000}-C-DMG on the lipid nanoparticle was used for the screening and confirmatory ADA assays. PEG_{2000}-C-DMG was coated onto the plate, and captured ADAs were detected using antihuman immunoglobulin G/immunoglobulin M antibodies. Serum samples were first analyzed with a screening assay. Samples testing positive for ADAs in the screening assay were further evaluated in a confirmatory assay. For the samples that tested positive for ADA in the confirmatory assay, ADA titer was then determined as the reciprocal of the highest dilution of the sample that yielded a positive ADA test result.

Analysis Population

The PD population was defined as all patients who were randomized and received at least 1 dose of patisiran or placebo. The PK population was defined as all patisiran-treated patients with ≥1 PK concentration measurement. The PK/PD population was defined as all patients who had both PK and PD data.

Statistical Methods

Plasma concentrations of ALN-18328 and DLin-MC3-DMA reached steady state (ss) after 24 weeks of treatment in the phase 2 open-label extension study.[32] Hence, in the APOLLO trial, steady state PK parameters were calculated by averaging concentrations obtained after 24 weeks as follows: $C_{min,ss}$ in weeks 36, 57, and 78, $C_{p,ss(30\ min)}$ in weeks 36 and 78, and $C_{max,ss}$ in week 57. Descriptive summary statistics were obtained for all PK parameters. Plasma concentrations below the LLOQ were treated as zero. Previous analysis of phase 2 studies demonstrated that plasma concentrations (C_{min}, C_{max}, and $C_{p(30\ min)}$) were linearly correlated with overall PK exposure (area under the plasma concentration-time curve during a dosing interval, AUC_τ) after the first dose[29] and at steady state[32] (Pearson correlation $R = 0.668$ to 0.794 and $P < 0.001$; data on file). Therefore, in the present analysis these PK concentration parameters at steady state ($C_{min,ss}$, $C_{p,ss(30\ min)}$, and $C_{max,ss}$) were used as surrogates of AUC_τ for PK subgroup analyses and PK exposure-response analyses.

Descriptive statistics for mean percent reduction from baseline for PD parameters were derived by (1) study visit, (2) averaging individual patient reductions using all postbaseline measurements over 18 months of treatment, and (3) averaging individual patient maximum reductions observed during the study. Baseline was defined as the average value for all PD measurements before the first dose at screening and on day 1. For PD subgroup analysis, mean reduction from baseline using all postbaseline measurements was used.

PK parameters, transthyretin change from baseline, and efficacy (difference between patisiran and placebo groups in mNIS+7 change from baseline at 18 months) were summarized across the following subgroups of intrinsic and extrinsic factors: sex (male vs female), age (<65 vs ≥65 years), V30M genotype status (yes vs no), race (white vs nonwhite), body weight (<100 vs ≥100 kg), prior use of transthyretin tetramer stabilizers (yes vs no), ADA status (positive vs negative), renal function (normal vs mild vs moderate impairment), and

Table 1. Baseline Demographic and Clinical Characteristics

Characteristic[a]	Placebo (n = 77)	Patisiran 0.3 mg/kg Once Every 3 Weeks (n = 148)
Age category, n (%)		
<65 years	44 (57.1)	86 (58.1)
≥65 years	33 (42.9)	62 (41.9)
Body weight, kg	67.5 ± 15.7 (40.8-99.0)	67.3 ± 16.6 (36.2-110.0)
Body weight category, n (%)		
<100 kg	77 (100.0)	141 (95.3)
≥100 kg	0 (0.0)	7 (4.7)
mNIS+7	74.6 ± 37.0 (11.0-154.0)	80.9 ± 41.5 (8.0-165.0)
TTR, mg/L	199 ± 58.1 (59.0-320.0)	197 ± 67.7 (52.0-411.0)
Vitamin A, μg/dL	37.0 ± 12.3 (14.0-67.0)	37.8 ± 13.7 (11.0-70.0)[b]
RBP, mg/dL	3.85 ± 1.41 (1.7-7.2)	3.89 ± 1.55 (1.7-8.0)
Hepatic function, n (%)		
Normal	75 (97.4)	138 (93.2)
Mild impairment	2 (2.6)	10 (6.8)
Renal function, n (%)		
Normal	49 (63.6)	104 (70.3)
Mild impairment	23 (29.9)	28 (18.9)
Moderate impairment	5 (6.5)	16 (10.8)

mNIS+7, modified Neuropathy Impairment Score+7; RBP, retinol-binding protein; TTR, transthyretin.

[a]Data are presented from baseline study visits and expressed as mean ± standard deviation (range) except where specified otherwise.

[b]n = 147.

hepatic function (normal vs mild impairment). Renal impairment subgroups were defined based on estimated glomerular filtration rate (eGFR) calculated using the Modification of Diet in Renal Disease equation[34]; eGFR ≥90 mL/min/1.73 m^2 indicated normal renal function, eGFR ≥ 60-<90 mL/min/1.73 m^2 indicated mild renal impairment, and eGFR ≥30–<60 mL/min/1.73 m^2 indicated moderate renal impairment. Hepatic impairment subgroups were defined by National Cancer Institute Organ Dysfunction Working Group criteria.[35] These criteria are based on serum aspartate transaminase (AST) and bilirubin (BIL) values[35]: BIL ≤ upper limit of normal (ULN) and AST ≤ ULN indicated normal hepatic function (BIL ≤ ULN and AST > ULN) or ULN < BIL ≤ 1.5 × ULN indicated mild hepatic impairment, and 1.5 × ULN < BIL ≤ 3 × ULN indicated moderate hepatic impairment.

For subgroup comparisons, the following statistical tests were performed[36]: for normally distributed variables, the 2-sample *t* test and analysis of variance were used to evaluate group differences for subgroups with 2 levels and more than 2 levels, respectively; for variables that were not normally distributed, the Wilcoxon rank sum test and Kruskal-Wallis test were used to evaluate group differences for subgroups with 2 levels and more than 2 levels, respectively. All tests were 2-sided at the .05 significance level. Type I error was controlled using a false discovery rate procedure that accounted for the correlated nature of the hypothesis tests, and adjusted *P* values are presented.[37,38]

To evaluate the impact of interpatient variability in PK on transthyretin reduction, clinical efficacy, and safety, PK exposure parameters from the PK/PD population were divided into 4 quartiles (≤25%, >25%-≤50%, >50%-≤75%, and >75%-≤100%). Serum transthyretin reduction from baseline over 18 months and change from baseline in mNIS+7 at 18 months versus placebo were summarized in each ALN-18328 PK exposure quartile. Similar analyses were performed for the lipid excipients (DLin-MC3-DMA and PEG_{2000}-C-DMG). Incidence of treatment-emergent AEs and SAEs, which were related or possibly related to study drug as determined by investigators, were summarized in each PK exposure quartile for ALN-18328 and the lipid excipients (DLin-MC3-DMA and PEG_{2000}-C-DMG).

PK and statistical analysis were performed using Phoenix WinNonlin software version 7.0 (Certara USA, Inc., Princeton, New Jersey) and SAS software version 9.4 (SAS Institute Inc., Cary, North Carolina), respectively. Graphical analysis was performed using Microsoft Office 365 (Microsoft Corporation, Redmond, Washington) and R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).[39]

Results

Patient Demographics and Baseline Characteristics

Baseline demographics and disease characteristics have been previously published[17]; additional patient characteristics are shown in Table 1. A total of 225 patients were randomly assigned in a 2:1 ratio to receive patisiran (n = 148) or placebo (n = 77). At baseline, mNIS+7 and serum levels of transthyretin, vitamin A, and RBP were similar in the 2 treatment groups (Table 1). All 225 patients were included in the PD analysis. The PK and PK/PD populations comprised 147 patients randomized to patisiran, as 1 patient received a partial dose in the first infusion and withdrew from the study shortly after it began.[17]

In the 148 patients in the patisiran group, mean body weight was 67.3 kg, and 7 patients (4.73%) weighed ≥100 kg; there were more male than female patients (109 vs 39), and the majority of patients (113 of 148 [76.4%]) were white.[17] Ten patients (6.76%) treated with patisiran had mild hepatic impairment, 28 (18.9%) had mild renal impairment, and 16 (10.8%) had moderate renal impairment. A total of 56 patients (37.8%) in the patisiran group had the V30M genotype, and 78

(52.7%) had received prior transthyretin tetramer stabilizer treatment.[17]

PK

Sparse plasma concentrations of ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG over 18 months are shown in Figure 1. PK parameters for ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG after the first dose and at steady state are summarized in Table 2.

For ALN-18328 and DLin-MC3-DMA, plasma C_{min} increased slightly from the first dose to 18 weeks and was stable thereafter, whereas plasma C_{max} and $C_{p(30min)}$ were similar across all measured times from the first dose to the last dose (Figure 1A,B, respectively, Table 2). At steady state, mean $C_{max,ss}$, $C_{p,ss(30\ min)}$, and $C_{min,ss}$ were 6.48, 4.97, and 0.0241 µg/mL for ALN-18328, respectively, and 36.9, 30.9, and 1.37 µg/mL for DLin-MC3-DMA, respectively (Table 2). For PEG_{2000}-C-DMG, no appreciable increases in plasma C_{max}, $C_{p(30\ min)}$, and C_{min} were observed during the 18-month treatment period (Figure 1C, Table 2). Interpatient PK variability, as measured by CV%, was low to moderate, ranging from 25.3% to 43.2% for ALN-18328 ($C_{max,ss}$ and $C_{p,ss(30\ min)}$), DLin-MC3-DMA, and PEG_{2000}-C-DMG ($C_{max,ss}$, $C_{p,ss(30\ min)}$, and $C_{min,ss}$); see Table 2. However, a significant number of ALN-18328 $C_{min,ss}$ values were below the LLOQ, resulting in a large interpatient variability of 547%.

The ALN-18328 PK parameters ($C_{max,ss}$, $C_{p,ss(30\ min)}$, and $C_{min,ss}$) did not show statistically significant differences across the following subgroups studied: age, sex, body weight, race, V30M genotype, renal function (normal, mild, and moderate impairment), hepatic function (normal and mild impairment), and prior use of transthyretin tetramer stabilizers (Figure 2). The mean ± SD $C_{max,ss}$ of ALN-18328 in patients with mild hepatic impairment (4.63 ± 1.14 µg/mL) was numerically lower than that in patients with normal hepatic function (6.57 ± 2.23 µg/mL), but this difference was not statistically significant after controlling for type I error ($P = .2208$). Similar findings were shown for PK parameters of the 2 lipid excipients, DLin-MC3-DMA and PEG_{2000}-C-DMG (Supplementary Table S1).

Across a wide range of body weights, from 36.2 to 110 kg, higher ALN-18328 PK exposure ($C_{max,ss}$ and $C_{p,ss(30min)}$) was seen with increasing body weight (Figure 3A, Supplementary Table S2). The PK exposures increased by 1.3- to 1.5-fold from body-weight quartile 1 (range, 36.2-56.6 kg) to quartile 4 (range, 79.5-110 kg). A similar trend of higher PK exposure ($C_{max,ss}$ and $C_{p,ss(30min)}$) with increasing body weight was seen with the 2 lipid excipients (DLin-MC3-DMA and PEG_{2000}-C-DMG) across the 4 body-weight quartiles (Supplementary Figure S1, Supplementary Table S2).

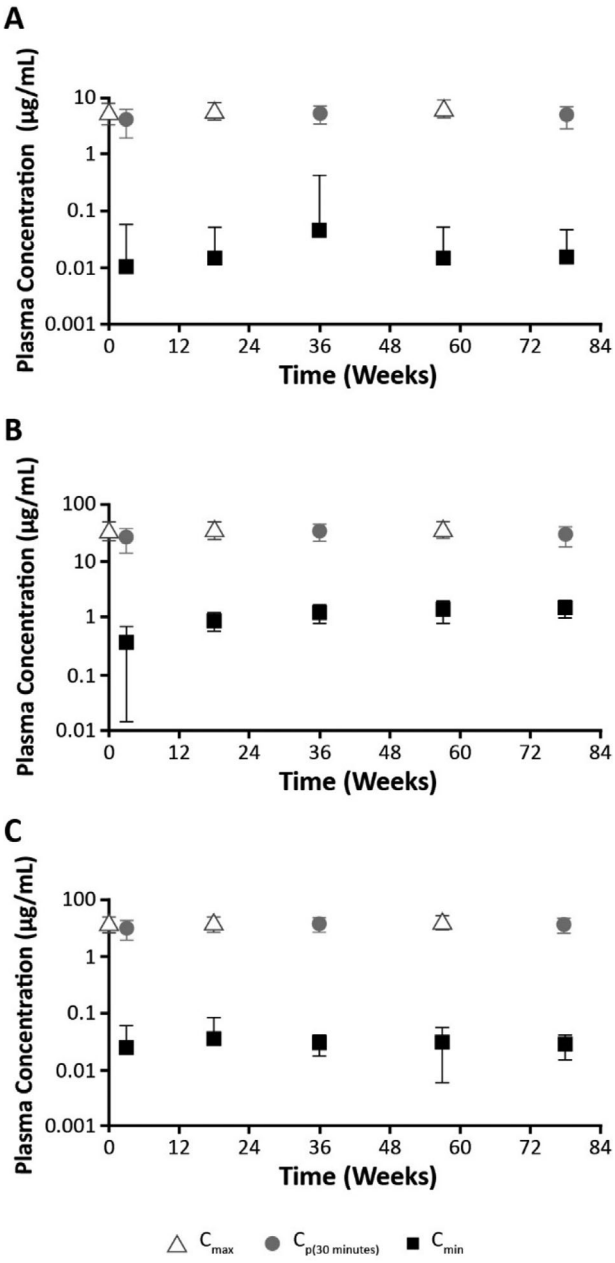

Figure 1. Mean plasma concentrations of (A) ALN-18328, (B) DLin-MC3-DMA, and (C) PEG_{2000}-C-DMG (semilog scale) after administration of patisiran 0.3 mg/kg once every 3 weeks over 18 months in patients (PK population). The error bars represent the ± standard deviation. Note: 1-sided error bars for ALN-18328 and some PEG_{2000}-C-DMG concentrations are presented because values are negative. ALN-18328, patisiran drug substance (small interfering RNA); C_{max}, maximum concentration observed at end of infusion; $C_{p(30\ minutes)}$, concentration observed 30 minutes postinfusion; C_{min}, observed predose concentration; DLin-MC3-DMA, lipid excipient (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate; PEG_{2000}-C-DMG, lipid excipient α-(3′-{[1, 2-di(myristyloxy)proponoxy]carbonylamino}propyl)-ω-methoxy, polyoxyethylene; PK, pharmacokinetic.

Table 2. Summary of PK Parameters for ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG After Intravenous Administration of Patisiran 0.3 mg/kg Once Every 3 Weeks

PK Parameter[a]	n	ALN-18328	n	DLin-MC3-DMA	n	PEG_{2000}-C-DMG
$C_{max,1}$, μg/mL	141	5.51 ± 2.32 (42.1%)	141	35.6 ± 12.8 (35.8%)	142	3.75 ± 1.15 (30.6%)
$C_{max,ss}$, μg/mL	135	6.48 ± 2.22 (34.3%)	135	36.9 ± 11.7 (31.6%)	135	4.07 ± 1.08 (26.5%)
R_{ac} for $C_{max,ss}$		1.18		1.04		1.09
$C_{p,2(30\ min)}$, μg/mL	139	4.02 ± 2.12 (52.9%)	139	25.6 ± 12.0 (46.9%)	140	3.08 ± 1.18 (38.3%)
$C_{p,ss(30\ min)}$, μg/mL	141	4.97 ± 1.59 (32.0%)	141	30.9 ± 9.46 (30.6%)	141	3.64 ± 0.92 (25.3%)
R_{ac} for $C_{p,ss(30\ min)}$	—	1.24	—	1.21	—	1.18
$C_{min,1}$,[b] μg/mL	139	0.010 ± 0.047 (470%)	138	0.365 ± 0.350 (95.9%)	140	0.0244 ± 0.0373 (153%)
$C_{min,ss}$,[c] μg/mL	141	0.0241 ± 0.132 (547%)	141	1.37 ± 0.456 (33.2%)	141	0.030 ± 0.013 (43.2%)
R_{ac} for $C_{min,ss}$	—	2.41	—	3.75	—	1.23

ALN-18328, patisiran drug substance (small interfering RNA); $C_{max,1}$, maximum concentration observed at end of infusion after first dose on day 1; $C_{max,ss}$, maximum concentration observed at end of infusion at steady state; $C_{p,2(30\ min)}$, concentration observed 30 minutes postinfusion after second dose on day 21; $C_{p,ss(30\ min)}$, concentration observed 30 minutes postinfusion at steady state; $C_{min,1}$, predose concentration observed at the end of first dosing interval and before the second dose; $C_{min,ss}$, predose concentration observed at steady state; PK, pharmacokinetics; R_{ac}, accumulation ratio (PK exposure at steady state: first dose); DLin-MC3-DMA, (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4 (dimethylamino) butanoate; PEG_{2000}-C-DMG, α-(3′-{[1,2-di(myristyloxy)propanoxy]carbonylamino}propyl)-ω-methoxy, polyoxyethylene; n, sample size.

[a]Data are expressed as mean ± standard deviation (% coefficient of variation).

[b]34.5% of $C_{min,1}$ values were below the lower limit of quantification and treated as zero.

[c]17.2% of $C_{min,ss}$ values were below the lower limit of quantification and treated as zero.

Pharmacodynamics

In the patisiran treatment group, serum transthyretin reduction was rapid and sustained over the 18-month duration of the study,[17] with a mean reduction of 82.6% and 84.3% at 9 and 18 months, respectively. The mean maximum serum transthyretin reduction was 87.8%. As expected, following patisiran treatment,[4,5] serum vitamin A and RBP levels also decreased in parallel with decreasing serum transthyretin levels, with mean reductions over 18 months of 62.4% for vitamin A and 45.3% for RBP. In the placebo group, there was no appreciable change in serum transthyretin levels over the 18-month duration of the study.[17] As expected, there were also no appreciable changes in vitamin A or RBP levels over 18 months of placebo treatment (Figure 4A,B, respectively). Furthermore, scatter plots showed that changes in vitamin A ($R^2 = 0.777$; $P < .001$) and RBP ($R^2 = 0.515$; $P < .001$) were linearly correlated with changes in transthyretin (data not shown).

There was no difference in mean or mean maximum serum transthyretin reduction from baseline over the 18-month duration of the study across the 4 body-weight quartiles (Figure 3B, Supplementary Table S2).

Subgroup Analysis of Efficacy and Transthyretin

In the patisiran group, mean serum transthyretin reduction from baseline over 18 months' treatment was not statistically significantly different across all subgroups analyzed (Figure 5A), ranging from 71.4% to 82%. The mean ± SD serum transthyretin reduction in patients with mild hepatic impairment (71.4% ± 12.6%) was numerically lower than in patients with normal hepatic function (78.0% ± 16.5%). However, the difference was not statistically significant ($P = .631$). A slightly higher mean ± SD percent reduction in transthyretin was seen in patients with prior use of tetramer stabilizers (82.0% ± 7.84%) compared with patients without prior use of tetramer stabilizers (72.8% ± 21.3%; $P = .0054$). However, the mean ± SD serum transthyretin level over 18 months of treatment was comparable in the 2 groups of patients (38.5 ± 24.6 vs 44.1 ± 43.0 μg/mL).

The mean mNIS+7 change from baseline compared with placebo was also similar across all subgroups analyzed (Figure 5B), ranging from −28.3 to −37.3 points.

Effect of Interpatient PK Variability on transthyretin and Efficacy

In the patisiran group, the mean reduction in serum transthyretin from baseline over 18 months of treatment was similar across the 4 PK exposure quartiles for all 3 PK parameters with ALN-18328 (Figure 6A), ranging from 71% to 81%. Similar results were seen for the 2 lipid excipients: mean transthyretin reduction from baseline ranged from 75.2% to 80.5% for DLin-MC3-DMA and from 74.6% to 80.3% for PEG_{2000}-C-DMG across the 4 PK exposure quartiles for all 3 PK exposure parameters analyzed (data not shown).

The mean change in mNIS+7 from baseline to 18 months compared with placebo was comparable across the 4 PK exposure quartiles for all 3 PK parameters with ALN-18328 (Figure 6B), ranging from −26.9 to −37.4 points. Similar mean changes in mNIS+7 from baseline to 18 months compared with placebo were noted for the 2 lipid excipients, ranging from

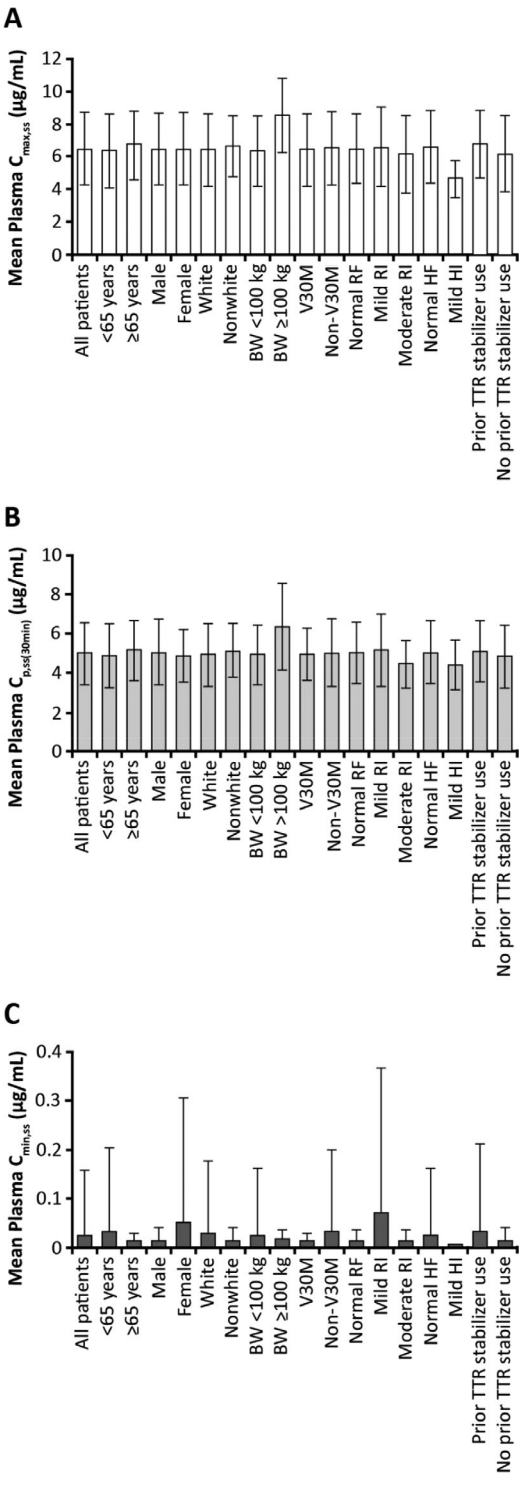

Figure 2. Mean steady-state plasma concentrations of ALN-18328 across patient subgroups. (A) $C_{max,ss}$, (B) $C_{p,ss(30\ min)}$, and (C) $C_{min,ss}$. The error bars represent the standard deviation. There were no statistically significant differences within subgroups after controlling for type I error. ALN-18328, patisiran drug substance (small interfering RNA); BW, body weight; $C_{max,ss}$, maximum concentration observed at end of infusion at steady state; $C_{p,ss(30\ min)}$, concentration observed 30 minutes postinfusion at steady state; $C_{min,ss}$, predose concentration observed at steady state; HF, hepatic function; HI, hepatic impairment; RI, renal impairment; RF, renal function; TTR, transthyretin.

−28.08 to −34.85 points for DLin-MC3-DMA and from −27.98 to −36.08 points for PEG_{2000}-C-DMG across the 4 PK exposure quartiles (data not shown).

Effect of Interpatient PK Variability on Safety

Primary safety results from the APOLLO trial have been reported previously.[17] Here we examined the effect of interpatient variability in PK on the incidence of treatment-emergent AEs and SAEs. The incidence of AEs or SAEs was similar across the 4 PK exposure quartiles for all 3 PK parameters for ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG (Supplementary Table S3).

Immunogenicity

Two of 224 patients (0.89%) tested positive for ADAs at baseline: 1 in the patisiran group and 1 in the placebo group. The overall incidence of treatment-emergent ADAs was 3.4% (5 of 145 patients) in the patisiran group and 1.3% (1 of 77 patients) in the placebo group. The 5 patisiran-treated patients tested positive for ADAs in week 3, with 2 patients also testing positive in week 18, but ADA positivity was transient, as all samples tested negative at subsequent visits through to end of the study. ADA titer ranged from 40 to 160.

Plasma PK exposures ($C_{max,ss}$, $C_{p,ss(30\ min)}$, and $C_{min,ss}$) for ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG were comparable in the ADA-positive and ADA-negative groups (Supplementary Table S4). The mean reduction from baseline in serum transthyretin levels over 18 months of treatment was similar in ADA-positive patients (n = 5 in week 3; n = 2 in week 18; range, 69%-79%) and ADA-negative patients (n = 134 to n = 138 from weeks 3 to 78; range, 73%-77%). The mean ± standard error of the mean difference between patisiran and placebo treatment in mNIS+7 change from baseline was also similar in ADA-positive (n = 6; −30.8 ± 9.46) and ADA-negative (n = 131; −32.2 ± 3.21) groups at 18 months. In the patisiran group, the patients with positive ADA status at any time postbaseline (n = 6) displayed a pattern of AEs and SAEs similar to that observed in the overall safety population.

Discussion

The present analyses of PK and PD data from the APOLLO study demonstrated that chronic dosing of patisiran 0.3 mg/kg every 3 weeks resulted in stable plasma concentrations of ALN-18328 and the 2 novel lipid excipients (DLin-MC3-DMA and PEG_{2000}-C-DMG) over 18 months of treatment. This dosing regimen elicited a rapid, robust, and sustained reduction in serum levels of the transthyretin protein. Subgroup analysis demonstrated that intrinsic and extrinsic factors had no meaningful impact on plasma

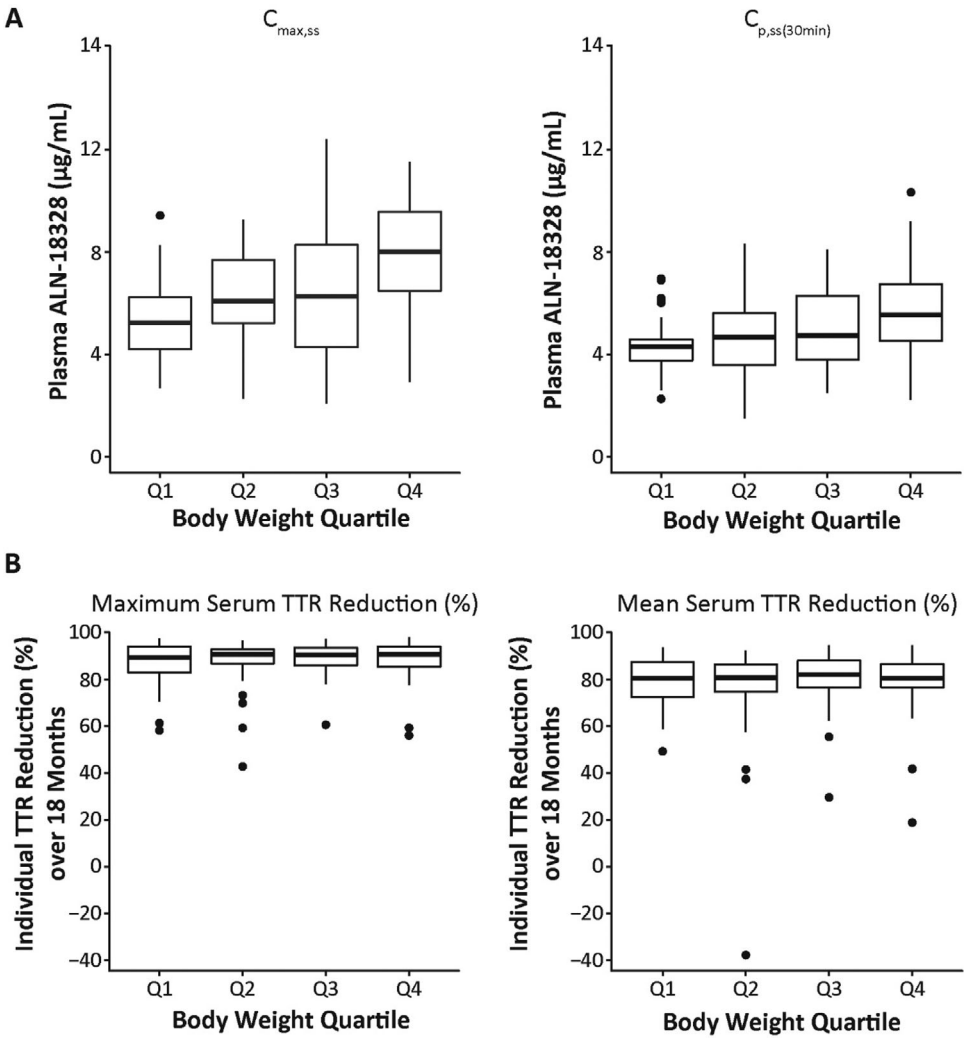

Figure 3. Effect of body weight on ALN-18328 PK exposure and TTR reduction. Box plots by body-weight quartiles for (A) $C_{max,ss}$ and $C_{p,ss(30min)}$ for ALN-18328 and (B) individual maximum TTR reduction and individual mean TTR reduction over 18 months. The lower and upper bounds of the rectangles represent the first and third quartiles, the horizontal line represents the median, the whiskers extend to the highest and lowest values within 1.5 × the the interquartile range, and data beyond the end of the whiskers are plotted as points. Body weights associated with the 4 quartiles are: Q1, 36.2-56.6 kg; Q2, 56.9-65.0 kg; Q3, 66.0-79.0 kg; and Q4, 79.5-110 kg. ALN-18328, patisiran drug substance (small interfering RNA); $C_{max,ss}$, maximum concentration observed at end of infusion at steady state; $C_{p,ss(30\ min)}$, concentration observed 30 minutes postinfusion at steady state; PK, pharmacokinetic; TTR, transthyretin.

PK exposures for all 3 analytes, serum transthyretin reduction, or clinical efficacy. Furthermore, interpatient variability in PK exposure for all 3 analytes did not result in differences in transthyretin reduction, clinical efficacy, or safety (AEs or SAEs).

ALN-18328 is a synthetic double-stranded oligonucleotide that is metabolized by nucleases to nucleotides of various lengths.[18] Nucleases belong to the hydrolase class of enzymes, which exist ubiquitously in the body and are not expected to be significantly different in patients with mild hepatic impairment. Hence, it is not surprising to see no effect of mild hepatic impairment on plasma ALN-18328 PK exposure. In addition, mechanisms of siRNA delivery to hepatocytes and RNAi-mediated suppression of transthyretin protein in the liver[21] were also not impacted, as similar transthyretin reduction from baseline was observed for patients with mild hepatic impairment versus those with normal hepatic function.

In previous studies, it was determined that negligible amounts of ALN-18328 (<1% of the dose) were excreted in the urine following patisiran dosing,[18] indicating that ALN-18328 is not renally cleared. Therefore, the lack of effect of mild and moderate renal impairment on ALN-18328 PK exposure is also as expected.

The similar ALN-18328 PK exposures and transthyretin reductions seen in V30M and non-V30M genotype patients in the present analysis are consistent with the observation in the APOLLO study that

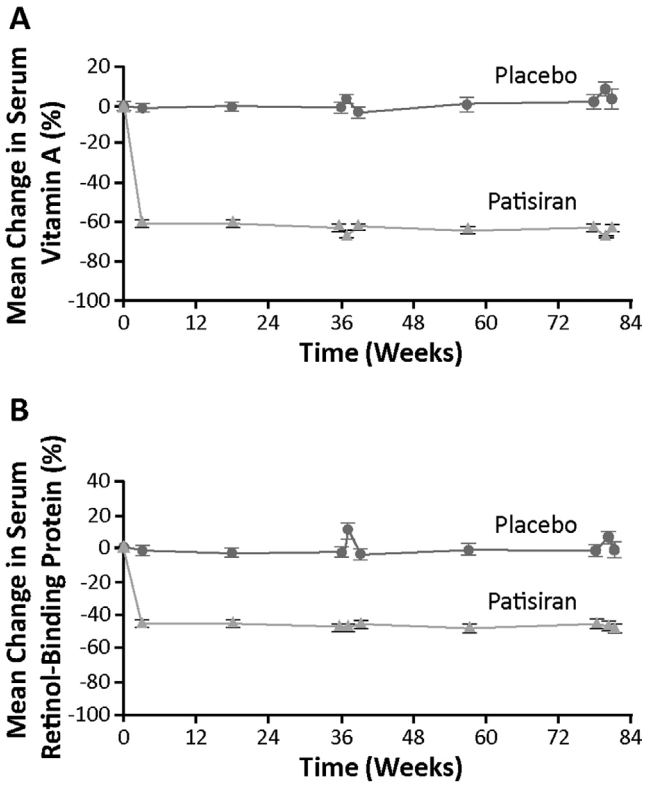

Figure 4. Mean change from baseline over time for serum levels of (A) vitamin A and (B) retinol-binding protein in the patisiran and placebo groups (PD population). The error bars represent the standard error of the mean. PD, pharmacodynamic.

change in mNIS+7 with patisiran was not affected by transthyretin genotype (V30M and non-V30M).[17] The similar results across different transthyretin mutations are expected because siRNA (ALN-18328) was designed to bind to a genetically conserved sequence in the 3′ untranslated region of transthyretin mRNA and thus mediate degradation of both wild-type and mutant transthyretin mRNA regardless of the specific pathogenic mutation.[21]

Patients with prior use of transthyretin stabilizers had higher baseline transthyretin levels in the present study compared with patients without prior use of transthyretin stabilizers. This is consistent with the finding in patients with transthyretin amyloid cardiomyopathy treated with an investigational transthyretin stabilizer (AG10) in which transthyretin levels were restored to the normal range.[40] The similar absolute serum transthyretin levels seen in patients with and without prior use of tetramer stabilizers over 18 months with patisiran treatment indicates similar PD effect of patisiran across these patients. The greater percentage reduction from baseline in patients with prior use of tetramer stabilizers is likely an artifact of higher baseline consequent to an inadequate washout period (≤14 days) in some patients prior to the start of patisiran dosing in the APOLLO trial. This was supported by higher mean ± SD baseline values in the patients with prior use of transthyretin stabilizers compared with those with no prior use (222 ± 65 vs 169 ± 59 μg/mL).

A clear trend toward higher PK exposures of ALN-18328 and the 2 lipid excipients was seen with increasing body weight (range, 36.2-110 kg). This can be attributed to the higher absolute dose that is administered to heavier patients. Despite the slight differences in PK exposure with body weight, observed serum transthyretin reductions were similar across the body-weight range studied. This is because the therapeutic dose of patisiran (0.3 mg/kg every 3 weeks) yields ALN-18328 concentrations that are in the plateau portion of the concentration-effect curve, resulting in 80% to 90% reduction in serum transthyretin,[33] where slight changes in PK concentrations do not result in meaningful differences in PD response.

A maximum absolute patisiran dose of 30 mg is proposed for patients weighing >100 kg, which results in a body-weight adjusted dose that is slightly lower than 0.3 mg/kg. The ALN-18328 plasma concentrations arising from the absolute dose of 30 mg given to patients weighing >100 kg are expected to be in the plateau portion of the concentration-effect curve, resulting in similar transthyretin lowering and mNIS+7 change as in patients weighing ≤100 kg receiving patisiran 0.3 mg/kg. This was indeed seen in the 7 patients who weighed >100 kg, in whom ALN-18328 PK exposure

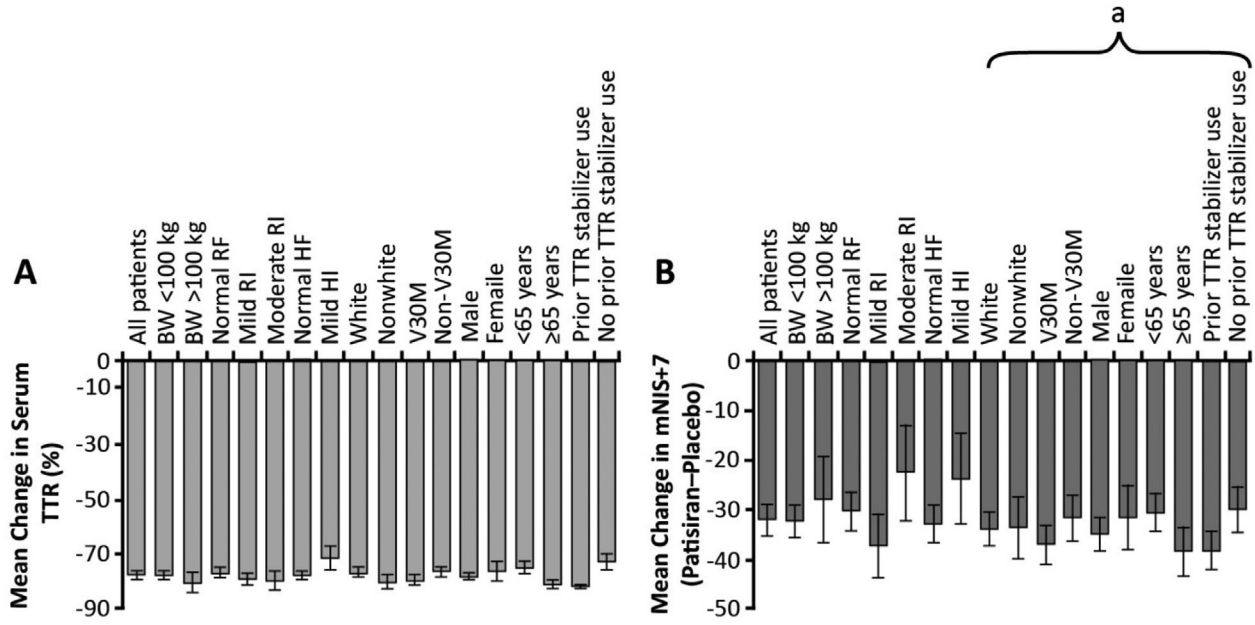

Figure 5. Analysis by intrinsic and extrinsic factors for change from baseline to 18 months with ALN-18328 in (A) mean serum TTR levels compared with baseline and (B) mean mNIS+7 from baseline at 18 months compared with placebo. The error bars represent the standard error of the mean. [a]Data from Adams et al 2018.[17] BW, body weight; HF, hepatic function; HI, hepatic impairment; mNIS+7, modified Neuropathy Impairment Score+7; RF, renal function; RI, renal impairment; TTR, transthyretin.

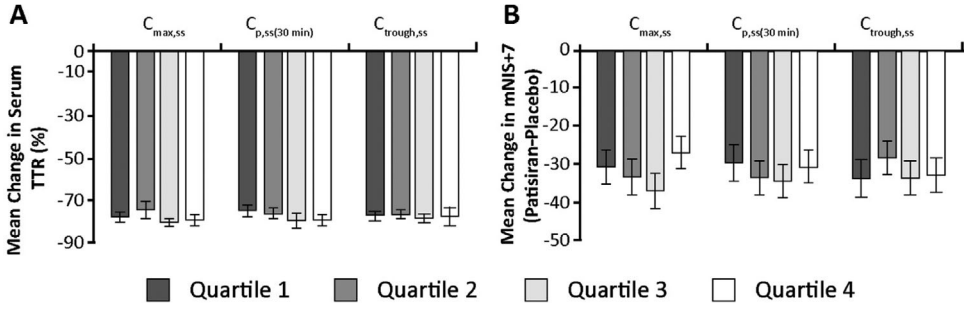

Figure 6. Effect of interpatient PK variability on (A) TTR change from baseline and (B) mNIS+7 change from baseline compared with placebo (PK/PD population). Quartile 1, 0%-≤25%; quartile 2, >25%-≤50%; quartile 3, >50%-≤75%; quartile 4, >75%-≤100%. The error bars represent the standard error of the mean. ALN-18328, patisiran drug substance (small interfering RNA); $C_{max,ss}$, maximum concentration observed at end of infusion at steady state; $C_{p,ss(30\ min)}$, concentration observed 30 minutes postinfusion at steady state; $C_{min,ss}$, predose concentration observed at steady state; mNIS+7, modified Neuropathy Impairment Score+7; PD, pharmacodynamic; PK, pharmacokinetic; TTR, transthyretin.

was about 35%-40% higher, yet serum transthyretin reduction and mNIS+7 change were similar to patients weighing ≤100 kg.

Exposure to ALN-18328 and serum transthyretin reduction was observed to be numerically lower in patients with mild hepatic impairment than in patients with normal hepatic function, although these differences were not statistically significant. Of the patients with mild hepatic impairment who provided data, 4 of 6 $C_{max,ss}$ values and 6 of 8 $C_{p,ss(30min)}$ values were in the first quartile range of body weight. This suggests that the lower PK exposure observed in patients with mild hepatic impairment compared with patients with normal hepatic function was likely a result of low body weight.

Interpatient variability in ALN-18328 PK exposure with patisiran 0.3 mg/kg every-3-week dosing in the APOLLO trial did not lead to differences in serum transthyretin reductions from baseline and clinical efficacy, confirming that 0.3 mg/kg every 3 weeks results in plasma concentrations in the plateau phase of the patisiran dose-response curve for transthyretin reduction, as reported in the phase 1 and 2 trials.[21,29,33] Comparable serum transthyretin reductions from baseline were also observed across the PK exposure range of DLin-MC3-DMA and PEG_{2000}-C-DMG, confirming that interpatient variability in the PK of the 2 lipid excipients had no impact on the magnitude of transthyretin reduction. In addition, similar transthyretin reduction across 4 PK exposure quartiles

of the 2 lipid excipients was an indirect indicator that the amount of siRNA (ALN-18328) that was delivered to the hepatocytes was not impacted by interpatient variability in the PK of the lipid excipients.

Population PK[33,41] and population PK/PD analyses[33,42] have previously been conducted on pooled data from the patisiran clinical program including single and multiple doses (0.01 to 0.5 mg/kg). The results from the present subgroup analyses are consistent with the covariate analyses of both population PK and population PK/PD models, which indicated that none of the studied intrinsic and extrinsic factors affected PK exposures of ALN-18328 and the 2 lipid excipients (DLin-MC3-DMA and PEG_{2000}-C-DMG), or the magnitude of serum transthyretin reduction.[33,41,42]

Likewise, the frequency of AEs and SAEs did not increase with increasing PK exposure to ALN-18328, DLin-MC3-DMA, or PEG_{2000}-C-DMG, indicating that the incidence of these events was independent of the PK concentrations of the 3 analytes. Furthermore, the lack of relationship between PK exposure and AE or SAE incidence was consistent with the similar incidence of AEs and SAEs observed in the patisiran and placebo arms of APOLLO.[17]

ADAs to patisiran were evaluated by measuring antibodies specific to PEG_{2000}-C-DMG, a lipid component exposed on the surface of the lipid nanoparticle. The current results show that treatment-emergent ADAs to PEG_{2000}-C-DMG occurred at a low frequency and were transient. ADAs specific to PEG_{2000}-C-DMG appeared to have no effect on PK, PD, safety, or efficacy. We noted from the literature that PEG is used in toothpaste, shampoo, moisturizers, colorants, foods, drinks, and deodorants[43]; therefore, environmental exposure to PEGylated products may be responsible for the positive ADA status of 2 patients at baseline.

In the APOLLO study, reductions in serum vitamin A and RBP levels paralleled the reductions in serum transthyretin levels. This observation is consistent with the role of transthyretin as a major transporter of the RBP-retinol complex in serum.[4,5,44,45] The transthyretin-RBP complex is a stable form of retinol transport, allowing its delivery to cells and preventing RBP from being filtered by the kidney.[5] Reduction in circulating transthyretin levels is expected to decrease circulating RBP and vitamin A levels. However, alternative mechanisms of vitamin A transport and tissue uptake can occur in the absence of transthyretin.[5] Although serum vitamin A levels are reduced because of reductions in serum transthyretin and RBP levels during treatment with patisiran, liver stores of vitamin A are not expected to be affected during treatment, as vitamin A uptake from the gastrointestinal tract to the liver is independent of serum transthyretin and RBP levels.[46] We noted that normal levels of hepatic retinol and retinyl ester were observed in transthyretin-deficient mice that had no transthyretin and very low levels of plasma retinol and RBP.[47,48] These transthyretin-deficient mice also had normal levels of retinol and retinyl esters in the peripheral tissues and did not develop symptoms of vitamin A deficiency.[47,48] Similar to the effects observed in the transthyretin-deficient mouse model, it is expected that in patients treated with patisiran, dietary uptake and transport of vitamin A to the liver will occur by the normal mechanisms that are independent of serum transthyretin levels and that hepatic stores of retinol will be normal.[46]

Conclusions

Dosing of patisiran at 0.3 mg/kg every 3 weeks over 18 months in patients with hATTR amyloidosis resulted in stable steady state plasma concentrations of the siRNA (ALN-18328) and 2 novel lipid nanoparticle constituents, with low to moderate interpatient variability. Incidence of ADAs was low and transient, with no effect on patisiran PK, transthyretin reduction, clinical efficacy, or the safety profile of patisiran. Various intrinsic factors and prior use of transthyretin tetramer stabilizers had no meaningful impact on the steady state PK exposure, serum transthyretin reduction, or clinical efficacy. Interpatient variability in ALN-18328 PK exposure did not result in differences in serum transthyretin reduction, clinical efficacy, or safety (AEs or SAEs). Overall results indicated that the patisiran dosing regimen of 0.3 mg/kg every 3 weeks, with a maximum dose of 30 mg for patients weighing $\geq$100 kg, is appropriate for patients with hATTR amyloidosis.

Acknowledgments

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Conflicts of Interest

The data in this article were obtained from the APOLLO trial funded by Alnylam Pharmaceuticals Inc. Authors are employees of Alnylam Pharmaceuticals Inc.

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Data-Sharing Statement

The data that support the findings of this study are available from the corresponding author (x.amy.zhang@gmail.com) on reasonable request.

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Supplemental Information

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