Population Pharmacokinetic–Pharmacodynamic Modeling of Inotersen, an Antisense Oligonucleotide for Treatment of Patients with Hereditary Transthyretin Amyloidosis

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A population pharmacokinetic (PK) and pharmacodynamic (PD) model was developed for inotersen to evaluate exposure–response relationships and to optimize therapeutic dosing regimen in patients with hereditary transthyretin (TTR) amyloidosis polyneuropathy (hATTR-PN). Inotersen PK and TTR level (PD) data were composed of one Phase 1 study in healthy subjects, one Phase 2/3 study in hATTR patients, and its one open-label extension study. Effects of intrinsic and extrinsic factors (covariates) on PK and PK/PD of inotersen were evaluated using a full model approach. Inotersen PK was characterized by a two-compartment model with elimination from the central compartment. The population PK analysis identified disease status and lean body mass (LBM) as significant covariates for inotersen PK. Nonetheless, the contribution of disease status and LBM on PK was small, as the difference in clearance (CL/F) was 11.1% between healthy subjects and patients with hATTR-PN and 38% between the lowest and highest LBM quartiles of the patient population. Age, race, sex, baseline renal function estimated glomerular filtration rate, and hepatic function markers (baseline albumin, bilirubin, and alanine aminotransferase values) were not statistically significant covariates affecting inotersen PK. An inhibitory effect indirect-response model (inhibition of TTR production) was used to describe the drug effect on TTR-time profiles, with baseline TTR included as a covariate. The overall population I_{max} and IC_{50}, together with 95% confidence interval, was estimated to be 0.913 (0.899–0.925) and 9.07 (8.08–10.1) ng/mL, respectively. V30M mutation showed no effect on the estimated IC_{50} value for hATTR patients. The final population PK and PK/PD model was used to simulate four different treatment regimens. The population PK/PD model developed well described the PK and PD of inotersen in patients with hATTR-PN and has been used for label recommendation and trial simulations.

Keywords: pharmacokinetics, pharmacodynamics, antisense, oligonucleotide, inotersen, hereditary TTR amyloidosis

Introduction

Hereditary transthyretin amyloidosis (hATTR) is a rare and fatal disease caused by mutations in the gene that codes for transthyretin (TTR) [1]. Single-point gene mutations destabilize the normal tetrameric structure of TTR protein causing its dissociation into free monomers and subsequent aggregation into insoluble extracellular deposits [2,3]. These deposits accumulate in multiple organs (including the peripheral nervous system, gastrointestinal tract, kidney, and heart) resulting in severe damage to cells, which causes the development of motor and sensory neuropathy,

autonomic neuropathy, nephropathy, and cardiomyopathy [4]. On average, death occurs within ~10 years from symptom onset (but is shorter when significant cardiomyopathy is present) and is primarily due to malnutrition and cachexia, renal failure, and cardiac disease [4]. The only treatment option for hATTR was liver transplant, until the approval of two treatment options in 2018: patisiran (Onpattro®; Alnylam Pharmaceuticals) and inotersen (Tegsedi™; Ionis Pharmaceuticals and Akcea Therapeutics), and both markedly changed the treatment landscape for this debilitating disease. Inotersen (also known as ISIS 420915) was approved by the European Commission, Canada, the U.S. Food and

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Drug Administration, and Brazil for the treatment of stage 1 or stage 2 polyneuropathy (PN) in adult patients with hATTR.

The strategy of treating hATTR patients with inotersen is to reduce the levels of mutated and wild-type TTR protein secreted by the liver, a primary organ for antisense oligonucleotide (ASO) distribution following systemic administrations. Inotersen is a 2′-O-(2-methoxyethyl) (2′-MOE) modified phosphorothioate oligonucleotide, which targets to the messenger RNA (mRNA) of human TTR via Watson–Crick base pairing and prevents expression of the encoded "disease-related" TTR protein. Inotersen demonstrated potent activity *in vitro* in reducing TTR mRNA levels in HepG2 cells and primary hepatocytes. Pharmacology studies conducted with inotersen in human TTR transgenic mice and cynomolgus monkeys demonstrated reproducible and robust reductions in liver TTR mRNA levels, which were accompanied by equally robust and significant reductions in plasma TTR protein levels.

The clinical experience included a Phase 1 double-blind, placebo-controlled, dose-escalation, first-in-human clinical study and a Phase 2/3 pivotal study in hATTR patients with open-label extension (OLE) up to 5 years. In the Phase 1 study, dose- and exposure-dependent reductions in plasma TTR levels were observed with an acceptable safety and tolerability profile when administered subcutaneously (SC) to healthy subjects up to 400 mg inotersen per week for 4 weeks. An exploratory pharmacokinetic (PK)/pharmacodynamic (PD) model was developed based on Phase 1 data [5] and was used to simulate TTR reduction at various dosing regimens. PK/PD simulations predicted that over 80% or 90% of patients would achieve TTR reduction of >60% or >50%, respectively, following 3 months of 300 mg inotersen once per week (QW). Therefore, the 300 mg once weekly dosing regimen was selected for the pivotal Phase 2/3 study. In the Phase 2/3 study in hATTR-PN patients, a 68%–74% mean (median: 75%–79%) reduction in TTR levels was achieved from week 13 to 65 of treatment with 300 mg inotersen QW and maintained throughout the 15-month treatment period in inotersen-treated subjects, consistent with results obtained in the inotersen Phase 1 healthy volunteer study [6]. The robust and persistent TTR reductions in hATTR patients were accompanied by clinically and statistically significant benefit in favor of inotersen treatment, as demonstrated by both primary end points [modified Neuropathy Impairment Score (mNIS) +7 and Norfolk Quality of Life Diabetic Neuropathy (QoL-DN)], as well as multiple prespecified sensitivity analyses, in multiple secondary, tertiary, and exploratory end points. Therefore, these results demonstrated that inotersen treatment provides clinical benefit to multiple aspects of the disease, including muscle weakness, large and small fiber sensation, and trends for benefit for autonomic symptoms. The overall safety profiles following chronic inotersen treatment were manageable. Taken together, these findings support the use of inotersen for the treatment of patients with hATTR.

The clinical PKs and PDs of inotersen have been evaluated in the first-in-human Phase 1 clinical study and the Phase 2/3 pivotal study with OLE by measuring plasma drug levels and serum TTR levels over time. The objective of this work was to develop a population PK and PK/PD model that describe the PKs and PDs of inotersen following SC administrations and evaluate the effect of intrinsic and extrinsic factors that potentially affect PK and PK/PD of inotersen and to perform alternative dose regimen simulations using the established PK/PD model.

Materials and Methods

Test compounds

Inotersen is a synthetic ASO of 20 nucleotides (ie, a 20-mer) in length that are connected sequentially by phosphorothioate linkages, with a total of ten 2′-MOE modified ribofuranosyl nucleotides. The sequence of inotersen is: $\underline{T^M CTTG}$ GTTA^{M}CATGAA $\underline{AT^M C^M C^M C}$ with 2′-MOE modifications at positions 1–5 and 15–20 (underlined). In addition, all the cytosines of the compound were modified to contain a 5-methyl group (5-methyl cytosine, ^{M}C). The molecular formula of inotersen is $C_{230}H_{299}N_{69}O_{121}P_{19}S_{19}Na_{19}$, and the molecular weight is 7600.8 amu. The molecular target of inotersen is within the 3′ untranslated region of the human TTR mRNA and binds to the mRNA using Watson and Crick base pairing.

Inotersen was formulated in water-for-injection (200 mg/mL) and supplied as a sterile solution in a 1 or 1.5 mL deliverable volume in a sealed glass vial or as a single-dose prefilled syringe in a 1.5 mL deliverable volume and contains no preservatives.

Clinical studies

All studies were performed according to the amended Declaration of Helsinki, and all patients provided written informed consent.

All three clinical studies conducted with inotersen by Ionis Pharmaceuticals were included in this population PK and PK/PD analysis.

The first study was a Phase 1 double-blind, placebo-controlled, dose-escalation study designed to assess the safety, tolerability, PKs, and PDs of single and multiple doses of inotersen administered SC to healthy subjects. Four single-dose cohorts (50, 100, 200, and 400 mg) and five multiple-dose cohorts (50, 100, 200, 400, and 300 mg) were evaluated sequentially with 3:1 or 8:2 randomization to inotersen or placebo, respectively. Subjects enrolled in the multiple-dose cohorts received a total of six doses of Study Drug (inotersen or placebo) administered by SC injection: three doses on alternate days during the first week (days 1, 3, and 5) and then once a week for the next 3 weeks (days 8, 15, and 22). All doses were administered as single SC injections with the exception of the 400 mg doses, which were administered as two 200 mg SC injections. The study enrolled 65 healthy volunteers (16 in the single-dose cohorts and 49 in the multiple-dose cohorts). Of the 65 subjects, 51 were administered inotersen and 14 were administered placebo.

Plasma samples for measurement of inotersen concentrations were collected at predose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h following the SC injection on day 1 for the single-dose cohorts and on days 1 and 22 for the multiple-dose cohorts. Plasma trough samples were collected before dose on days 3, 5, 8, 15, and 22 in the multiple-dose cohorts. In addition, post-treatment plasma samples for determination of elimination half-life were collected during the post-treatment follow-up period, on days 4 and 8 in the single-dose cohorts and on days 29, 36, 50, 64, 78, and 92 in the multiple-dose cohorts. Serum samples for TTR analysis (PD) was collected at screening, predose, 24 h postdose, and on days 4 and 8 in the single-dose cohorts and at predose and 24 h postdose on days 1, 3, 5, 8, 15, and 22 and during post-treatment period on days 29, 36, 43, 50, 64, 78, and 92 in the multiple-dose cohorts.

The second study was a pivotal Phase 2/3 randomized, double-blind placebo-controlled study to assess the efficacy and safety of inotersen in patients with hATTR. The primary objective was to evaluate the efficacy of inotersen compared to placebo, given for 65 weeks, as measured by the change from baseline in the mNIS +7 and in the Norfolk QoL-DN questionnaire total score, in patients with hATTR. Patients were randomized in a 2:1 ratio to receive 300 mg inotersen or placebo. Study Drug (inotersen or placebo) was administered thrice on alternate days during week 1 (days 1, 3, and 5) and once weekly thereafter. A total of 172 subjects were dosed; 112 were administered inotersen and 60 received placebo. If required for safety or tolerability reasons, temporary dose reductions or interruptions were permitted. Plasma trough PK and PD samples were collected from all patients during the 65-week treatment phase and post-treatment follow-up period (if available). In addition, serial plasma samples were collected on days 1, 240, and 449 (weeks 1, 35, and 65, respectively) from a small number of subjects (a total of 10 subjects) at selected sites enrolled in the PK subgroup, for the assessment of inotersen PK in patients with hATTR.

The third study was an OLE study for eligible patients who had satisfactorily completed the pivotal Phase 2/3 as described above, and patients received 300 mg inotersen SC once weekly for up to 260 weeks (5 years) in the OLE study. Subjects who had a dose reduction or schedule change in the pivotal Phase 2/3 study were permitted to continue with the adjusted dose level or schedule in the OLE study. A total of 108 subjects had at least one evaluable PK sample and were included in the analysis. Plasma trough PK and PD samples were collected periodically from patients in the OLE study.

There were total of 202 subjects in the study dataset, with 74.8% ($N=151$) of the analysis population being patients with hATTR and 25.2% ($N=51$) being healthy subjects (Table 1). Most individuals were Caucasian (87.6%), with 7.43% African Americans and 3.47% Asians. There were more males (69.8%) than females in the analysis population. There was no use of nonsteroidal anti-inflammatory drugs (NSAID), antirheumatic products, or renin-angiotensin agent in the hATTR population; however, roughly a third of the hATTR population used diuretics (30.5%) or antithrombotic agents (36.4%), and 83.4% of the hATTR patients used non-NSAID analgesic agents. Healthy volunteers from the Phase 1 study were younger on average, had slightly higher baseline TTR levels, and had lower total bilirubin levels than hATTR patients in the pivotal Phase 2/3 study.

Analytical methods

Quantitation of inotersen concentrations in plasma. All plasma samples were analyzed using a quantitative, sensitive, hybridization-based enzyme-linked immunosorbent assay method, a variation on the method reported previously [7]. The assay was validated for precision, accuracy, selectivity, sensitivity, hook effect, metabolite cross-reactivity and interference, stability, and lack of antidrug antibody (ADA) interference in the quantitation of inotersen before analysis of the human plasma samples.

Plasma sample analyses were conducted at Covance Laboratories (West Trenton, NJ) 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 inotersen. The quantitation range was 1.00–200 ng/mL, with the lower and higher ends of the range defining the lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ), respectively. There were no active metabolites for inotersen present in plasma.

Quantitation of TTR in serum. Serum samples for TTR (aka prealbumin) were analyzed by South Bend Medical Foundation (South Bend, IN) using a standardized immunoturbidimetric assay on Roche automated clinical chemistry analyzers. Anti-prealbumin antibodies reacted with the antigen in the sample to form antigen/antibody complexes, which were determined turbidimetrically after agglutination.

TABLE 1. SUMMARY OF SUBJECT DEMOGRAPHICS AND BASELINE LABORATORY VALUES

Parameter	Mean (SD)	Minimum	Median	Maximum	Units	$N_{Subjects}$
Age	55.4 (13.8)	25.0	57.0	81.0	Years	202
Height	172 (9.43)	144	173	196	Cm	202
Ideal body weight	66.2 (10.0)	38.1	68.3	89.2	Kg	202
Lean body mass	51.7 (8.44)	31.3	51.6	80.3	Kg	202
Lean body weight	54.8 (10.0)	31.4	54.9	82.9	Kg	202
Weight	72.7 (16.2)	37.0	71.8	140	Kg	202
BMI	24.6 (4.45)	13.3	24.5	40.2	kg/m^2	202
BSA	1.85 (0.223)	1.30	1.83	2.61	m^2	202
TTR	22.0 (5.95)	3.80	22.1	39.7	mg/dL	202
Albumin	4,249 (314)	3,167	4,233	5,000	mg/dL	202
ALT	23.1 (10.8)	6.00	20.7	83.2	U/L	202
AST	25.5 (7.68)	12.3	24.0	60.0	U/L	202
Total bilirubin	0.586 (0.306)	0.0800	0.517	2.18	mg/dL	202
CrCL	100 (34.0)	35.2	97.9	220	mL/min	202
eGFR	93.2 (19.9)	41.3	93.9	148	$mL/min/1.73\,m^2$	202

$N_{Subjects}$ refers to the number of subjects over which the parameter statistics were computed and included five subjects who received treatment but had no PK observations.

PK, pharmacokinetic; BMI, body mass index; TTR, transthyretin; BSA, body surface area; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CrCL, creatinine clearance; eGFR, estimated glomerular filtration rate.

The quantitation range of the assay was from 3 to 80 mg/dL (ie, 30–800 ng/mL), with the lower and higher ends of the range defining the LLOQ and ULOQ, respectively.

Data analyses

A population model using nonlinear mixed-effects modeling software, NONMEM® (version 7.2; ICON Plc, Hanover, MD), was developed to quantitatively characterize the PKs and PDs of inotersen to evaluate effects of covariates on inotersen PK/PD and to determine the inter- and intrasubject variability. A sequential modeling approach was used where a population-based PK model was established first to describe the kinetics of inotersen and, subsequently, using the *post hoc* PK as input to develop the population-based PD model. The first order conditional estimation method with interaction was used throughout model development.

Model features (eg, lag times, compartments, covariances, and so on) were added or removed based on evaluation of diagnostic plots, consideration of biological plausibility, and statistical tests. For hierarchical (or nested) models, the significance of adding or removing a parameter was assessed using the likelihood ratio test. For these models, the difference in the objective function value (ΔOFV) is $\sim \chi^2$ distributed with n degrees of freedom. As such, considering a significance level (α) of 0.05, the proposed change to the model was considered to improve the model fit only if ΔOFV is at least 3.84 when $n = 1$ and 5.99 for $n = 2$. In contrast, for non-nested models, the Akaike Information Criterion (AIC) and/or the Bayesian Information Criterion (BIC) were used to compare models following the principle that the lower the AIC and/or BIC, the better the model.

Population PK modeling

The population PK analysis included pooled data from three clinical studies in healthy volunteers and patients with hATTR-PN, as described above. The analysis dataset consisted of 202 subjects (51 healthy volunteers and 151 patients with hATTR-PN), among which 5 patients with hATTR-PN did not have PK data yet at the time of analysis. Therefore, available PK data from 197 subjects (51 healthy volunteers and 146 patients with hATTR-PN) with a total of 3,602 observations were used for the population PK analysis, to estimate the population PK parameters and the associated intersubject variability and residual error, to identify significant covariates, and to estimate their impact on exposure.

After visual inspection of the PK data following SC administration, plasma inotersen concentrations declined in a biphasic manner with time (Supplementary Fig. S1). In addition, one, two, and three-compartment models were fit to the naive pooling of all available data, and the two-compartment model was confirmed as the most appropriate base model structure for inotersen population PK analysis. The model was parameterized for absorption rate constant (K_a), clearance (CL), central volume of distribution (V_c), intercompartmental clearance (Q), and peripheral volume of distribution (V_p) (Fig. 1) and included interindividual variability (IIV) on CL, V_c, and V_p, with additive residual error model on log transformed data.

The dose nonlinearity on clearance was observed in the Phase 1 data [5]. Thus, the dose nonlinearity was modeled by increasing the central clearance as the dose decreased using a
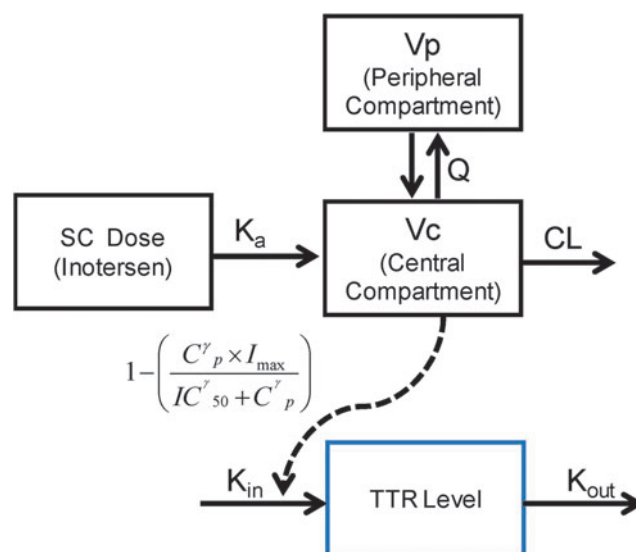

FIG. 1. Base PK and PK-PD model schematic. PK, pharmacokinetic; PD, pharmacodynamic.

simplified exponential model with dose administered being normalized to 300 mg dose.

Following the identification of the base model, population PK covariate relationships were systematically examined using the Gastonguay full model approach [8], with backward elimination of candidate covariates. The covariates assessed were body size [body weight, lean body mass (LBM), body surface area, and body mass index], age effects (>65 years old), sex, race, baseline estimated glomerular filtration rate (eGFR), baseline albumin, baseline bilirubin normalized by upper limit of normal (ULN), baseline alanine aminotransferase (ALT), baseline TTR, TTR V30M mutation, disease state, frequently used concomitant medications (≥20% of population), including diuretics, antithrombotic, and non-NSAID analgesics, and region of the world from which the participants originated from.

ADAs were detected following chronic treatment with inotersen and were characterized by a late onset (median onset >6 months) and low antibody titers (median peak titer ≤800) in the pivotal Phase 2/3 and OLE studies (internal data). The presence of ADA affected plasma trough concentrations of inotersen (C_{trough}) to various degrees; however, the presence of ADA did not change other PK parameters (including $C_{\max}$, area under the curve [AUC], $t_{1/2\lambda z}$, and CL_{ss}/F). In addition, the presence of ADA did not have any effect on PD (serum TTR levels), clinical primary efficacy end points (mNIS +7 and Norfolk QoL-DN Score), nor incidence or seriousness of all safety measures evaluated. These findings suggest that anti-inotersen antibodies are binding antibodies but not neutralizing antibodies and did not affect pharmacological response, clinical benefit, or safety of inotersen. Because of these reasons, the PK data collected post the onset of ADA were excluded from the population PK model, in an effort to develop a population PK model and characterize potential effects of various covariates without confounding effects from ADA.

Candidate models were compared with state-of-the-art diagnostics, the selected final PK model was qualified with visual predictive checks, and the precision of the final model

parameters was estimated using covariance and nonparametric bootstrap analyses.

Population PD modeling

The population PD model of inotersen was developed with the aim to understand the changes in TTR response following the administration of inotersen in patients with hATTR. Based on the mechanism of action of inotersen as described previously, the counter-clockwise hysteresis loop of the time-ordered TTR level versus inotersen plasma trough concentrations and visual inspection of the TTR over time data following SC administration (Supplementary Fig. S2), an indirect response model with an inhibition function on the input rate was used as the base PD model to describe the effect of inotersen treatment on serum TTR levels:

$$\frac{dTTR}{dt} = k_{in}\left(1 - \frac{I_{max}C_p}{IC_{50} + C_p}\right) - k_{out}TTR$$

Where k_{in} and k_{out} represent the zero-order synthesis rate and first order turnover of TTR, respectively. I_{max} and IC_{50} describe the maximum inhibition of TTR and plasma concentration of inotersen corresponding to 50% of maximum drug effect, respectively.

The population PD model was developed following an iterative model building process using a nonlinear mixed effects approach. Modeling was performed on log-transformed TTR data, which were proved to be more stable and led to more consistent model convergence. Individual PK parameters obtained from the final population PK model were used to generate individual drug input for the PK/PD model. Covariates evaluated for the PD model included disease status (healthy volunteers vs. hATTR patients), baseline TTR levels, and TTR mutation (V30M and non-V30M). Final PD model was qualified with visual predictive checks, and the precision of the final model parameters was estimated using covariance and nonparametric bootstrap analyses.

Simulations

Simulations were conducted based on the final population PK and PD model to simulate concentration-time profiles of inotersen and TTR, as well as steady-state exposure PK parameters in patients with hATTR for the following dosing regimens:

- 300 mg QW for 65 weeks
- 300 mg once every other week (QOW) for 65 weeks
- 150 mg QW for 65 weeks
- 300 mg with loading dose on days 1, 3, and 5 during the first week, followed by QW for an additional 64 weeks

A total of 500 replicated datasets were produced for each simulation, with parameter uncertainty included using the variance–covariance matrix from the final model as the prior distribution. In each replicated dataset, the 151 patients from the original dataset were used along with their demographic variables, thus total of 75,500 patients were simulated. Steady-state exposure measures (AUC$_{0-\tau}$, C_{max}, C_{trough}) from these simulations were summarized by geometric mean [90% confidence interval (CI)] following the first dose on day 1 and the last dose after 65 weeks of dosing. The predicted TTR levels from these simulations were plotted as median (90% prediction interval [PI]).

Results

Population PK model

Following the identification of the base model as a two-compartment linear model with first order absorption following SC administration and first order elimination from the central compartment, dose nonlinearity term was added, which resulted in a decrease in the objective function of 57.12 points, as well as a reduction of IIV in CL/*F*, V_c/*F*, and V_p/*F*. The final population PK model suggests that the apparent dose nonlinearity is driven by a higher plasma clearance (primarily distributional clearance) at the lowest dose level of 50 mg. This apparent nonlinearity, however, will not be clinically relevant because the projected clinical dose levels are in the range of 150–300 mg.

Then, a full covariate approach followed by stepwise backward elimination was undertaken, incorporating remaining predetermined covariates, and the full covariate model was then subject to backward elimination process to exclude statistically and clinically insignificant covariates. The effect of four different measures of body size (body weight, LBM, body surface area, and body mass index) on the clearance and central volume of distribution was evaluated individually. LBM was chosen as the measure of body size based on the largest drop in the OFV. The model included interindividual variability on clearance, central volume, and peripheral volume and with additive residual error model on log transformed data. As shown in Figs. 2 and 3, only LBM and disease state showed clinical significance and remained in the model, where LBM remained as covariate on the central and intercompartmental clearances and the central and peripheral volumes and disease status on the central compartment clearance and central volume.

Bayesian *post hoc* estimates of exposures showed that the difference in the steady-state AUC$_\tau$ or C_{max} was small between LBM quartile categories. For example, the geometric mean ratio for steady-state AUC$_\tau$ and C_{max} was 1.38 and 1.34, respectively, between the first quartile (Q1) of LBM (LBM in the range of 31.1–45.7 kg) and the fourth quartile (Q4) (LBM in the range of 56.0–80.3 kg). The difference in geometric mean of C_{trough} level between Q1 and Q4 was even less, ~10%. Similarly, small differences (<30%) in the exposure are observed based on body weight quartile categories (Q1 in the range of 37.0–58.2 kg vs. Q4 in the range of 80.8–140 kg). Thus, overall the effect of body weight or LBM on inotersen exposure was considered to be small and may not be clinically relevant.

There were differences in some of the baseline clinical measures across disease groups, for example, hATTR patients had higher bilirubin levels and slightly lower baseline TTR levels; however, there was a larger range of baseline TTR levels in the hATTR population. The hATTR patients, as describe earlier, were in general an older population that had lower creatinine clearance levels compared to healthy volunteers. Given these correlations between disease status and other variables, disease status was selected preferentially as the covariate of interest if two covariates appeared to be correlated in the final model.
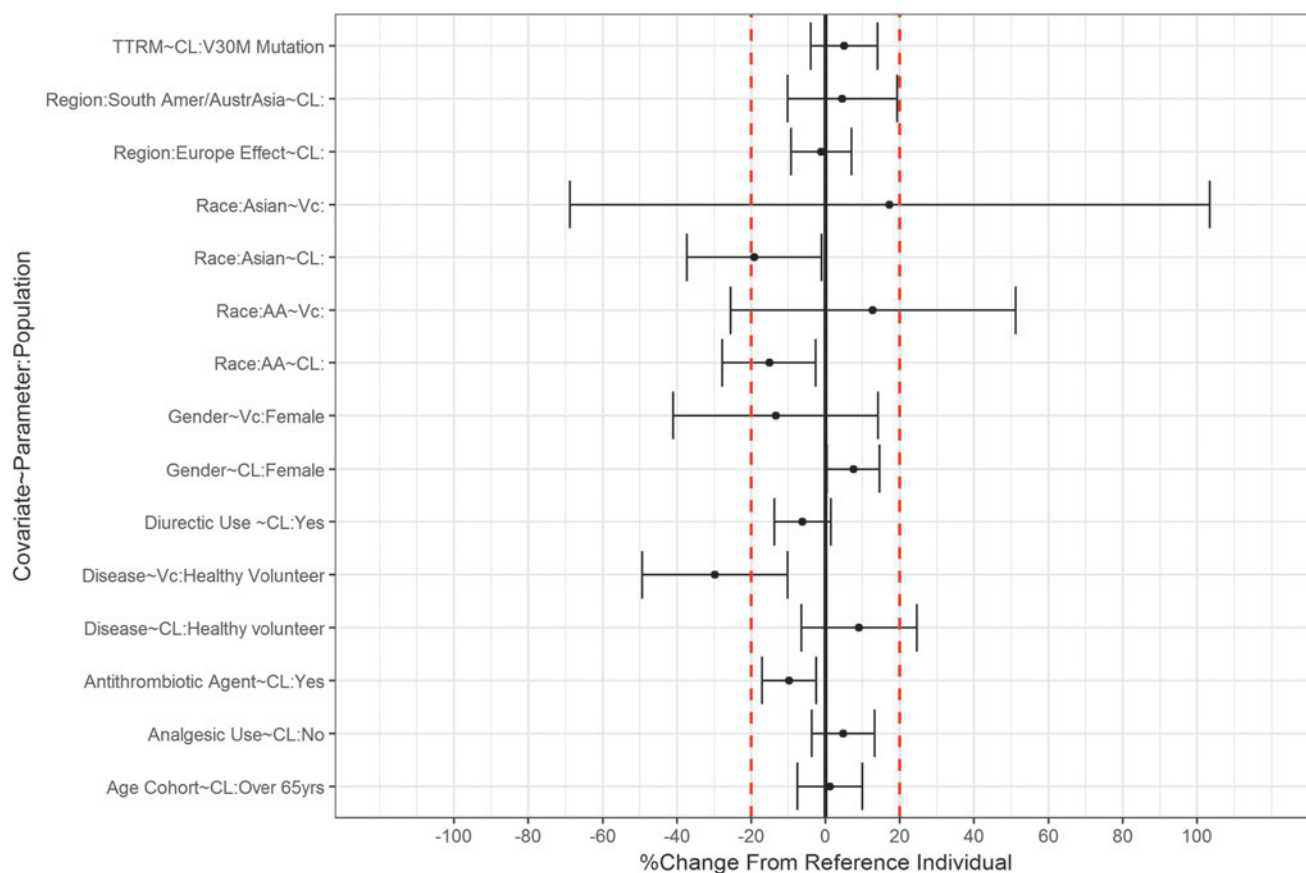
FIG. 2. Clinical significance testing for categorical covariates in full model: distribution of percentage change from reference individual. *Vertical red dashed lines*, boundaries representing the 20% clinical significant test; *closed circle*, median percentage change from the reference category; Whiskers, 5th and 95th CI of the percentage change from the reference category. Cl, apparent clearance, CL/F; Vc, apparent central volume, Vc/F; Amer for America, AA for African American.

Other statistically and clinically insignificant covariates were excluded from the model through backward elimination process, which included age effects (>65 years old), sex, race, baseline eGFR, baseline albumin, baseline bilirubin normalized by ULN, baseline ALT, baseline TTR, TTR V30M mutation, concomitant use of diuretics, antithrombotic, and non-NSAID analgesics, and region of the world. It was noted that the apparent differences in observations due to race should be interpreted with caution; as 87.6% of the subjects were Caucasians, these apparent differences could be the result of small sample size.

The final model was qualified using visual predictive checks and nonparametric bootstrap analysis. As indicated by the goodness-of-fit, residual diagnostic plots and prediction corrected visual predictive checks, the model provided an adequate characterization of the observed inotersen plasma concentration-time course (Supplementary Figs. S3 and S4). The structural model parameters were generally estimated with good precision (% RSE <30%), except for the covariate effect of disease status on CL/F and V_c/F. The final model parameter estimates are in good agreement with bootstrapping results and well within the 95% CIs of parameter estimates for each parameter (Table 2). None of the random effects had shrinkage values >30% or had the center of their distributions statistically different from zero.

Based on final estimated population PK parameters, the calculated alpha and beta (terminal) half-lives are 3.91 hs and 26.9 days, respectively, consistent with the PK property of inotersen being biphasic profile with an initial rapid disposition phase followed by a slower elimination phase with apparent terminal elimination half-life of ∼4 weeks. The calculated accumulation ratio based on beta and dosing interval is 6.07, in agreement with the simulation results (Table 4).

Population PD modeling

As described earlier, there was a slight difference in baseline TTR levels between healthy volunteers and hATTR patients; therefore, disease effect on baseline was added as a covariate to the estimated baseline. V30M mutation showed no effect on the estimated IC_{50} value for hATTR patients.

The robustness and precision of the final model parameter estimates were confirmed by the 95% CIs from the bootstrap results (Table 3). The overall population I_{max} and IC_{50}, together with 95% CI, was estimated to be 0.913 (0.899–0.925) and 9.07 (8.08–10.1) ng/mL, respectively. The final model was qualified using visual predictive checks and nonparametric bootstrap analysis. As indicated by the goodness-of-fit, residual diagnostic plots and prediction corrected visual predictive
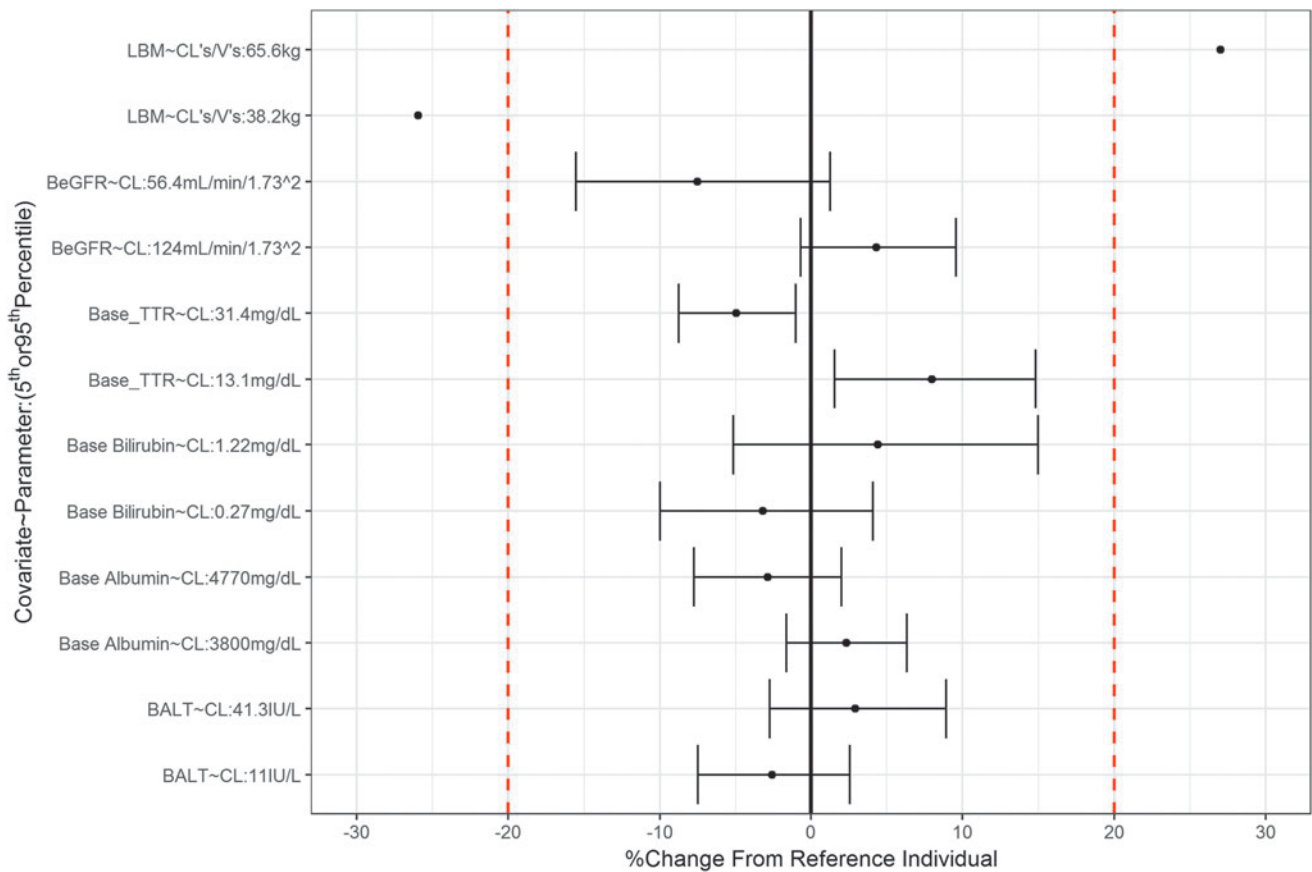
FIG. 3. Clinical significance testing for continuous covariates in full model: distribution of 5th and 95th confidence intervals of percentage change from reference individual. *Y Axis Labels*, values of the 5th and the 95th percentile of the continuous covariate being tested are displayed as separate categories on the X-axis; *Vertical Red Dashed Lines*, boundaries representing the 20% cinicalsignificanfe test; Closed Circle, median percentage change from the reference category *Whisker*. 5th and 95th percentile of the percentage change from the reference category. B or Base refter to baseline. Cl, apparent clearance, CL/F and Apparent inter-compartmental clearence Q/F; Vc, apparent central volume, Vc/F; Vp, periphereal volumes Vp/F.

TABLE 2. PARAMETER ESTIMATES FOR FINAL POPULATION PHARMACOKINETIC MODEL

Parameter	Covariate model	Estimate	% RSE	95% CI
Clearance, CL (L/h)		3.4	5.23	3.05–3.75
Central volume, V_c (L)		20.7	7.87	17.5–23.9
Absorption rate constant, K_a (1/h)		0.261	7.73	0.222–0.301
Peripheral volume, V_p (L)		230	13.8	167–292
Intercompartmental clearance, Q (L/h)		0.266	9.61	0.216–0.315
Dose effect ~ CL	Exponential	3.97	25.2	2.01–5.93
Lean body mass ~ CL/Q	Power-centered on median	1 FIXED	NA	NA
Lean body mass ~ V_c/V_p	Power-centered on median	1 FIXED	NA	NA
Disease ~ CL	Proportional	0.111	46.9	0.009–0.214
Disease ~ V_c	Proportional	−0.284	31.4	−0.459 to −0.109
Covariance, V_c ~ CL		0.049	48.4	0.003–0.096
Covariance, CL ~ V_p		−0.145	27.1	−0.222 to −0.068
$\omega^2 V_c$		0.335	27	0.158–0.512
ω^2 CL		0.071	21.3	0.041–0.101
$\omega^2 V_p$		0.677	21	0.399–0.955
Residual variability, σ^2	Log additive error	0.168	5.29	0.151–0.186

Base model did not have any covariates; reduced and final models had covariates removed as result of analysis. Covariances of parameter displayed as "ω^2 parameter."

% RSE, relative standard error (%) calculated as standard error divided by parameter estimate; CI, confidence interval; NA, not applicable; AUC, area under the curve.

TABLE 3. FINAL POPULATION PHARMACOKINETIC/PHARMACODYNAMIC MODEL PARAMETER ESTIMATES

Parameter	Units	Final model estimates	95% CI
I_{max}		0.913	(0.899–0.925)
IC_{50}	ng/mL	9.07	(8.08–10.1)
Estimated baseline	mg/dL	20.4	(19.7–21.1)
∼Disease effect on baseline (healthy volunteers)		0.269	(0.178–0.360)
k_{out}	h^{-1}	0.00308	(0.00289–0.00327)
ω^2 IC50		0.953	(0.702 − 1.20)
Covariance, IC50 ∼ k_{out}		−0.182	(−0.297 to −0.0669)
ω^2 k_{out}		0.288	(0.187 − 0.389)
ω^2 Baseline		0.0537	(0.0404 − 0.0670)
Residual variability (log space), (SD)			
Proportional error		0.13	(0.117 − 0.143)
Additive error		1.35	(1.26 − 1.45)

Disease effect covariate was the proportional shift in baseline TTR levels of healthy volunteers compared to hATTR patients. The 95% CI was determined from the successful covariance step of the PD model.

PD, pharmacodynamic; hATTR, hereditary transthyretin amyloidosis.

check plots (1,000 simulations), the model provided an adequate characterization of the observed TTR concentration–time course (Supplementary Figs. S5 and S6). The 5th, 50th, and 95th percentiles of the simulations were consistent with the observed data. In addition, the observed data (blue circles) were well within 90% of the prediction interval. Although the prediction variability was larger than the observed data, the predicted median values were mainly used when comparing various simulated dosing regimens. Therefore, these support the adequacy of the model and its suitability to investigate alternative dosing regimens using simulation.

Simulations

The final population PK and PD model was used to simulate four different dosing regimens of inotersen, from which the geometric mean along with 90% CI was calculated for $AUC_{0-\tau}$, C_{max}, and C_{trough} for day 1 and steady state (Table 4). Plots of the median plasma trough concentration and TTR profiles over time along with 90% prediction interval were created for each dosing regimen (Figs. 4 and 5). PK simulations showed that there was little accumulation in $AUC_{0-\tau}$ and C_{max} for all dosing regimens. There was also little difference in $AUC_{0-\tau}$ between the different 300-mg regimens, while the 150-mg

regimen produced $AUC_{0-\tau}$ approximately half of the 300-mg regimens. A similar trend was seen with C_{max}.

The use of 300 mg loading doses achieved trough levels of ∼65.1% and 85.6% of the steady-state level after 1 and 3 months of 300 mg QW treatment, compared with 47.4% and 81.5%, respectively, without the use of loading doses. At steady state, the C_{trough} levels following 300 mg QOW and 150 mg QW treatment regimen were 14.8 (13.3–16.8) ng/mL and 17.2 (15.5–19.1) ng/mL, respectively, approximately one half of the C_{trough} level [34.4 (31.0–38.2) ng/mL] following 300 mg QW treatment regimen. Loading-dose regimen did not alter steady-state trough levels, but reduced time to reach approximately two-thirds of the steady-state C_{trough} (∼23 ng/mL) level from 2 months to 1 month (Fig. 4).

Similarly, the use of 300 mg loading doses followed by 300 mg QW achieved mean percent change from baseline TTR levels of approximately −45.1%, −57.5%, and −69.0% at 2 weeks, 1 month, and 3 months, respectively, compared to approximately −34.8%, −51.2%, and −67.9% with no loading doses (Fig. 5; Table 5). A dosing regimen of 150 mg QW resulted in mean percent change from baseline TTR levels of approximately −28.1%, −42.6%, and −58.6% at 2 weeks, 1 month, and 3 months, respectively. For 300 mg QOW, the values were −24.7%, −38.0%, and −53.8%, respectively.

TABLE 4. SIMULATED MEASURES OF EXPOSURE FOR ADDITIONAL DOSING REGIMENS

Regimen	Day/type	$AUC_{0-\tau}$ (μg · h/mL)	C_{max} (μg/mL)	C_{trough} (ng/mL)
300 mg QW	1	84.4 (77.6–91.6)	6.35 (5.61–7.17)	5.62 (4.92–6.24)
300 mg QOW	1	85.5 (78.4–92.5)	6.33 (5.66–7.18)	4.28 (3.82–4.74)
Loading + 300 mg QW	1	85.5 (78.5–92.8)	6.36 (5.76–7.26)	25.7[a] (20.9–31.8)
150 mg QW	1	42.3 (38.8–45.9)	3.16 (2.81–3.57)	2.81 (2.45–3.11)
300 mg QW	449	89.9 (82.4–97.4)	6.39 (5.65–7.20)	34.3 (31.0–38.2)
300 mg QOW	449	90.1 (82.6–97.5)	6.34 (5.67–7.20)	14.8 (13.3–16.8)
Loading + 300 mg QW	449	90.0 (82.8–97.8)	6.34 (5.72–7.24)	34.3 (30.6–38.3)
150 mg QW	449	45.0 (41.4–48.8)	3.18 (2.83–3.59)	17.2 (15.5–19.1)
300 mg QW	AR	1.06 (1.06–1.07)	1.01 (1.00–1.01)	6.11 (5.26–7.30)
300 mg QOW	AR	1.05 (1.05–1.06)	1.00 (1.00–1.00)	3.45 (2.99–4.08)
Loading + 300 mg QW	AR	1.05 (1.05–1.06)	0.996 (0.991–0.999)	NA
150 mg QW	AR	1.06 (1.06–1.07)	1.01 (1.00–1.01)	6.14 (5.27–7.26)

Data presented are geometric mean (90% CI).

[a]C_{trough} after the dose on day 1 is 48 h time point during loading dose period.

AUC, area under cureve; QW, once weekly; QOW, once every other week; AR, accumulation ratio.
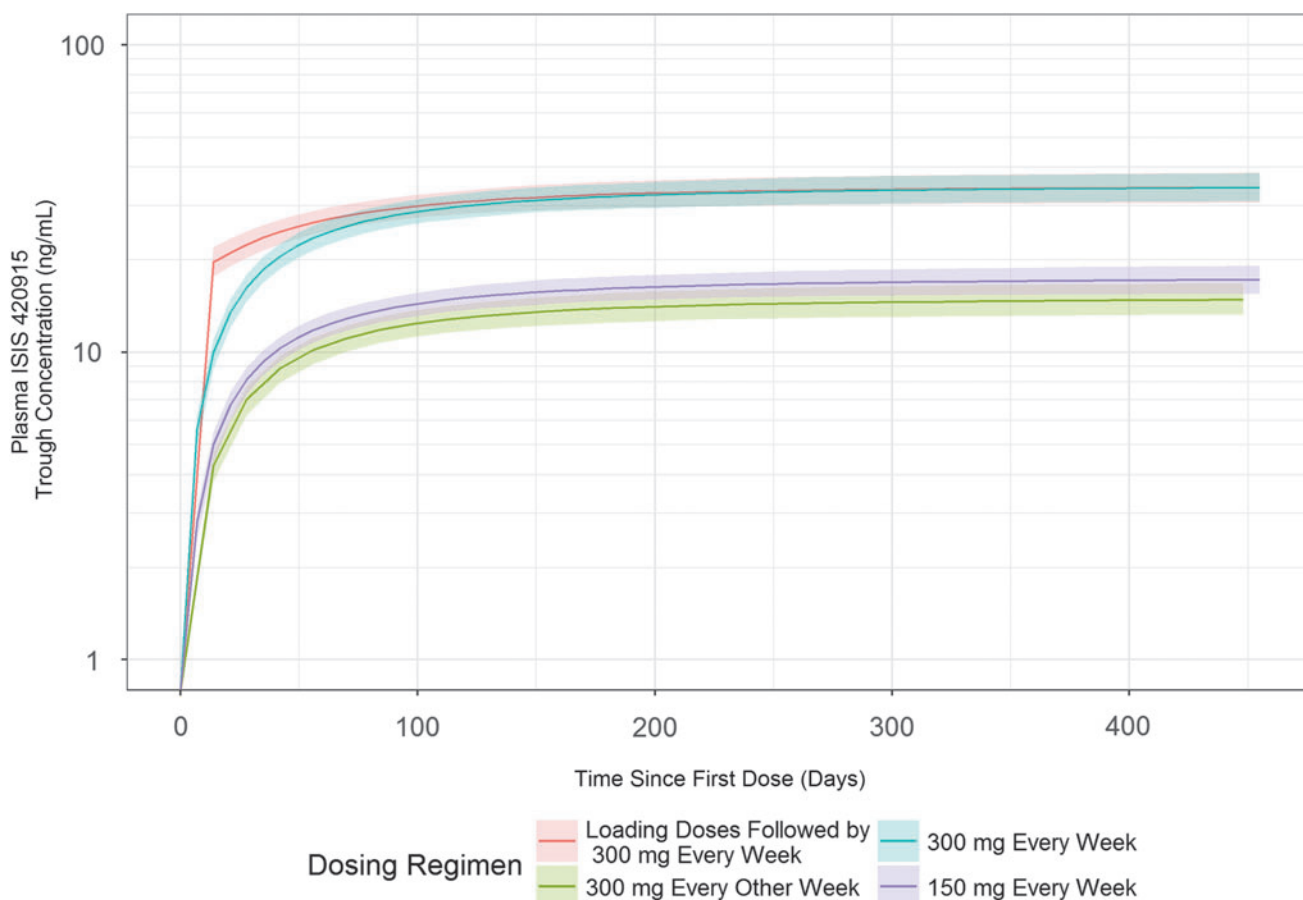
FIG. 4. Simulated plasma trough concentration profile for ISIS 420915 for various dosing regimens.

Overall, 300 mg loading doses followed by 300 mg QW would result in a slightly faster onset of TTR reduction than without loading doses, and the same predicted geometric mean reduction of approximately −71.9% at steady state.

Discussion

We have developed and reported the first population PK and PK/PD model for an ASO therapeutic, inotersen. Inotersen PK is well described by a two-compartment linear PK model, and its pharmacological effect on TTR is well described using an inhibitory effect indirect response PD model, where TTR formation is modulated by inotersen exposure.

Out of the demographic parameters evaluated, including age, gender, body weight, and race, only body weight or body size was shown to be a significant covariate on inotersen clearance and volume of distribution. However, its overall impact on PK exposure (C_{max}, AUC, or C_{trough}) appeared to be small (<30%) by body weight and 38% by LBM. Nonetheless, the clinical relevance of body weight on plasma exposure, selected safety (platelet), and PD (TTR reduction) measures was further evaluated by graphical analysis. Results showed a weak association between platelet reduction and inotersen plasma exposures (C_{max} and AUC), but less with C_{trough} (Supplementary Fig. S7) among patients at the same dose level. Similarly, a weak association was observed between body weight and platelet reduction, but not with TTR reduction (internal data). These results suggest that dose

reduction, if warranted, would be helpful in reducing the risk of platelet reduction without comprising the PD activity.

The clearance and central volume of inotersen were not affected by baseline TTR level or TTR mutation. However, disease status was retained as a covariate on central compartment clearance and central volume. Nonetheless, the contribution of the disease status on PK was small. For example, the population typical value for central clearance (CL/F) was 3.40 and 3.18 L/h in patients with hATTR-PN, based on *post hoc* analysis, while the apparent volume of distribution (V_{ss}/F) was estimated to be 250.7 L as the typical value and 293 L in patients with hATTR.

Even though the PK of inotersen is not affected by hepatic function, no conclusions can be drawn for patients with hepatic impairment due to the absence of such population in the clinical program. The population PK analysis showed that renal function, as represented by baseline eGFR, is not a statistically significant covariate for inotersen clearance, in the range of 41.3–127 mL/min/1.73 m². Thus, no dose adjustment is necessary in patients with mild-to-moderate renal impairment. Given the lack of experience in patients with low moderate and severe renal impairment for inotersen, no conclusions on the safety in such populations can be drawn, even though the PK of inotersen is expected to be similar across a range renal function impairment based on almost exclusive clearance of inotersen through ubiquitous nucleases in tissues.

Commonly used concomitant medications in the Phase 2/3 study included diuretics (30.5%), antithrombotic (36.4%),
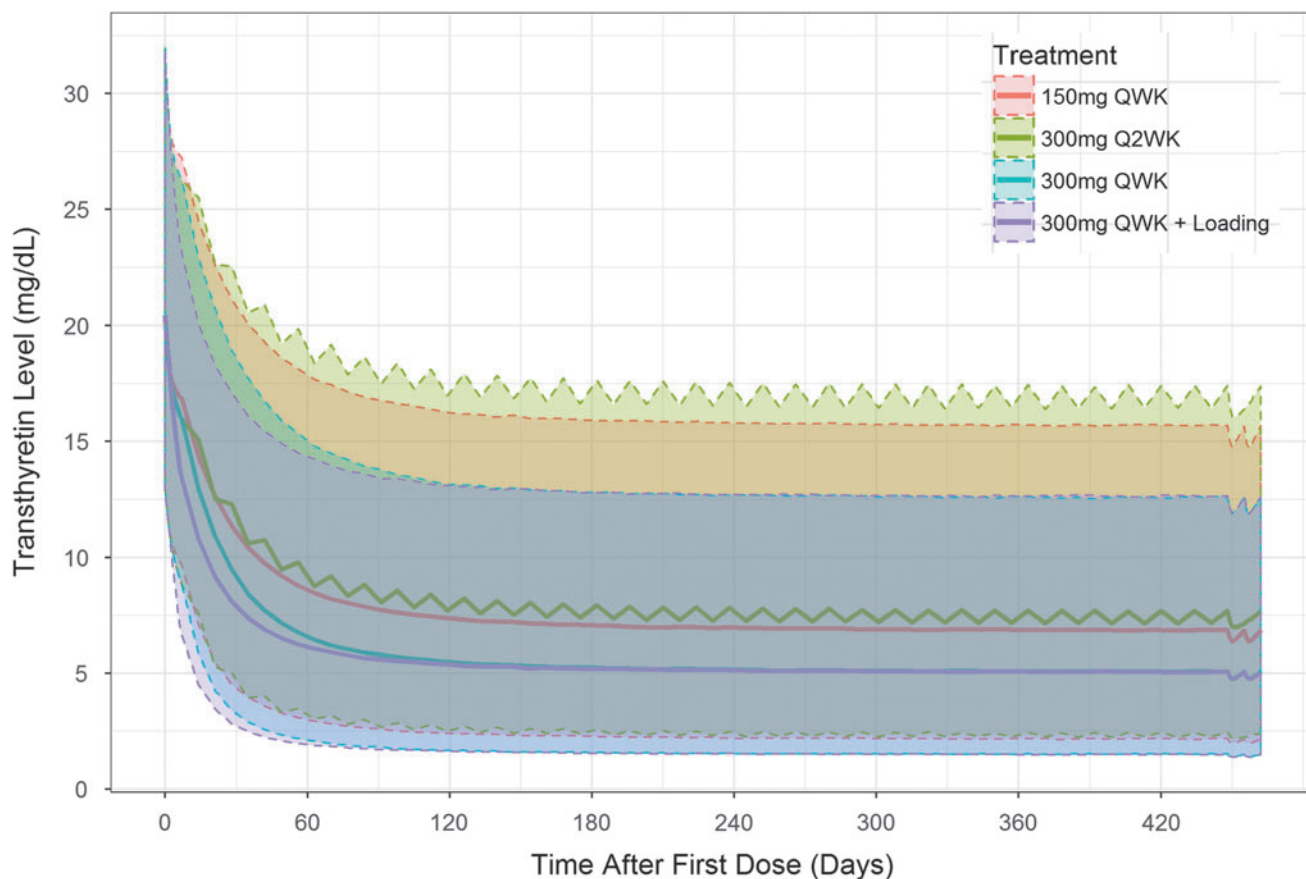
FIG. 5. Predicted transthyretin levels for various simulated dosing regimens. *Solid lines* represent medians, and *shaded regions* represent 90% PI.

TABLE 5. SIMULATED TRANSTHYRETIN LEVELS OVER TIME FOR VARIOUS DOSING REGIMENS

Dose	Day	TTR level (mg/dL)	TTR % change from baseline (%)
300 mg QW	Baseline	20.4 (19.7–21.2)	NA
	Day 7	15.7 (15.0–16.4)	−20.2 (−23.0 to −17.5)
	Day 14	12.4 (11.8–13.1)	−34.8 (−37.9 to −32.0)
	Day 28	8.91 (8.33–9.53)	−51.2 (−54.1 to −48.4)
	Day 85	5.56 (5.12–6.02)	−67.9 (−70.2 to −65.5)
	Day (steady state)	4.82 (4.41–5.22)	−71.9 (−74.0 to −69.7)
300 mg QW with loading dose	Baseline	20.4 (19.7–21.1)	NA
	Day 7	13.0 (12.4–13.7)	−32.5 (−35.3 to −29.2)
	Day 14	10.3 (9.72–11.0)	−45.1 (−47.8 to −42.3)
	Day 28	7.67 (7.10–8.26)	−57.5 (−60.4 to −54.8)
	Day 85	7.39 (6.84–7.96)	−69.0 (−71.1 to −66.8)
	Day (steady state)	4.78 (4.39–5.22)	−71.9 (−74.1 to −69.8)
300 mg Q2W	Baseline	20.4 (19.7–21.2)	NA
	Day 7	15.7 (15.0–16.4)	−20.2 (−23.0 to −17.5)
	Day 14	14.6 (13.9–15.3)	−24.7 (−27.8 to −21.8)
	Day 28	11.7 (11.0–12.6)	−38.0 (−41.1 to −35.0)
	Day 85	8.29 (7.70–8.92)	−53.8 (−56.7 to −50.9)
	Day (steady state)	7.24 (6.67–7.82)	−58.8 (−61.5 to −56.1)
150 mg QW	Baseline	20.4 (19.7–21.2)	NA
	Day 7	16.6 (15.9–17.4)	−15.9 (−18.6 to −13.3)
	Day 14	13.9 (13.3–14.6)	−28.1 (−31.5 to −25.3)
	Day 28	10.8 (10.1–11.4)	−42.6 (−45.6 to −39.6)
	Day 85	7.39 (6.84–7.96)	−58.6 (−61.2 to −56.0)
	Day (steady state)	6.47 (5.95–6.98)	−63.1 (−65.7 to −60.5)

and non-NSAID analgesics (83.4%) in patients with hATTR. None of these concomitant medications showed any clinically significant effect on clearance of inotersen and was not included in the final population PK model. Therefore, dose adjustment for inotersen in consideration of PK interaction when used concomitantly with these agents is not necessary, consistent with different metabolism pathways for inotersen (ubiquitous nuclease mediated metabolism) and small molecule drugs (mainly metabolized by cytochromes P450 [CYPs]).

There was a slight difference between TTR baseline levels for healthy volunteers and patients with hATTR; therefore, disease effect on TTR baseline was added as a covariate to the estimated baseline in the population PK/PD model. There was little difference in the estimated IC_{50} for patients with (8.17 ng/mL) and without (9.19 ng/mL) V30M mutation. The model determined k_{out} was 0.00308/h, corresponding to a half-life of ~ 9 days, which is much longer than the ~ 2-day half-life of plasma TTR (aka, prealbumin) as reported in the literature (turnover rate of 0.296/day) [9]. This discrepancy may be explained by the PD profiles being driven by inotersen PK, which is the rate-limiting step.

The change from baseline for mNIS +7 score on week 66 (day 449)plotted against day 85 TTR levels showed clear distinction between placebo and inotersen groups, and the majority of patients on inotersen had >50% reduction in TTR levels (Supplementary Fig. S8a). Similarly, the change from baseline for Norfolk QoL-DN score on week 66 plotted against day 85 TTR levels showed similar results as noted for mNIS +7 (Supplementary Fig. S8b). These results suggest that a TTR reduction of approximately >50% is associated with clinical benefit.

TTR reduction is dose- and exposure dependent. PK/PD simulations showed that 300 mg weekly dosing would produce the best numeric TTR reduction; however, the range of TTR reduction is largely overlapping among the three dosing regimens simulated, that is, 300 mg weekly, 300 mg biweekly, and 150 mg weekly, suggesting that the two lower doses would be effective in TTR reduction for the majority of patients. Loading dose shortened the time to steady state, but showed little effect on TTR reduction at 3 months and beyond. Therefore, loading dose may not be necessary for chronic treatment with inotersen.

In summary, the population PK and PK/PD models developed in this study may be useful for dose optimization in patients receiving inotersen and for development of an inotersen follow-on in the same patient population.

Author Disclosure Statement

R.Z.Y., S.H., R.G., B.M., S.H., and Y.W. are employees of Ionis Pharmaceuticals, Inc. and hold stock in the company. E.J.A. is an employee of Avidity Biosciences, San Diego, CA and was a former employee of Ionis Pharmaceuticals and holds stock in the company. J.W.C. is currently an employee of GlaxoSmithKline and was employed with Parexel at the time of work.

Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Figure S4
Supplementary Figure S5
Supplementary Figure S6
Supplementary Figure S7
Supplementary Figure S8

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