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APPLICATION NUMBER:

211172Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology Review

NDA Number	211172
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Submission Date	Nov 6, 2017
Submission Type	505 (b)(1), Priority Review
Brand Name	TEGSEDI™
Generic Name	Inotersen
Dosage Form and Strength	Sterile, (b) (4) injection, 284 mg/1.5 mL (300 mg inotersen sodium/1.5 mL) in single use prefilled syringe
Route of Administration	Subcutaneous injection
Proposed Dose/Regimen	300 mg once weekly (b) (4)
Proposed Indication	Treatment of hereditary transthyretin amyloidosis with polyneuropathy (hATTR-PN)
Applicant	Ionis Pharmaceuticals, Inc.
Associated IND	113968
OCP Review Team	Mariam Ahmed, Atul Bhattaram, Theingi Thway, Hobart Rogers, Christian Grimstein, Kevin Krudys, and Sreedharan Sabarinath
OCP Final Signatory	Mehul Mehta

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1. EXECUTIVE SUMMARY

Hereditary transthyretin amyloidosis with polyneuropathy (hATTR-PN) is a rare and fatal disease caused by mutations in the gene that codes for transthyretin (TTR) protein. Mutant TTR protein is thermodynamically less stable and tends to dissociate into monomers, which can then misfold and subsequently aggregate into amyloid deposits. This causes cell degeneration and death. Inotersen is a 2'-O-(2-methoxyethyl) [2'-MOE] chimeric phosphorothioate antisense oligonucleotide inhibitor of both mutant and wild type TTR protein production.

The applicant, Ionis Pharmaceuticals, Inc., is seeking approval for inotersen (TEGSEDI™) for the treatment of patients with hATTR-PN. TEGSEDI™ will be available as a single-use prefilled syringe (PFS) containing 300 mg/1.5 mL inotersen sodium for subcutaneous (SC) injection.

The applicant is relying on a pivotal, double-blind, placebo-controlled, Phase 2/3 clinical study (Study CS2) of 65 weeks duration in patients with hATTR-PN as the basis for approval. This study evaluated the efficacy and safety of only one dose level of 300 mg (inotersen sodium salt) once weekly, with three 300 mg loading doses in the first week. As per the applicant's analyses, the changes from baseline in the co-primary endpoints (Modified Neuropathy Impairment Score +7 composite score (mNIS+7) and Norfolk Quality of Life-Diabetic Neuropathy total score (Norfolk QoL-DN)) demonstrated statistically significant benefit in favor of inotersen treatment at Week 65 ($p<0.001$). The benefit was evident as early as Week 35 ($p<0.05$). Please refer to the review by Dr. Massie Tristan, Division of Biometrics I, Office of Biostatistics for additional details.

At the evaluated dose level of 300 mg, inotersen was associated with >30% reduction in platelet count from baseline in about 70% of subjects as compared to 5% of subjects in the placebo group. About 23 % patients on inotersen had a platelet count $<100x10^9$/L. Severe (Grade 4) thrombocytopenia occurred in three inotersen-treated subjects as compared to none in placebo. Therefore, the applicant is proposing a Risk Evaluation and Mitigation Strategy (REMS) with Elements to Assure Safe Use (ETASU) to be implemented to ensure that the benefit of TEGSEDI™ outweighs the risk. Please refer to the safety review by Dr. Evelyn Mentari.

The primary objectives of this review are:

1) to evaluate the appropriateness of the proposed dose (b) (4)
2) to assess the effect of immunogenicity on inotersen pharmacokinetics, pharmacodynamics, efficacy, and safety
3) to evaluate the adequacy of labeling statements based on population pharmacokinetic analyses

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed the information contained in this NDA and recommends approval from a clinical pharmacology perspective. The review focus with specific recommendations and comments are summarized below.

Review Summary	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Primary evidence of effectiveness was established from a single pivotal Phase 2/3 placebo-controlled study in patients with hATTR-PN.
General dosing instructions	The recommended dosing regimen is 300 mg of inotersen sodium injected subcutaneously once weekly by a single-dose, prefilled syringe. (b) (4) This dosing regimen is appropriate given the implementation of the appropriate risk mitigation strategy for thrombocytopenia.
Dosing in patient subgroups (intrinsic and extrinsic factors)	No dose adjustments are required. Hepatic/renal impairment is not expected to affect inotersen exposures. Drug-drug interaction liability with inotersen is considered low.
Labeling	The labeling concepts proposed by the applicant are generally adequate.
Bridge between the to-be-marketed and clinical study formulations	The to-be-marketed formulation is the same as the one used in the pivotal efficacy study.

1.2 Post-Marketing Requirements and Commitments

None.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Mechanism of Action: Inotersen is a 2'-O-(2-methoxyethyl) [2' MOE] phosphorothioate antisense oligonucleotide (ASO) inhibitor of both mutant and wild type human TTR production. Inotersen selectively binds to TTR messenger RNA (mRNA) causing degeneration of both mutant and wild type TTR mRNA.

Absorption: Following subcutaneous administration, median time to maximum plasma concentration (T_{max}) was 1.5 to 4 hours.

Distribution: Inotersen distributes rapidly to the liver and kidney. Inotersen is >94% bound to human plasma proteins.

Metabolism: Inotersen is metabolized by endonucleases to form shorter, inactive oligonucleotides that are further metabolized by exonucleases.

Elimination: The mean terminal elimination half-life in plasma ranges between 2 to 4 weeks. It is mainly excreted in the urine as chain-shortened nucleotides that are not considered active. Urinary recovery of unchanged inotersen is limited to less than 0.05% after a single dose administration within the first 24 hours and < 1.1% was excreted in urine after the 6th dose.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The recommended dosing regimen includes [redacted] 300 mg once weekly [redacted] Inotersen cannot be initiated in subjects with platelet count <100 x10^9/L or urine protein to creatinine ratio (UPCR) ≥ [redacted]). Plasma vitamin A (retinol levels) below the lower limit of normal should be corrected and any ocular symptoms or signs of vitamin A deficiency should have resolved [redacted].

2.2.2 Therapeutic individualization

No therapeutic individualization is required for inotersen based on extrinsic or intrinsic factors. Inotersen is administered subcutaneously and is not a substrate, inhibitor, or inducer of major CYP enzymes or transporters. Intrinsic factors like hepatic/renal impairment are not expected to affect inotersen exposures.

2.3 Outstanding Issues

The benefit/risk profile of the recommended dose/regimen for inotersen may improve with a [redacted]

[redacted] Efficacy was demonstrated at this dose level. However, this study showed a reduction in platelet count (<100x10^9/L) in about 23% of inotersen treated subjects as compared to 2% of placebo subjects. A REMS with ETASU is required to balance the benefit and risk at the recommended dose of inotersen. Treatment emergent reduction in platelet count can trigger dose pausing or interruptions and require intense platelet count monitoring (please refer to Dr. Evelyn Mentari's safety review for details). This may lead to more patients discontinuing inotersen therapy and there are no approved alternate treatments available for hATTR-PN at this time.

Previous experience with ASOs suggests that risk for some types of thrombocytopenia can be dose dependent and doses greater than 175 mg per week are associated with increased risk for thrombocytopenia. Studies CS1 in healthy subjects and CS2 in hATTR-PN patients showed that less frequent dosing with inotersen resulted in similar pharmacodynamic response (i.e., reduction of TTR levels) as compared to the once weekly dosing. A relationship between TTR reduction and the primary efficacy endpoint(s) was not apparent from the pivotal Study CS2, which used only the 300-mg weekly regimen, possibly because of the lack of a dose range studied. However, the PK/PD of inotersen support the possibility that a lower dose or reduced dosing frequency than studied in CS2 may improve the benefit/risk profile of inotersen (for more details, please refer to Sections 3.3.2 and 4.3).

2.4 Summary of Labeling Recommendations

The labeling concepts proposed by the applicant are generally adequate. The Office of Clinical Pharmacology suggests revisions to Section 6.2 Immunogenicity to include updated anti-drug

antibody (ADA) information and Sections 7 & 12.3 to incorporate potential interference between inotersen and laboratory tests for serum vitamin A.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

Inotersen is available as (b) (4) injection and is supplied in a glass prefilled syringe (PFS) delivering 300 mg inotersen sodium. Inotersen received Orphan Drug Designation for the treatment of familial amyloid polyneuropathy and Fast-track designation by FDA in 2012. The clinical development program consists of 2 completed and 1 ongoing clinical studies. Study CS1 was a first-in-human Phase 1 single and multiple dose escalation study that evaluated the safety, PK and reduction of TTR levels as a pharmacodynamic (PD) measure of inotersen in healthy subjects. Study CS2 was the pivotal Phase 2/3 study followed by the ongoing, Phase 3, open-label extension study (CS3) in patients with hATTR-PN.

Dedicated *in vivo* drug-drug interaction studies were not conducted given the lack of any significant *in vitro* findings of inotersen as an inducer or inhibitor of CYP enzymes or transporters. Renal and hepatic impairment studies were not conducted. However, given the mechanism of inotersen clearance, it is not likely that renal or hepatic dysfunction will significantly affect inotersen pharmacokinetics.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	Inotersen is a 2'-O-(2-methoxyethyl) [2'-MOE] antisense oligonucleotide that targets human TTR mRNA. Hybridization to the cognate TTR mRNA results in the RNase H1-mediated degradation of the TTR mRNA preventing the production of the TTR protein.
QT Prolongation	TQT study was waived. No clinically relevant changes in ECG parameters in inotersen-treated subjects in CS2 or CS3 were reported. For more information, please refer to the QT-IRT review.
General Information	
Bioanalysis	Plasma inotersen concentrations were measured using two validated hybridization ELISAs. Serum TTR concentrations were measured using an automated clinical chemistry analyzer. Details are described in Section 4.1.
Healthy Volunteers vs. Patients	PK is similar between hATTR patients and healthy subjects.
Dose Proportionality	Dose-proportional over a dose range of 150-400 mg.
Variability	Inter-individual variability in plasma $AUC_{0\text{-last}}$ (%CV) for inotersen ranges from 28% to 44%. Inter-individual variability in plasma C_{trough} (%CV) levels for inotersen ranges from 17% to 66%.
Immunogenicity	No immunogenicity assessment was performed in Study CS1 with healthy subjects. Antibodies to inotersen were formed in about 30%

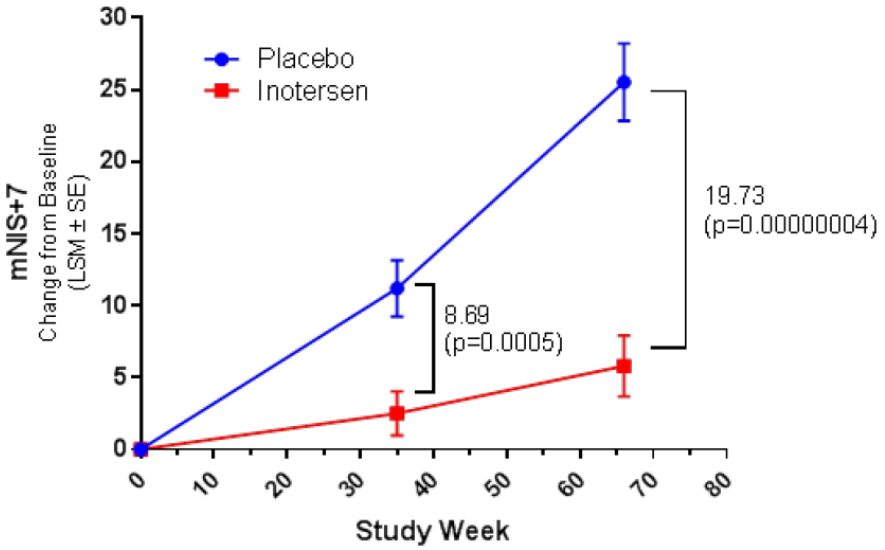
	of subjects treated with inotersen in Study CS2 and 40 % in CS3. Immunogenicity data were evaluated from Studies CS2 and CS3 (open label extension) combined. Overall, 40% (45/114) of treated subjects were positive for treatment-emerged ADA. For more details, refer to Section 4.4.
Absorption	
T_{max}	1.5 to 4 hours after SC injection.
Distribution	
Volume of Distribution	The apparent volume of distribution at steady state is 293 L.
Protein Binding	> 94%.
Substrate of transporter systems	Not a substrate or inhibitor for BCRP, P-gp, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, and BSEP *in vitro.*
Elimination	
Terminal Elimination half-life	The elimination half-life ranges from 2 to 4 weeks.
Metabolism	
Primary Metabolizing enzymes	Endonucleases and exonucleases.
Inhibitor/Inducer	Not an inhibitor or inducer for any of the major CYP enzymes or transporters.
Excretion	
Primary excretion pathways	Excreted by the kidneys as chain-shortened oligonucleotides, which are not considered pharmacologically active. Less than 0.05% of administered dose was excreted unchanged in urine within 24 hours after a single dose.

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

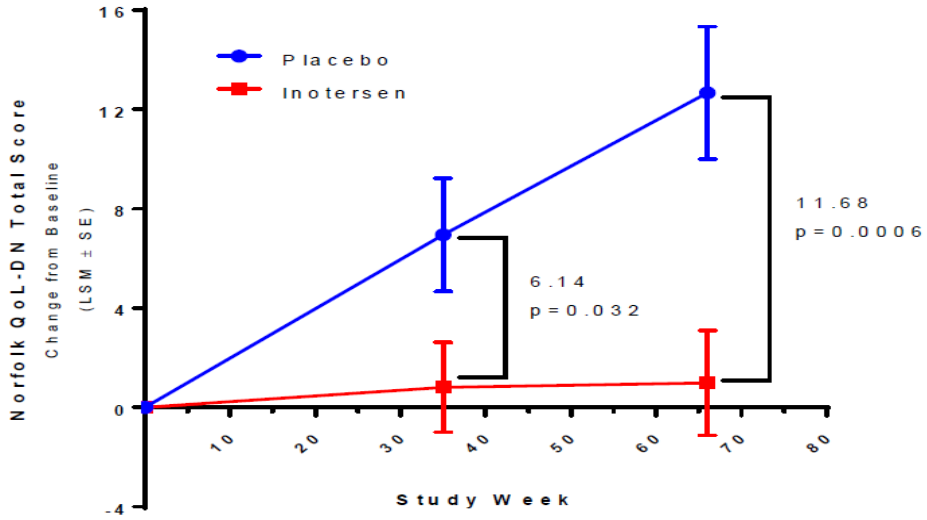
The primary evidence of the efficacy of inotersen is from a pivotal, placebo-controlled Phase 2/3 study (CS2). Study CS2 evaluated the efficacy and safety of a fixed dose level of 300 mg inotersen in subjects with Stage 1 or Stage 2 hATTR-PN. As per applicant's analyses, changes from baseline in both co-primary endpoints: mNIS+7 composite score and Norfolk QoL-DN total score were statistically significant in favor of inotersen compared to placebo at Week 66. The differences in least squares means (LSMs) between treatment groups for mNIS+7 composite score and Norfolk QoL-DN total score, were -19.73 and -11.68, respectively (Figure 1 and Figure 2). Please refer to the review by Dr. Massie Tristan (Division of Biometrics I, Office of Biostatistics) for more information.

Figure 1: On-Treatment LSM Change from Baseline in mNIS+7 Composite Score (CS2 Full Analysis Set).



Source: Table 2.01, CS2 CSR, Module 5.3.5.1. Abbreviations: SE=standard error

Figure 2: On-Treatment LSM Change from Baseline in Norfolk QoL-DN Total Score (CS2 Full Analysis Set).



Source: Table 2.02, CS2 CSR, Module 5.3.5.1. Abbreviations: SE=standard error.

Pharmacodynamic Effect (Serum TTR Levels):

Consistent with the proposed mechanism of action, a persistent reduction in the TTR level was observed in the CS2 study for subjects treated with inotersen 300 mg once weekly. The maximum reduction in TTR levels was observed by about Week 13 (Figure 3). The inotersen treated group showed approximately 70% reduction in TTR levels from baseline as compared to about 8% reduction in the placebo arm. These findings support the mechanism of action of inotersen. It should be noted that the relationship between TTR reduction and the clinical endpoints is not yet established.

Source: Figure 2.11, CS2 CSR, Module 5.3.5.1, and applicant's proposed US label.
Abbreviations: SE=standard error.

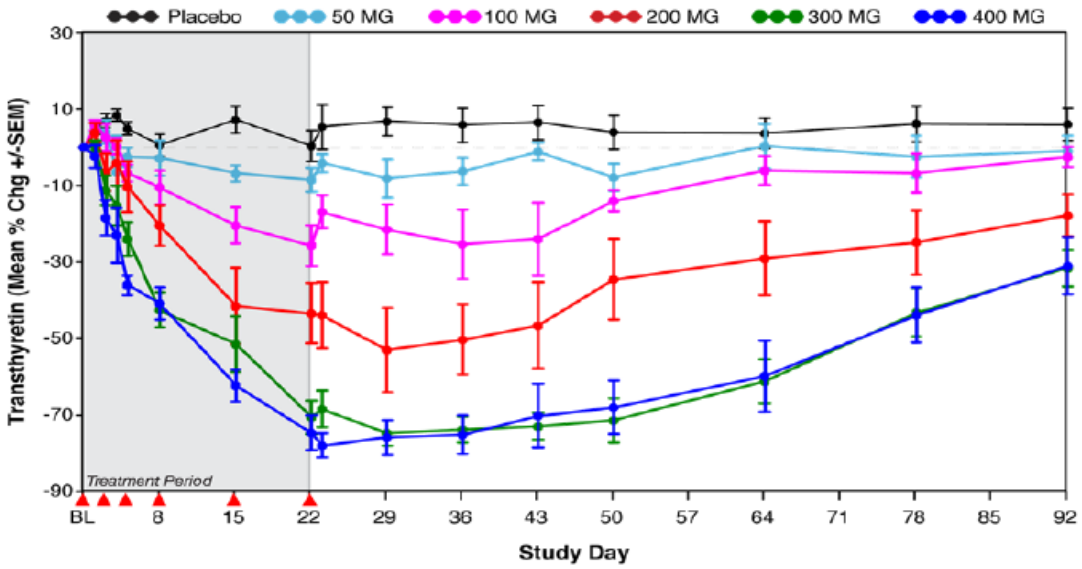
3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes, the proposed dosing regimen with the appropriate risk mitigation strategies for managing thrombocytopenia is acceptable. The proposed dosing regimen is the same that was evaluated in the pivotal efficacy/safety study (CS2). A Risk Evaluation and Mitigation Strategy (REMS) with Elements to Assure Safe Use (ETASU) is proposed for TEGSEDI™ to ensure that benefit outweighs the risk.

Dose and dosing regimen for the pivotal study were selected based on the observed TTR reduction in healthy volunteers in Study CS1. The multiple dose cohorts of Study CS1 evaluated 5 dose levels (50, 100, 200, 300, and 400 mg) administered once weekly over 3 weeks. Three loading doses given every other day in the first week were administered for all dose groups. After 3 weeks of inotersen administration, both the 300-mg and 400-mg dose levels were associated with similar reductions (75% and 76%, respectively) in the mean percent change of

TTR levels at Day 29 as compared to lower doses (Figure 4). At steady-state, a TTR reduction of ~80% was predicted with both 300 mg/week and 400 mg/week regimens.

Figure 4: Mean TTR Reduction by Dose (CS1 Study).



Source: CS1 Study. Abbreviations: BL=baseline; Chg=change; SEM=standard error of the mean.

A persistent reduction in TTR levels was observed for about two weeks or more after the last dose of inotersen in Study CS1 (Figure 4). This is consistent with the reported elimination half-life of 2-4 weeks for inotersen. (b) (4)

As detailed in Section 3.3.1, Study CS2 demonstrated a statistically significant improvement in primary efficacy endpoints favoring inotersen. As illustrated in Figure 3, sustained reduction in TTR levels in patients treated with inotersen was observed in this study. Consistent with the findings from the CS1 study in healthy subjects, persistent reduction in the TTR levels were seen even in subjects who discontinued inotersen treatment for at least 1 week for any reason (see Figure 18 in Section 4.3, for representative subjects;). Additionally, no apparent exposure-response relationship between C_{trough} and the primary endpoints (mNIS+7 composite score and Norfolk QoL-DN total score) was demonstrated (see Section 4.3; Figure 19 and Figure 20). Similarly, a relationship between TTR reduction and the primary efficacy endpoint in Study CS2 was not established. These findings may be expected given that only a single dose level was tested in this study.

Reduction in platelet count was one of the major treatment emergent adverse event reported for inotersen. About 70% of inotersen-treated patients as compared to 5% in the placebo arm (see Section 4.3 for more details) showed >30 % reduction in platelet count from baseline. Of these, about 23 % of patients on inotersen has platelet count <100x10^9/L at some point. Severe

fatal thrombocytopenia cases were also reported in ~3% of inotersen treated subjects. Various strategies were implemented to mitigate the risk of thrombocytopenia in CS2. These included more frequent monitoring for platelet count, treatment interruptions and dose reductions, as well as administration of corticosteroids.

Previous experience with ASOs with the same phosphorothioate backbone indicates that these drugs can cause thrombocytopenia. The mechanism of ASOs-induced thrombocytopenia is not fully understood. However, a dose-dependent[1] thrombocytopenia has been described for ASOs, in general. Although platelet count reduction is mostly mild, instances of severe thrombocytopenia have been also reported. In the inotersen program, 3 subjects in Study CS2 developed severe Grade 4 thrombocytopenia that resulted in death of 1 subject and permanent drug discontinuation in the other 2 subjects. However, Study CS2 included only one dose level and therefore there is no sufficient data to determine the exposure-thrombocytopenia relationship for inotersen.

For these reasons, the applicant may consider evaluating the efficacy of a lower dose or less frequent regimen to improve the benefit/risk profile of inotersen. This is supported by the reported PK/PD and observed safety profile of inotersen.

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

No. Dose adjustments are not necessary based on age, race, sex, body weight, renal and hepatic impairment. Inotersen is metabolized by nucleases and its exposure is not expected to be impacted by these intrinsic factors. Also, no patient attributes were identified from clinical studies that may significantly affect the exposure to inotersen.

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

No. Given its subcutaneous route of administration, food-drug interaction is not expected. Inotersen is an antisense oligonucleotide and *in vitro* studies showed that potential for drug-drug interactions mediated by CYP enzymes or transporters is low.

[1] Chi, Xuan, Philip Gatti, and Thomas Papoian. "Safety of antisense oligonucleotide and siRNA-based therapeutics." *Drug discovery today* 22, no. 5 (2017): 823-833.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

4.1.1 Pharmacokinetics

For study CS1, plasma inotersen concentrations were determined using a validated hybridization ELISA (Method # TCAM-051) in which complementary cuttings probe dual-labeled with biotin and digoxigenin was used to hybridize to inotersen as well as capture reagent to NeutrAvidin-coated plate. Alkaline phosphatase-labeled anti-digoxigenin antibody was used as detection reagent (Validation report 420915-MV04). Calibration standard was prepared by spiking inotersen in 100% K_2EDTA plasma. Method validation and sample analysis for measurement of plasma inotersen were performed at (b) (4) and summarized in the Table 1 below.

Table 1: Summary Review of Bioanalytical Method Measuring Plasma Inotersen.

Bioanalytical method review summary	Method was adequately validated to support clinical study CS1.		
Material for calibration curve & Concentration	ISIS 420915 (ISIS Pharmaceuticals, Lot: WSS-420915-01, 1152 mcg/mL)		
Validated assay range	1.0 (LLOQ) – 200 (ULOQ) ng/mL in 100% K_2EDTA plasma		
Source of reagents	Dual-labeled cutting probe (ISIS Pharmaceuticals, Lots# 53977094/93494828) Alkaline phosphatase-labeled anti-digoxigenin antibody (Varies)		
Regression model & weighting	4 Parameter logistic auto-estimate with $1/Y^2$		
Validation parameters	**Method validation summary (Validation report 420915-MV04)**		**Acceptability**
Standard curve performance during accuracy & precision	Number of standard levels including LLOQ to ULOQ	9	Yes
	Cumulative accuracy (%bias) in standard calibrators	-9.0 to 10.9%	Yes
	Cumulative precision (%CV)	≤ 4.4%	Yes
QCs performance during accuracy & precision	Cumulative accuracy (%bias) in 5 QCs	-18.5 to -4.7%	Yes
	Inter-assay precision (%CV)	≤ 14.5%	Yes
	Total error (TE) in percent	≤ 29.4% (≤ 33.0 for LLOQ)	Yes
Selectivity & matrix effect	10 plasma lots tested. At least 90% of the lots within 25 % bias		Yes
Interference & specificity	The presence of ≥1 mcg/mL rabbit anti- ISIS 420915 antibodies inhibited the quantification of ISIS 420915 at >59% (negatively bias).		Yes. Expected.

	Cross-reactivity to [redacted] (b) (4) in the method with 50% reduced immunoreactivity. No cross-reactivity to [redacted] (b) (4)	
Hemolysis effect	Not tested	No*
Lipemic effect	Not tested	NA
Dilution linearity & hook effect	Linear within 2- to 16,000-fold dilutions. Tested with 200 mg/mL No hook effect	Yes
Bench-top/process stability	Stable at room temperature or at 5°C for 21.7 hours in plasma	Yes
Freeze-Thaw stability	Up to 8 cycles	Yes
Long-term storage stability	At nominal -80°C for 496 days	Yes
Method performance in study CS1		
Assay passing rate	• 84 out of 110 runs met the method acceptance criteria	Yes
Standard curve performance	• Cumulative bias range: -6.2 to 6.2% • Cumulative precision: ≤ 3.8% CV	Yes
QC performance	• Cumulative bias range: -0.9 to 8.3% • Cumulative precision: ≤ 10.5% CV • TE: ≤ 18%	Yes
Method reproducibility	• Incurred sample reanalysis was performed in 175 out of 1763 (10%) study samples and 88% of samples were within 30% of average concentration.	Yes
Study sample stability	All samples analyzed within the established storage stability.	

*Impacted samples are less than 5% and are not expected to impact the results

The method TCAM-051 was later modified, partially validated, and transferred to [redacted] (b) (4). For the study CS2, plasma inotersen concentrations were determined with the modified methods, #TCAM-110 and #TLIAM-14212 at [redacted] (b) (4) respectively. For the study CS3, plasma inotersen concentrations were determined with TLIAM-14212 at [redacted] (b) (4). Modification and lab-to-lab cross-validation results of methods TCAM-110 and TLIAM-14212 were described in Table 2 below.

Table 2: Summary Review of Modified Bioanalytical Methods Measuring Plasma Inotersen.

Cross-validation review summary	The modified methods TCAM-110 and TLIAM-14212 measured total inotersen, unbound and bound to ADAs. Partial method validations were performed in both laboratories (Validation reports 420915-MV11 and 420915-MV13, respectively). Lab-to-lab cross validation was also performed using both spiked and incurred samples as two laboratories were involved in analyzing samples from study ISIS 420915-CS2. The cross-validation results were adequate (Validation report 420915-MV13).		
Changes in method description	A step to include digestion of proteins using proteinase K was added to method TCAM-051.01 to eliminate inhibition of anti- ISIS 420915 antibodies for measurement of inotersen (Validation report 420915-MV11).		
Validated assay range	1.0 (LLOQ) – 200 (ULOQ) ng/mL in 100% K_2EDTA plasma		
Validation parameters	**Partial- or cross- validation performance (Validation reports 420915-MV11[a] or 420915-MV13[b])**		**Acceptability**
Standard curve performance during accuracy & precision	Cumulative accuracy (%bias) in standard calibrators	-9.8 to 10.7%[a] -7.6 to 6.5%[b]	Yes
	Cumulative precision (%CV) in standard calibrators	≤ 6.3%[a] ≤ 5.2%[b]	Yes
QCs performance during accuracy & precision	Cumulative accuracy (%bias) in 5 QCs	-4.3 to 4.1%[a]	Yes
	Inter-assay %CV	≤ 8.1%[a]	Yes
	% Total error (TE)	≤ 12%[a]	Yes
Interference	No interference in the presence of rabbit anti- ISIS 420915 antibodies at up to 10 mcg/mL		Yes, acceptable but the impact of ADA for PK measurement could not be determined.
Method performance in study CS2			
Assay passing rate	• 34 out of 39 runs met the method acceptance criteria at (b) (4) • 83 out of 89 runs met the method acceptance criteria at (b) (4)		Yes
Standard curve performance (site-combined)	• Cumulative bias range: -8.3 to 9.5% (site combined) • Cumulative precision: ≤ 6.4% CV		Yes
QC performance (site-combined)	• Cumulative bias range: -7.4 to 1.2% • Cumulative precision: ≤ 8.2% CV		Yes

	• TE: ≤ 13%	
Method reproducibility	• Incurred sample reanalysis was performed in 98 out of 867 (11%) study samples at [redacted] (b) (4) and 140 out of 1811 (8%) study samples at [redacted] (b) (4) • In total, 233 out of 2678 samples were reanalyzed and 94% of samples were within 30% of average concentration.	Yes
Study sample stability note	Nine out of 868 samples were in compromised conditions upon arrival at the bioanalytical site. We recommend excluding the results from these samples in data analysis.	
Method performance in study CS3		
Assay passing rate	• 46 out of 48 runs met the method acceptance criteria.	Yes
Standard curve performance	• Cumulative bias range: -2.9 to 4.8% • Cumulative precision: ≤ 6.9% CV	Yes
QC performance	• Cumulative bias range: -9.0 to -1.2% • Cumulative precision: ≤ 7.4% CV • TE: ≤ 15%	Yes
Method reproducibility	• Incurred sample reanalysis was performed in 79 out of 752 study samples (11%) and 98% of samples were within 30% of average concentration.	Yes
Study sample stability note	All samples analyzed within established storage stability.	

[a] Validation report 420915-MV11
[b] Validation report 420915-MV13

4.1.2 Pharmacodynamics

For studies CS1 and CS2, serum TTR concentrations were determined with immunoturbidimetric assay using an automated [redacted] (b) (4). Agglutination reaction Anti-prealbumin antibodies in reagent R2 reacted with TTR in serum to form antigen/antibody complexes resulting in turbidimetric. The method performance was summarized in Table 3 below.

Table 3: Summary Review of Bioanalytical Method Measuring Serum TTR.

Bioanalytical review summary	Method was sufficiently validated to support clinical studies CS1 and CS2 as a secondary endpoint.
Material for calibrator & QC	Calibrator for automated systems (PAC, Cat # 03555941) QC were MAS Chemtrak levels 1 (Ref#05947626) and 2 (Ref#0594774)
Reportable range	3.0 to 80.0 mg/dL in serum
Ranges of QCs in daily runs	QC level 1: 25.2 – 26.9 mg/dL QC level 2: 19.5 – 21.4 mg/dL
Inter-assay CV%	≤ 3.5%
Sample stability	• 3 days at 2 °-8 °C • 6 months at -15° to -25 °C
Interference	No significant interference from; • Hemolyzed samples (up to H index 500), • Lipemic (up to 1730 mg/dL), • Rheumatoid factor (<100 IU/mL) and • Bilirubin (up to I index 60) IgM may interfere.
Sample analysis	Serum samples were analyzed within the pre-established sample stability.

4.2 Population PK and/or PD Analyses

Study Report: Population Pharmacokinetic Meta-Analysis of Inotersen

Objectives:

- To develop a model that describes PK of inotersen in plasma following single and multiple SC administrations to healthy volunteers and patients with hATTR-PN.
- To identify statistically significant covariates that contribute to the inter-individual variability (IIV) in the PK of inotersen in patients with hATTR-PN and to determine if these covariates are clinically relevant.

Clinical Studies and PK Sampling

Brief description of the 3 studies included in the analysis is provided in Table 4.

Table 4: Studies Included in the Population PK Analysis.

Study	Study Title	Nominal Dose	Number of Subjects	Sampling Schedule
ISIS 420915-CS1, Amendment 2	A Double Blind, Placebo-Controlled, Dose-Escalation, Phase 1 Study to Assess the Safety, Tolerability and Pharmacokinetics of Single and Multiple Doses of ISIS 420915 Administered Subcutaneously to Healthy Volunteers	**SAD cohorts** (SC on D1): 50 mg or placebo 100 mg or placebo 200 mg or placebo 400 mg or placebo **MAD cohorts** (SC on D1, 3, 5, 8, 15 and 22): 50 mg or placebo 100 mg or placebo 200 mg or placebo 400 mg or placebo	**SAD cohorts:** 12 on active drug; 4 on placebo **MAD cohorts:** 39 on active drug; 10 on placebo	**SAD cohorts PK Sampling:** **Day 1:** Predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 hours post SC injection **Day 1:** Amount of ISIS 420915 excreted in urine over a 24 hour time period **Day 2:** 24 hours post SC injection **Day 4 and 8:** ±24 hour window **MAD cohorts PK Sampling:** **Day 1:** Predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 hours post SC injection **Day 2:** 24 hours post SC injection **Day 3**: One sample that is both pre-dose and 48 hours post Day 1 SC injection **Day 4:** 24 hours post Day 3 SC injection **Days 5, 8, and 15:** Predose **Day 22:** Predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 hours post SC injection **Day 23:** 24 hours post Day 22 SC injection **Day 29 (or early term):** ±48 hour window **Days 36, 50, 64, 78, and 92:** ±48 hour window **TTR PD Sampling:** **SAD Cohorts**: screening, pre-dose, 24 hours post dose, and ±24 hour window on Days 4 and 8 **MAD Cohorts**: pre-dose and 24 hours post-dose on SC injection Days 1, 3, 5, 8, 15, and 22; ±24 hour window on Days 29, 36, 43, 50, 64, 78, and 92 **No Immunogenicity Sampling for any patient**

Study	Description	Dose and Administration	Number of Subjects	Sampling Schedule
ISIS 420915-CS2, Amendment 7	A Phase 2/3 Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of ISIS 420915 in Patients with Familial Amyloid Polyneuropathy	Placebo and 300 mg ISIS 420915 multiple subcutaneous doses administered three times on alternate days during Week 1 (Days 1, 3, and 5), and then once weekly during Weeks 2-65 (for a total of 67 doses)	Randomized 173 patients (2:1 active:placebo allocation) with 172 patients dosed and approximately 20 patients enrolled in an extensive PK sampling subgroup	**Trough PK sampling for all patients except for PK subgroup::** Nominal Days 1, 15, 29, 50, 85, 120, 155, 197, 240, 281, 323, 365, 407, and 449:Predose Post-treatment Days 491, 533, and 631: ±7 Day window **Extensive PK sampling for PK subgroup:** Nominal Day 1:Predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours post SC injection Nominal Days 240 and 449:Predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours, and 3 and 7 days post SC injection Nominal Days 3, 5, 15, 29, 50, 85, 120, 155, 197, 281, 323, 265, and 407:Predose Post-treatment Days 491, 533, and 631: ±7 Day window **TTR PD Sampling for all patients:** Screening and Nominal Days 1, 15, 29, 50, 85, 120, 155, 197, 240, 281, 323, 365, 407, and 449:Predose Post-treatment days 491, 533, and 631: ±7 Day window **Immunogenicity Sampling for all patients:** Nominal Days 1, 29, 85, 197, 323, and 449:Predose Post-treatment Days 491 and 631: ±7 Day window
ISIS 420915-CS3, Amendment 2	An Open-Label Extension Study to Assess the Long-Term Safety and Efficacy of ISIS 420915 in Patients with Familial Amyloid Polyneuropathy (FAP)	300 mg ISIS 420915 multiple subcutaneous doses administered once weekly for 156 weeks (3 years)	Eligible patients who have satisfactorily competed study ISIS 420915-CS2; At the time of CS3 data cut-off (DCO) for the current submission (28 February 2017), a total of 114 subjects had enrolled into CS3; 40 subjects had received placebo and 74 subjects had received inotersen in CS2.	**Trough PK sampling for all patients :** Nominal Days 1, 43, 85, 120, 176, 267, 358, 449, 540, 631, 722, 813, 904, 995, and 1086:Predose Post-treatment Day 1177: ±10 Day window **TTR PD Sampling for all patients:** Nominal Days 1, 43, 85, 120, 141, 176, 267, 358, 449, 540, 631, 722, 813, 904, 995, and 1086:Predose Post-treatment Day 1177: ±10 Day window **Immunogenicity Sampling for all patients:** Nominal Days 1, 43, 85, 120, 176, 267, 358, 449, 540, 631, 722, 813, 904, 995, and 1086:Predose Post-treatment Day 1177: ±10 Day window

Source: Table 1, PK01 report, Page 20-21, Module 5.3.3.5.

Applicant's Analysis

Structural Model Building

Population PK modeling was performed using NONMEM® (Version 7.2). The first-order conditional estimation with interaction method was used during all stages of the model development process. Data exploration was performed to understand the PK disposition (mono- or bi-exponential) and PK linearity across evaluated doses. A preliminary two-compartment population PK model with first order absorption was developed using data from the Phase 1 study (CS1).

Data Exclusions

Missing PK values, concentrations with missing preceding dose information, and PK values below the limit of quantitation (BLQ) were excluded from the analysis per the "M1" methodology. Additionally, outliers in the PK dataset plausibly caused by errors in dosing or timing of samples/assessments or identified by diagnostic variables (i.e. absolute value of

conditional weighted residuals (CWRES) in the population PK model larger than 5) were excluded from the analysis.

Covariate Analysis

The full model approach was implemented, where all covariate-parameter relationships of interest were entered in the model simultaneously.

The following covariates were explored for inclusion in the final population PK model:

- Subject demographics
    - Categorical variables: race, gender, and age (< 65 years old, ≥ 65 years old)
    - Continuous variables: baseline body size [body weight (WT), body surface area (BSA), lean body mass (LBM), body mass index (BMI)]
- Disease status (healthy volunteer vs. hATTR-PN patients)
- Baseline clinical chemistry [creatinine clearance (CrCL) estimated by Cockcroft-Gault formula, estimated glomerular filtration rate (eGFR) estimated by modification of diet in renal disease (MDRD) equation, alanine aminotransferase (ALT), aspartate aminotransferase(AST), total bilirubin and albumin]
- Renal impairment status (severity) based on baseline eGFR:
    - 90 mL/min/1.73m^2
    - 60 to 90 mL/min/1.73m^2
    - 45 to 60 mL/min/1.73m^2
    - 30 to 45 mL/min/1.73m^2
- Concomitant medications, classified as present or absent:
    - Diuretics
    - Analgesics
    - Anti-thrombotic agents
- Geographic location (North America, Europe, South America/Australasia)
- TTR mutation (V30M and non-V30M)

Continuous covariates were included in the model in either a linear or power model with or without centering based on a summary statistic (typically the median of the covariate). On the other hand, categorical covariates were included in the model using proportional form.

Simulations Based on Final Population PK Model

The final model was also used to simulate concentration-time profiles for the following dosing regimens to estimate relevant PK exposure parameters:

- 300 mg once per week (QW) for 65 weeks
- 300 mg once every other week (QOW) for 65 weeks
- 150 mg once per week (QW) for 65 weeks

- 300 mg with loading doses (Days 1, 3, and 5) during the first week, followed by once per week (QW) for another 64 weeks (i.e. same dosing regimen as in Study CS2)

Five hundred replicated datasets were produced for each scenario. Each dataset contained the original 151 patients studied in CS2 and CS3 along with their demographic variables. Steady-state exposure measures ($AUC_{0-\tau}$, C_{max}, C_{trough}) from these simulations were summarized by geometric mean (90% confidence interval) following the first dose on day 1, and the last dose after 65 weeks of dosing.

Results

The source data contained a total of 3,602 post first dose plasma concentrations from 202 individuals who were treated with inotersen, across three different studies. Five (5) individuals were treated with inotersen but had no observed plasma concentrations. A total of 580 records were excluded per Table 5.

Table 5: Reasons and Number of Excluded Observations.

Reason for Exclusion	Number of Observations Excluded	Percentage Excluded
Below the lower limit of quantification (BLQ)	112	3.11
PK observations occurred after the formation of anti-drug antibodies	452	12.5
Concentrations were greater than 50-fold higher than similarly timed PK observations	5	0.14
Trough concentrations were greater than 50-fold higher than typical value	5	0.14
Concentration observations were higher than previous BLQ concentrations in Subject (b) (6)	2	0.06
C_{max} observation was nearly 2-fold higher than any other concentration in Subject (b) (4)	1	0.03
Absolute conditional weighted residuals were greater than 5	3	0.08
Total	580	16.1

Subject's baseline continuous and categorical covariates are presented in Table 6 and Table 7.

Table 6: Summary Statistics of Baseline Continuous Covariates of the Final Analysis Dataset.

Parameter (Unit)	Mean (SD)	Minimum	Median	Maximum
Age (years)	55.4 (13.8)	25.0	57.0	81.0
Albumin (mg/dL)	4249 (314)	3167	4233	5000
ALT (U/L)	23.1 (10.8)	6.00	20.7	83.2
AST (U/L)	25.5 (7.68)	12.3	24.0	60.0
Total Bilirubin (mg/dL)	0.586 (0.306)	0.0800	0.517	2.18
BMI (kg/ m^2)	24.6 (4.45)	13.3	24.5	40.2
BSA (m^2)	1.85 (0.223)	1.30	1.83	2.61
CrCL (mL/min)	100 (34.0)	35.2	97.9	220
eGFR (mL/min/1.73m^2)	93.2 (19.9)	41.3	93.9	148
Height (cm)	172 (9.43)	144	173	196
Ideal Body Weight (kg)	66.2 (10.0)	38.1	68.3	89.2
Lean Body Mass (kg)	51.7 (8.44)	31.3	51.6	80.3
Lean Body Weight (kg)	54.8 (10.0)	31.4	54.9	82.9
TTR (mg/dL)	22.0 (5.95)	3.80	22.1	39.7
Weight (kg)	72.7 (16.2)	37.0	71.8	140

Source: Table 5, PK01 report, Page 37, Module 5.3.3.5 (Modified).

Table 7: Summary Statistics of Baseline Categorical Covariates of the Final Analysis Dataset.

Categorical Parameter	$N_{all\ Subjects}$ (%)	$N_{hATTR\text{-}PN}$ (%)
Age < 65	136 (67.3%)	85 (56.3%)
Age ≥ 65	66 (32.7%)	66 (43.7%)
eGFR ≥ 90	116 (57.4%)	73 (48.3%)
eGFR 60 – < 90	72 (35.6%)	64 (42.4%)
eGFR 45 – < 60	12 (5.94%)	12 (7.95%)
eGFR 30 – < 45	2 (0.990%)	2 (1.32)
Missing Diuretic Information	51 (25.2%)	0 (0.00%)
No Diuretics	105 (52.0%)	105 (69.5%)
Diuretics	46 (22.8%)	46 (30.5%)
Missing Analgesic Information	51 (25.2%)	0 (0.00%)
No Analgesics	25 (12.4%)	25 (16.6%)
Analgesics	126 (62.4%)	126 (83.4%)
Missing Antithrombotic Information	51 (25.2%)	0 (0.00%)
No Antithrombotic	96 (47.5%)	96 (63.6%)
Antithrombotic	55 (27.2%)	55 (36.4%)
Healthy Volunteers	51 (25.2%)	0 (0.00%)
hATTR-PN Patients	151 (74.8%)	151 (100%)
North America	127 (62.9%)	76 (50.3%)
Europe	51 (25.2%)	51 (33.8%)
South America/Australasia	24 (11.9%)	24 (15.9%)
Caucasian	177 (87.6%)	142 (94.0%)
Black	15 (7.43%)	3 (1.99%)
Asian	7 (3.47%)	3 (1.99%)
Other	3 (1.49%)	3 (1.99%)
Male	141 (69.8%)	106 (70.2%)
Female	61 (30.2%)	45 (29.8%)
Non-V30M Mutation	124 (61.4%)	73 (48.3%)
V30M Mutation	78 (38.6%)	78 (51.7%)
Missing Vitamin A Deficiency Information	51 (25.2%)	0 (0.00%)
No Vitamin A Deficiency	114 (56.4%)	114 (75.5%)
Vitamin A Deficiency	37 (18.3%)	37 (24.5%)

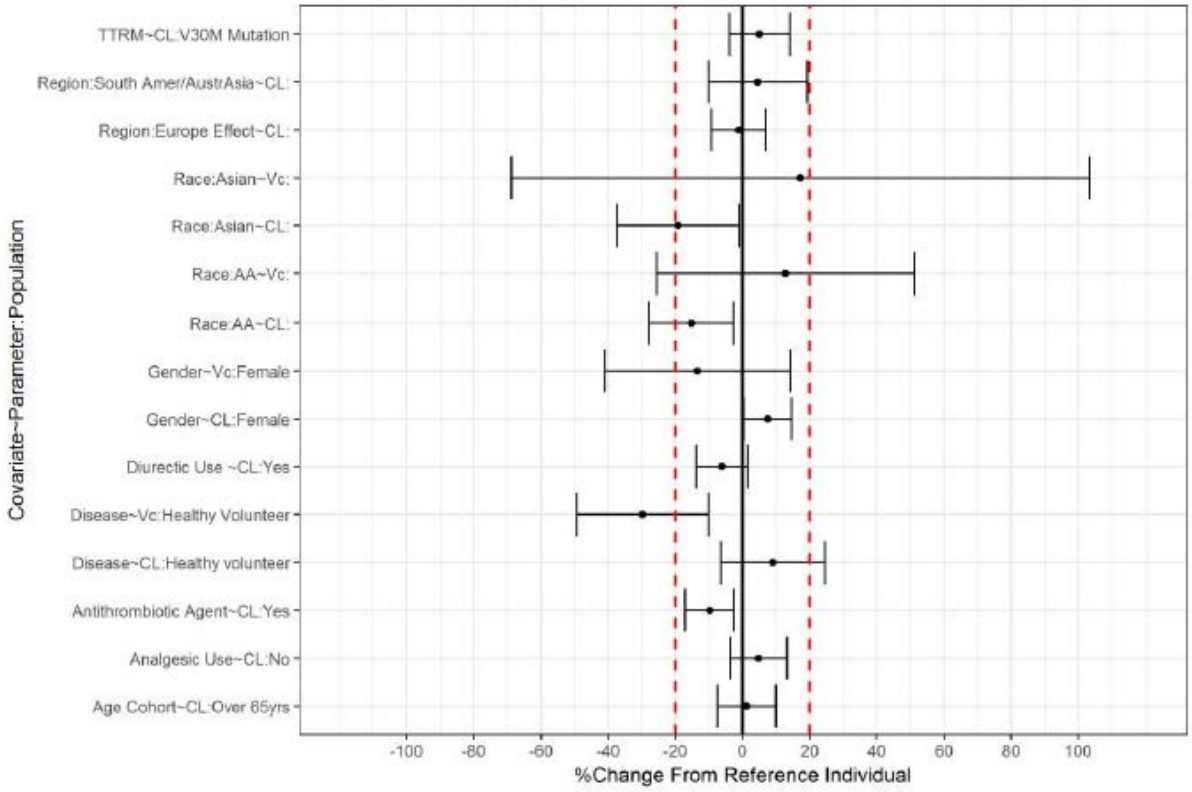
Source: Table 8, PK01 report, Page 40, Module 5.3.3.5 (Modified).

The base model was a two-compartment model with first-order absorption and first-order elimination with IIV on clearance, central volume, and peripheral volume, and with additive residual error model on log transformed data. Dose non-linearity was applied to doses less than 150 mg using a simplified exponential model with the dose administered being normalized to

the 300-mg dose. The influence of lean body mass on PK parameters was evaluated using a power model with a power value fixed to 1. The base model also contained an off-diagonal correlation block with correlation between V_c/F and CL/F and between CL/F and the apparent peripheral volume of distribution (V_p/F).

The full covariate model included the following covariates on CL/F: disease status, V30M mutation, baseline transthyretin (TTR) levels, baseline eGFR, gender, race, baseline albumin levels, baseline alanine aminotransferase levels, baseline bilirubin levels, age effects (> 65 years old), concurrent medication status (diuretics, analgesic, or anti-thrombotic medications), and region of the world from which the participant originated from. Additionally, disease status, gender, and race were assessed on V_c/F. Based on the parameter estimates of the full covariate model and their associated uncertainties, only a few parameters appear to be statistically significant (i.e. 95% confidence interval excluded the null value) on CL/F and V_c/F. No parameter was estimated with enough precision to be found conclusively clinically relevant (i.e. greater than 20% change from the reference population); however, some of the covariates could not be conclusively ruled out as being non-clinically relevant (Figure 5 and Figure 6).

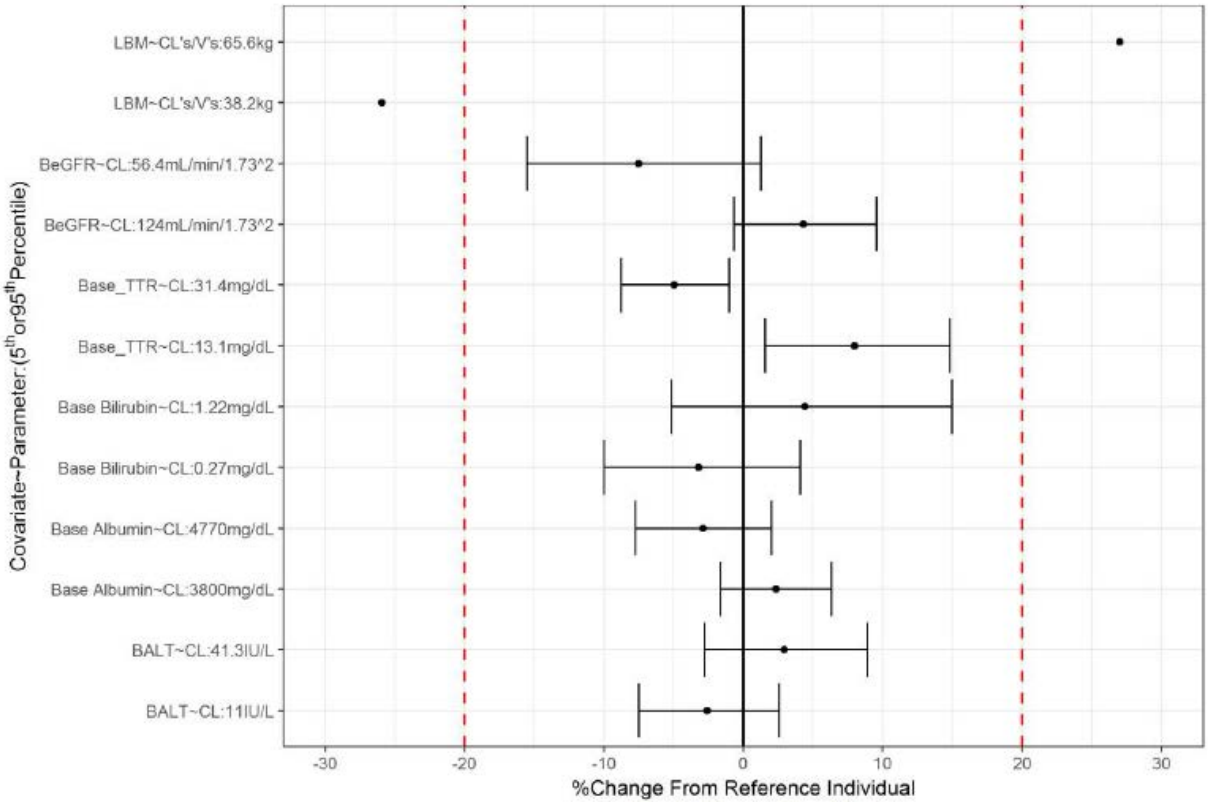
Figure 5: Clinical Significance Testing for Categorical Covariates in Full Model: Distribution of Percentage Change from Reference Individual



Note: vertical Red Dashed Lines: Boundaries representing the 20% clinical significance test; Closed Circle: Median percentage change from the reference category; Whiskers: 5*th* and 95*th* CI of the percentage change from the reference category; Abbreviations: CL is Apparent Clearance: CL/F, V_c is the Apparent Central Volume: V_c/F, Amer for America, AA for African American.

Source: Figure 9, PK01 report, Page 52, Module 5.3.3.5.

Figure 6: Clinical Significance Testing for Continuous Covariates in Full Model: Distribution of 5th and 95th Confidence Intervals of Percentage Change from Reference Individual.



Source: Figure 10, PK01 report, Page 53, Module 5.3.3.5.

Table 8 shows the estimates of parameters from the final population pharmacokinetic model.

Table 8: Parameter Estimates and Standard Errors from the Final Population Pharmacokinetic Model.

Parameter	Units	Estimate	%RSE	95% CI	Bootstrap Estimate	Bootstrap 95% CI
Clearance, CL/F	*L/h*	3.40	5.23	(3.05, 3.75)	3.44	(3.12, 3.79)
Central Volume, *Vc/F*	*L*	20.7	7.87	(17.5, 23.9)	21.5	(16.4, 30.6)
Absorption Rate Constant, *ka*	h^{-1}	0.261	7.73	(0.222, 0.301)	0.264	(0.228, 0.511)
Peripheral Volume, *Vp/F*	*L*	230	13.8	(167, 292)	234	(186, 300)
Inter-Compartmental Clearance, Q/F	*L/h*	0.266	9.61	(0.216, 0.315)	0.271	(0.227, 0.326)
Dose ~ Effect on Clearance		3.97	25.2	(2.01, 5.93)	4.21	(2.76, 6.37)
Lean Body Mass ~ CL/F, Q/F		1 (Fixed)	–	–	1 (Fixed)	–
Lean Body Mass ~ *Vc/F*, *Vp/F*		1 (Fixed)	–	–	1 (Fixed)	–
Disease State ~ CL/F		0.111	46.9	(0.00899, 0.214)	0.121	(0.026, 0.229)
Disease State ~ *Vc/F*		-0.284	31.4	(-0.459, -0.109)	-0.270	(-0.466, 0.117)
ω^2*Vc/F*		0.335	27.0	(0.158, 0.512)	0.301	(0.126, 0.491)
Covariance Vc/F~CL/F		0.0492	48.4	(0.00252, 0.0958)	0.0465	(0.00677, 0.0998)
ω^2CL/F		0.0712	21.3	(0.0414, 0.101)	0.0685	(0.0457, 0.105)
Covariance CL/F~Vp/F		-0.145	27.1	(-0.222, -0.0682)	-0.139	(-0.234, -0.0775)
ω^2 *Vp/F*		0.677	21.0	(0.399, 0.955)	0.654	(0.432, 0.955)
Residual Variability, σ^2		0.168	5.29	(0.151, 0.186)	0.167	(0.148, 0.187)
Bootstrap Success Rate, n = 1300		82.1%				

NOTE: Bootstrap "n" refers to the number of replicate datasets. 95% CI calculated as Estimate ± 1.96*Standard Error, %RSE = (Standa Error/Absolute Value (Estimate)) * 100%

Source: Table 13, PK01 report, Page 55, Module 5.3.3.5.

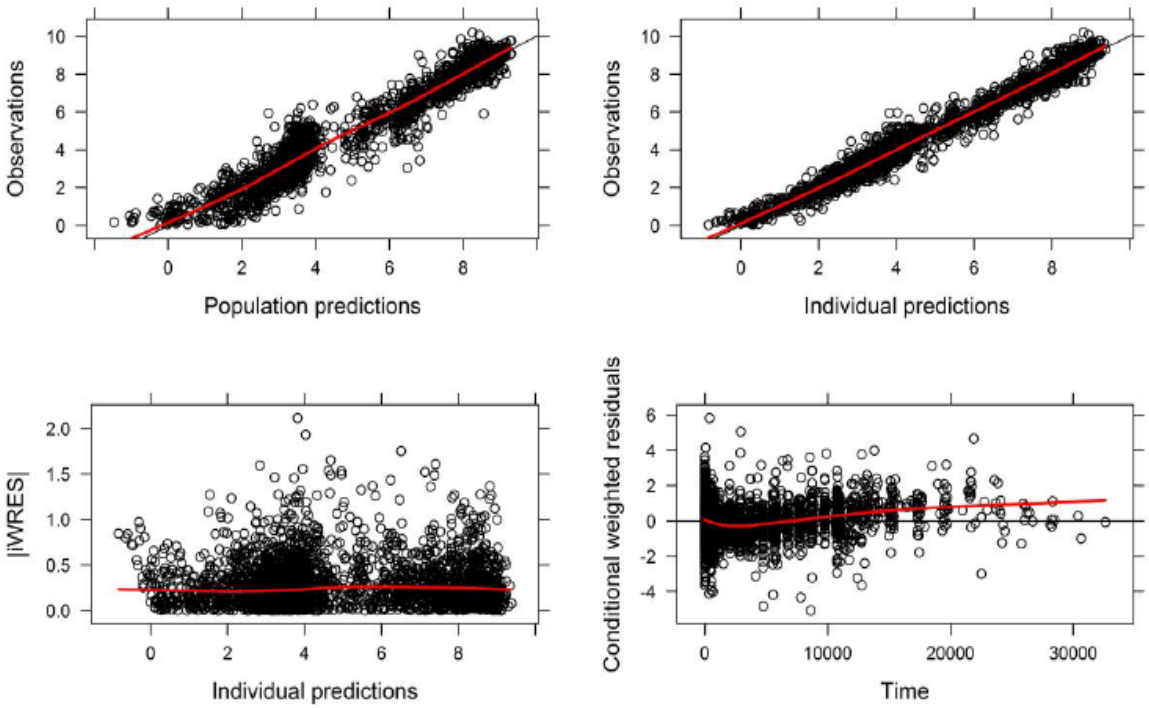
Reviewer Comment

The model parameters are in agreement with the estimated clearance and half-life from non-compartmental analysis in healthy volunteers.

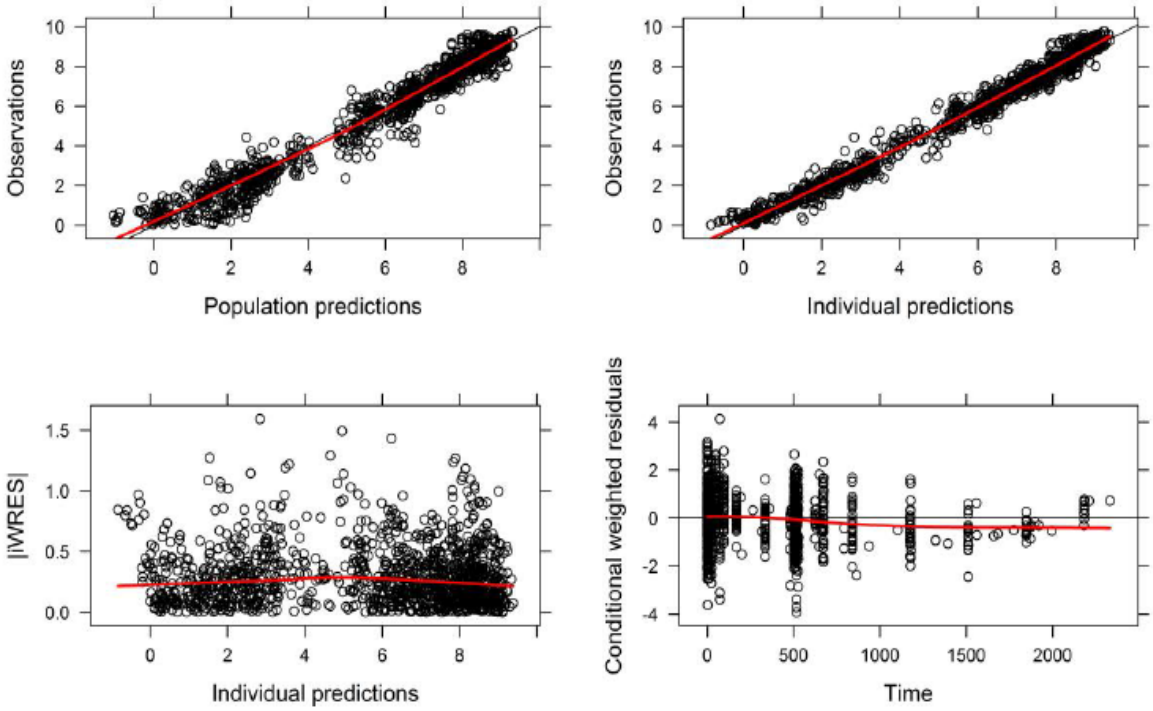
The goodness of fit plots for the overall population stratified by disease state (HV versus hATTR-PN) are shown in Figure 7.

Figure 7: Goodness of Fit Plots for the Final Population PK Model.

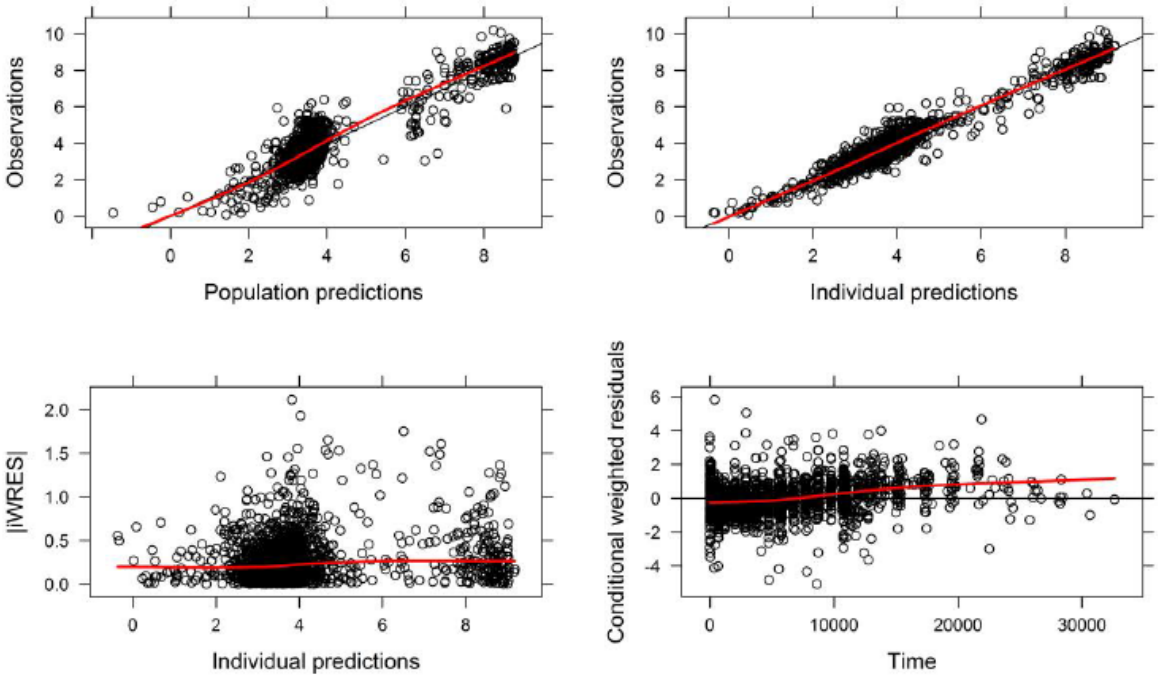
(a) Healthy Volunteers and hATTR-PN Patients



(b) Healthy Volunteers Only



(c) hATTR-PN Patients Only



Note: values are displayed as open circles with a red Loess fit line.
Source: Figure 11, PK01 report, Page 56-58, Module 5.3.3.5.

Reviewer Comment

The goodness-of-fit plots appear reasonable indicating that the model describes the data reasonably well.

Simulations

The final population PK model was used to simulate four different dosing regimens of inotersen. Table 9 shows the geometric mean along with 90% CI for $AUC_{0-\tau}$, C_{max}, and C_{trough} for Day 1 and steady-state. Simulated plasma trough concentrations for various dosing regimens are presented in Figure 8.

Table 9: Simulated Measures of Exposure for Additional Dosing Regimens.

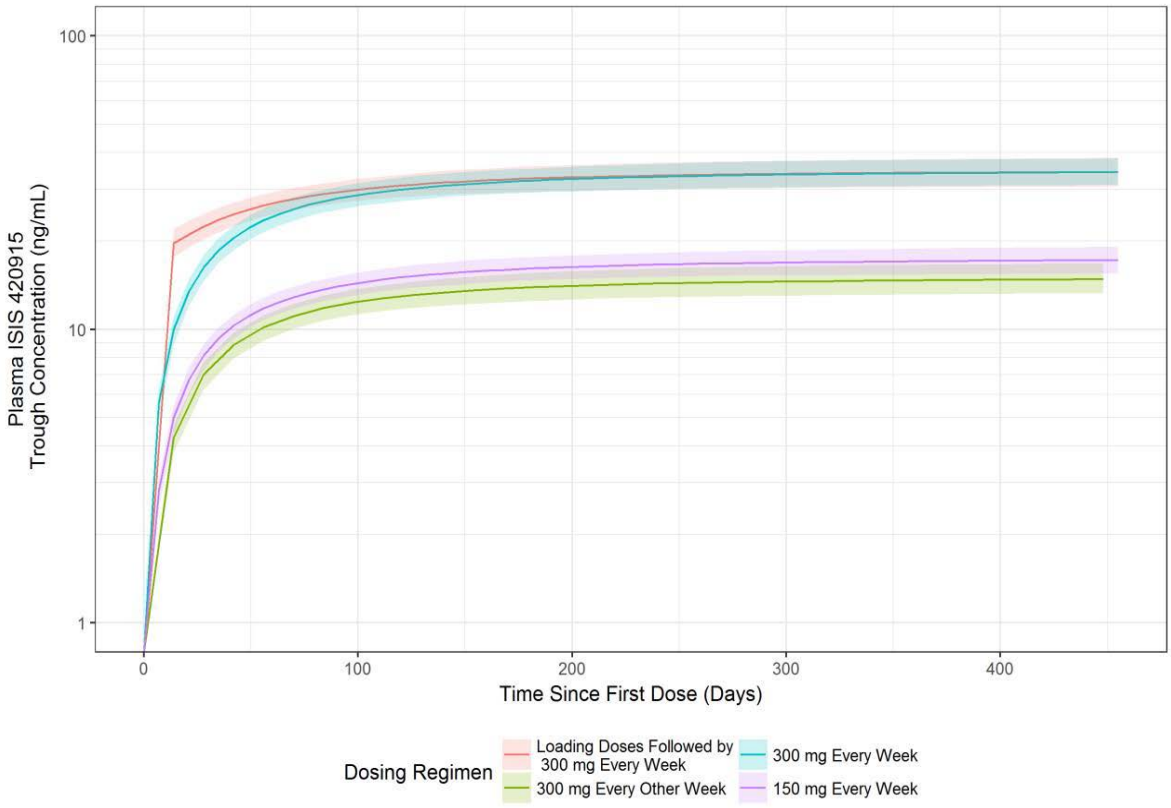
Regimen	Day/ Type	$AUC_{0-\tau}$ (µg*h/mL)	Cmax (µg/mL)	Ctrough (ng/mL
300 mg QW	1	84.4 (77.6, 91.6)	6.35 (5.61, 7.17)	5.62 (4.92, 6.24)
300 mg QOW	1	85.5 (78.4, 92.5)	6.33 (5.66, 7.18)	4.28 (3.82, 4.74)
Loading + 300 mg QW	1	85.5 (78.5, 92.8)	6.36 5.76, 7.26)	25.7[a] (20.9, 31.8)
150 mg QW	1	42.3 (38.8, 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)

NOTE: QW, QOW, and AR are shorthand for once weekly dosing, every other week dosing, and Accumulation Ratio.

[a] Ctrough after the dose on Day 1 is 48 h time point during loading dose period.

Source: Table 19, PK01 report, Page75, Module 5.3.3.5.

Figure 8: Simulated Plasma Trough Concentration Profile for ISIS 4020915 for Various Dosing Regimens.



Source: Figure 15, PK01 report, Page 76, Module 5.3.3.5.

Reviewer Comment

Use of 300-mg loading doses achieved trough levels of approximately 65% and 86% of the steady-state level after one and three months of 300-mg QW treatment, compared with 47% and 82%, respectively, without the use of loading doses.

Reviewer Analysis

Aim

To verify population pharmacokinetic analyses evaluating different intrinsic and extrinsic factors effects on the PK of inotersen.

Data

The dataset (\\cdsesub1\evsprod\nda211172\0001\m5\datasets\420915-ppk01\analysis\legacy\datasets\229880-nonmem-2017-07-28-v1-2-csv.txt) submitted by the applicant were used for the analysis.

Software

NONMEM® Version 7.3 was used for the analysis.

Analysis Strategy

- Execute the base and final population PK models to verify applicant reported pharmacokinetic parameters.
- Analyze other sources of information that support findings from population PK analysis.

Findings

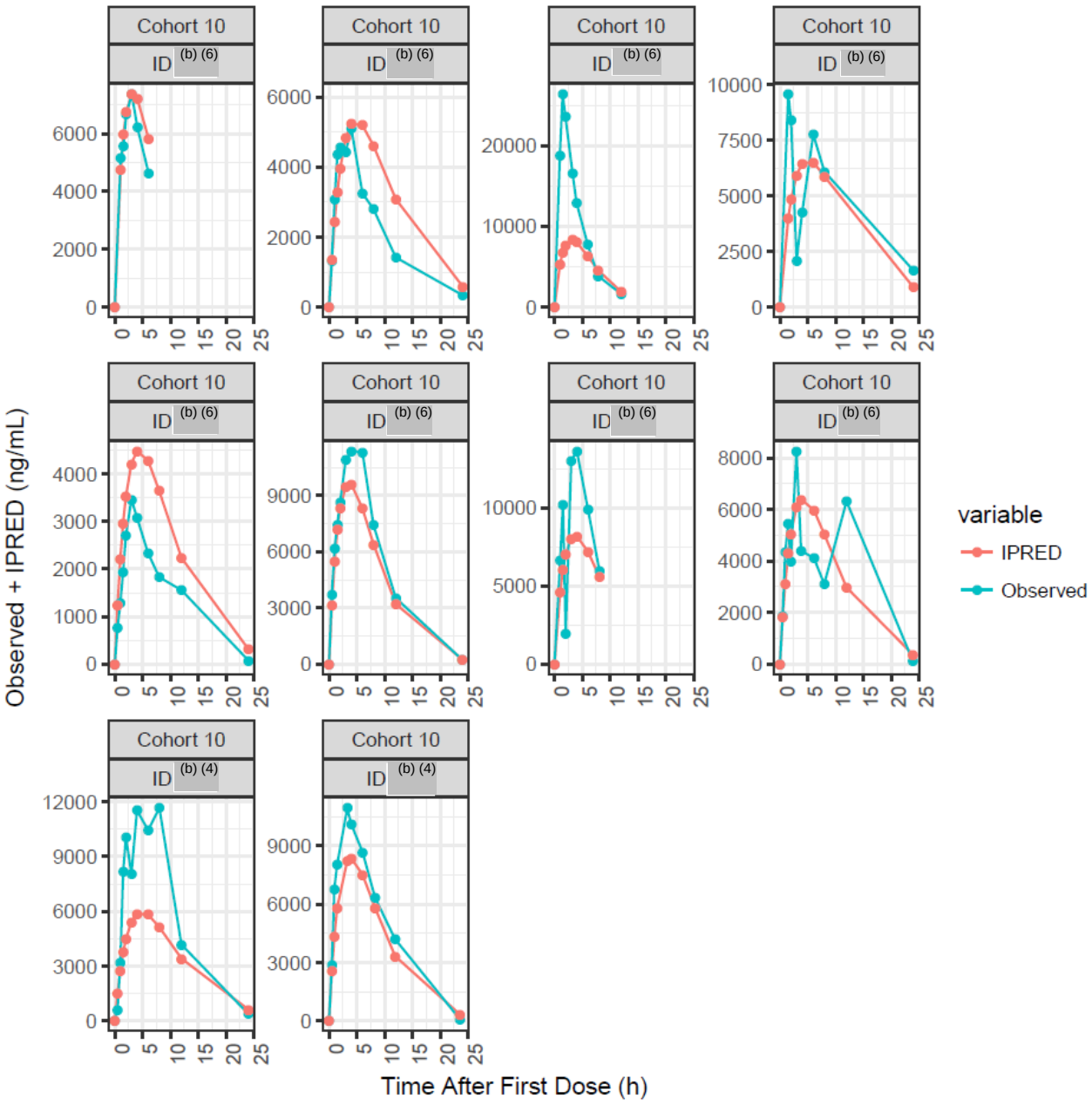
The reviewer confirmed the applicant's reported pharmacokinetic parameters from the base and final models. Additionally, the reviewer re-analyzed the data without exclusion of any data points except the BLQ data (M1 is considered an acceptable methodology given that its percentage is less than 5% of the whole dataset). As expected, similar parameter estimates were obtained.

Figure 9 shows the individual predictions (IPRED) time profile overlaid on the observed inotersen concentration time profile after the first dose for the PK subset in CS2.

Reviewer Comment

The findings of the population PK model are acceptable. However, C_{max} was underestimated in some subjects (Figure 9).

Figure 9: Individual Predictions/Observed Concentrations for Time 0-24 Hours for Subjects with Rich PK Sampling in CS2 Study.



Acceptability of applicant's proposed labeling statements regarding the influence of covariates (body weight, age, gender and race) on inotersen pharmacokinetics:

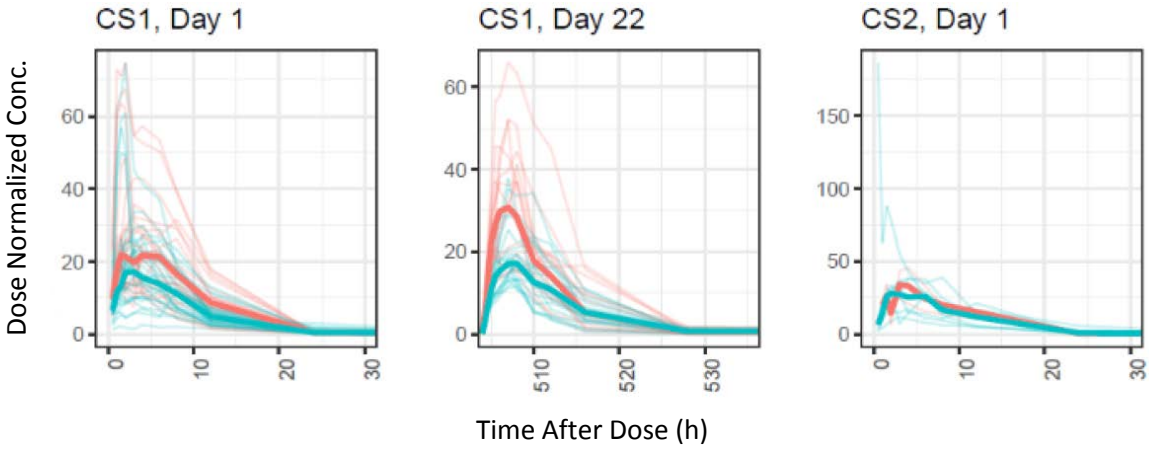
To address the adequacy of labeling statements, the reviewer conducted graphical analyses in addition to the model-based analyses that were done by the applicant (see above for the applicant's analysis).

Graphical Analyses:

Effect of Body Weight on Inotersen Pharmacokinetics

Figure 10 shows the individual level and median concentration-time profile of inotersen by body weight category in HV and hATTR-PN patients from CS1 and CS2 studies. Weight was categorized into 2 categories (≤73.5 kg and >73.5 kg) based on the overall median body weight.

Figure 10: Individual Level Inotersen Plasma Concentrations by Weight.

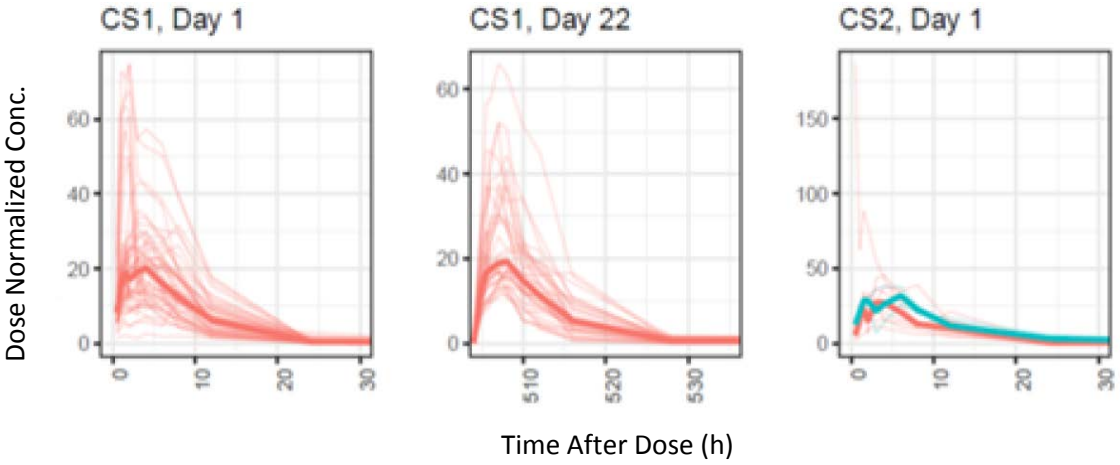


Weight Category: ≤73.5 [red] and >73.5 kg [blue]. Unit of Dose Normalized Conc. is ng/mL/mg.

Effect of Age on Inotersen Pharmacokinetics

Figure 11 shows the individual level and median concentration-time profile of inotersen by age category, in HV and hATTR-PN patients from CS1 and CS2 studies. Age was categorized into 2 categories (≤65 years and >65 years).

Figure 11: Individual Patient-Level Inotersen Plasma Concentrations by Age Category.

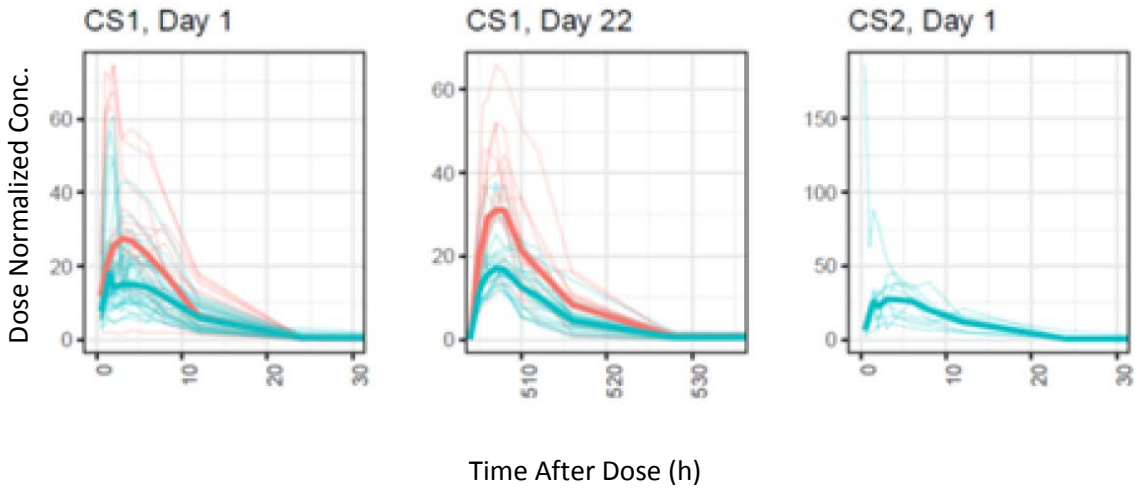


Age Category: ≤ 65 [red] and > 65 [blue] years. Unit of Dose Normalized Conc. is ng/mL/mg.

Effect of Sex on Inotersen Pharmacokinetics

Figure 12 shows the individual level and median concentration-time profile of inotersen by sex category in HV and hATTR-PN patients from CS1 and CS2 studies.

Figure 12: Individual Patient-Level Inotersen Plasma Concentrations by Sex Category.

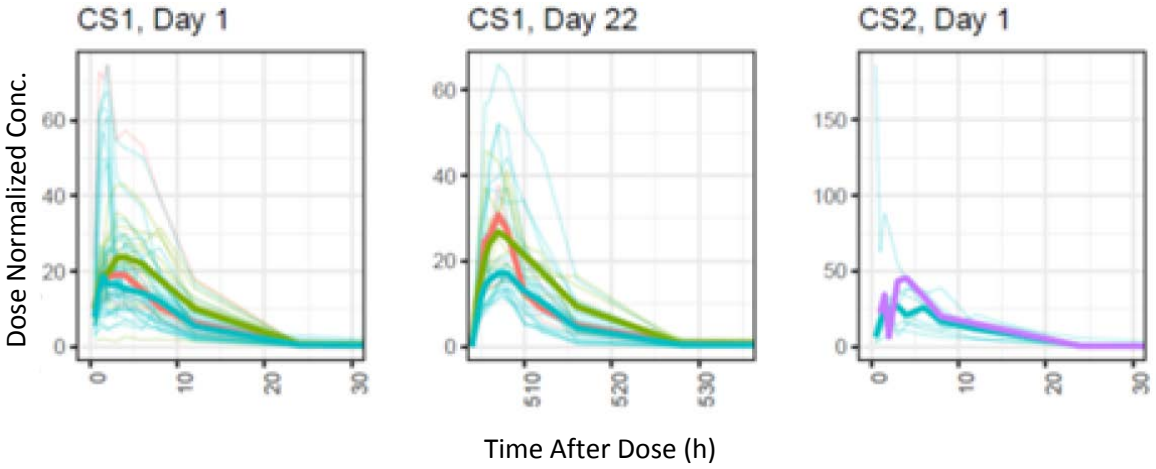


Sex Category: Female [red] and Male [blue]. Unit of Dose Normalized Conc. is ng/mL/mg.

Effect of Race on Inotersen Pharmacokinetics

Figure 13 shows the individual level and median concentration-time profile of inotersen by race category in HV and hATTR-PN patients from CS1 and CS2 studies.

Figure 13: Individual Patient-Level Inotersen Plasma Concentrations by Race Category.



Race Category: Asian [red], Black [green], Caucasian [blue], Others [purple]. Unit of Dose Normalized Conc. is ng/mL/mg.

Reviewer Comment

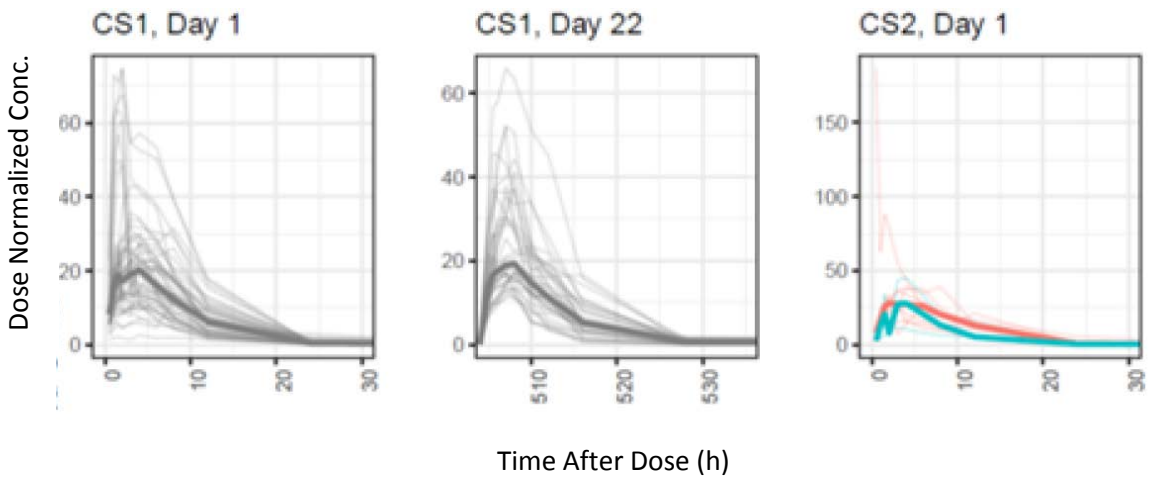
Consistent with the applicant's analyses, the above figures indicate the exposure profiles of inotersen are not affected by the evaluated covariates. Therefore, we agree with the applicant that dose adjustments are not needed based on body weight, age, sex or race.

Acceptability of applicant's proposed labeling statements regarding the influence of concomitant medications (diuretics, antithrombotic, and NSAID analgesics) on inotersen pharmacokinetics:

Effect of Diuretics on Inotersen Pharmacokinetics

Figure 14 shows the effect of concomitant administration of diuretics on the individual level and median concentration-time profile of inotersen in HV and hATTR-PN patients from CS1 and CS2 studies, respectively. PK profiles from study CS1 are also included to show the similarity in exposure between HV and hATTR-PN patients.

Figure 14: Individual Patient-Level Inotersen Plasma Concentrations by Concomitant Diuretics Administration.

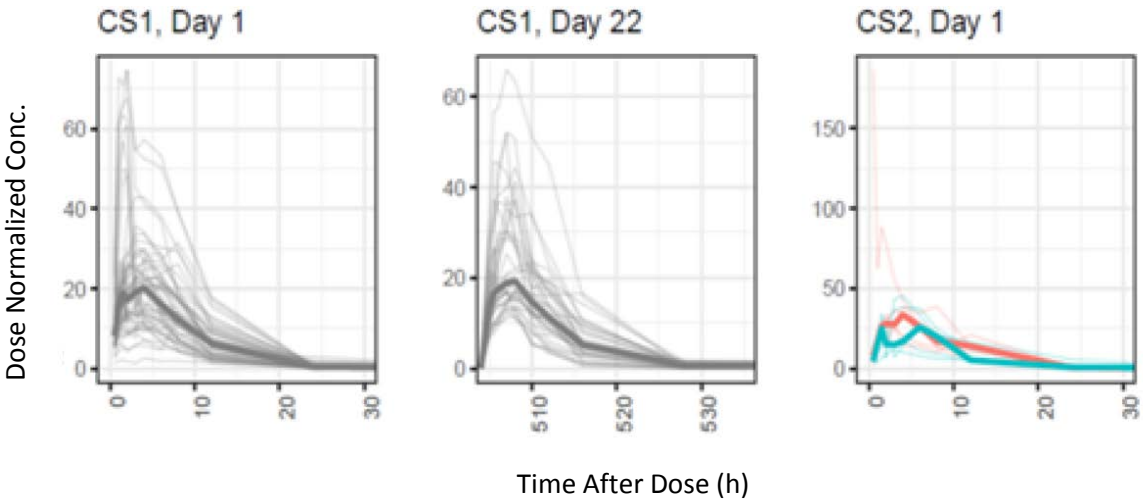


Concomitant Diuretics Administration [red: no diuretics, blue: diuretics, grey: NA]. Unit of Dose Normalized Conc. is ng/mL/mg.

Effect of Antithrombotic on Inotersen Pharmacokinetics

Figure 15 shows the effect of concomitant administration of antithrombotic on the individual level and median concentration-time profile of inotersen in HV and hATTR-PN patients from CS1 and CS2 studies.

Figure 15: Individual Patient-Level Inotersen Plasma Concentrations by Concomitant Antithrombotic Administration

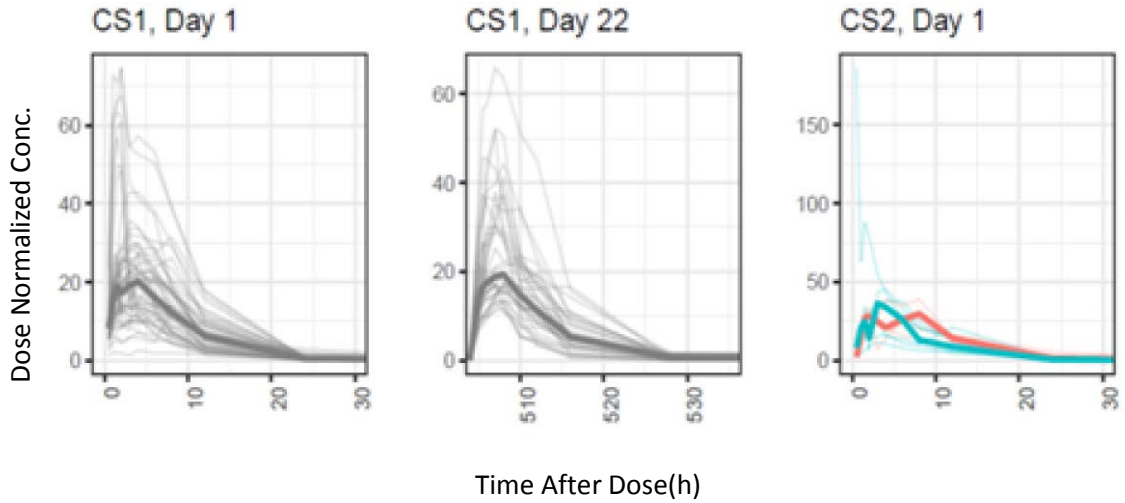


Concomitant Antithrombotic Administration [red: no antithrombotic, blue: antithrombotic, grey: NA]. Unit of Dose Normalized Conc. is ng/mL/mg.

Effect of Analgesics on Inotersen Pharmacokinetics

Figure 16 shows the effect of concomitant administration of analgesics on the individual level and median concentration-time profile of inotersen in HV and hATTR-PN patients from CS1 and CS2 studies.

Figure 16: Individual Patient-Level Inotersen Plasma Concentrations by Concomitant Analgesics Administration



Concomitant Analgesics Administration [red: no analgesics, blue: analgesics, grey: NA]. Unit of Dose Normalized Conc. is ng/mL/mg.

Reviewer Comment

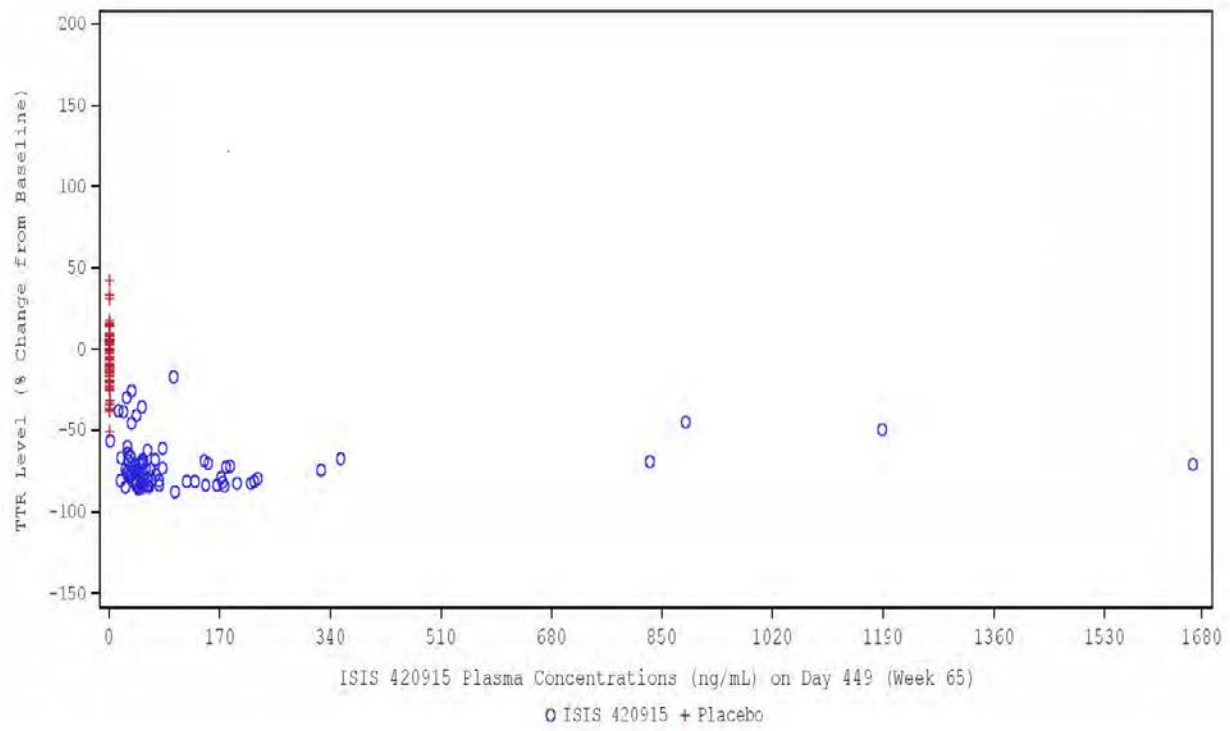
The applicant evaluated the effect of commonly used concomitant medications on inotersen plasma concentrations. While the applicant did not include information regarding dose and timing of concomitant medications in the analysis, it is expected that inclusion of such information would improve the description of drug interaction potential of these drugs with inotersen. Given the PK characteristics of inotersen and the results of the *in vitro* studies, the potential of drug-drug interactions is low.

4.3 Exposure-Response Analyses

Effects of inotersen on TTR Levels

Figure 17 shows the relationship between C_{trough} levels at Day 449 and serum TTR level at Day 449 (Week 65).

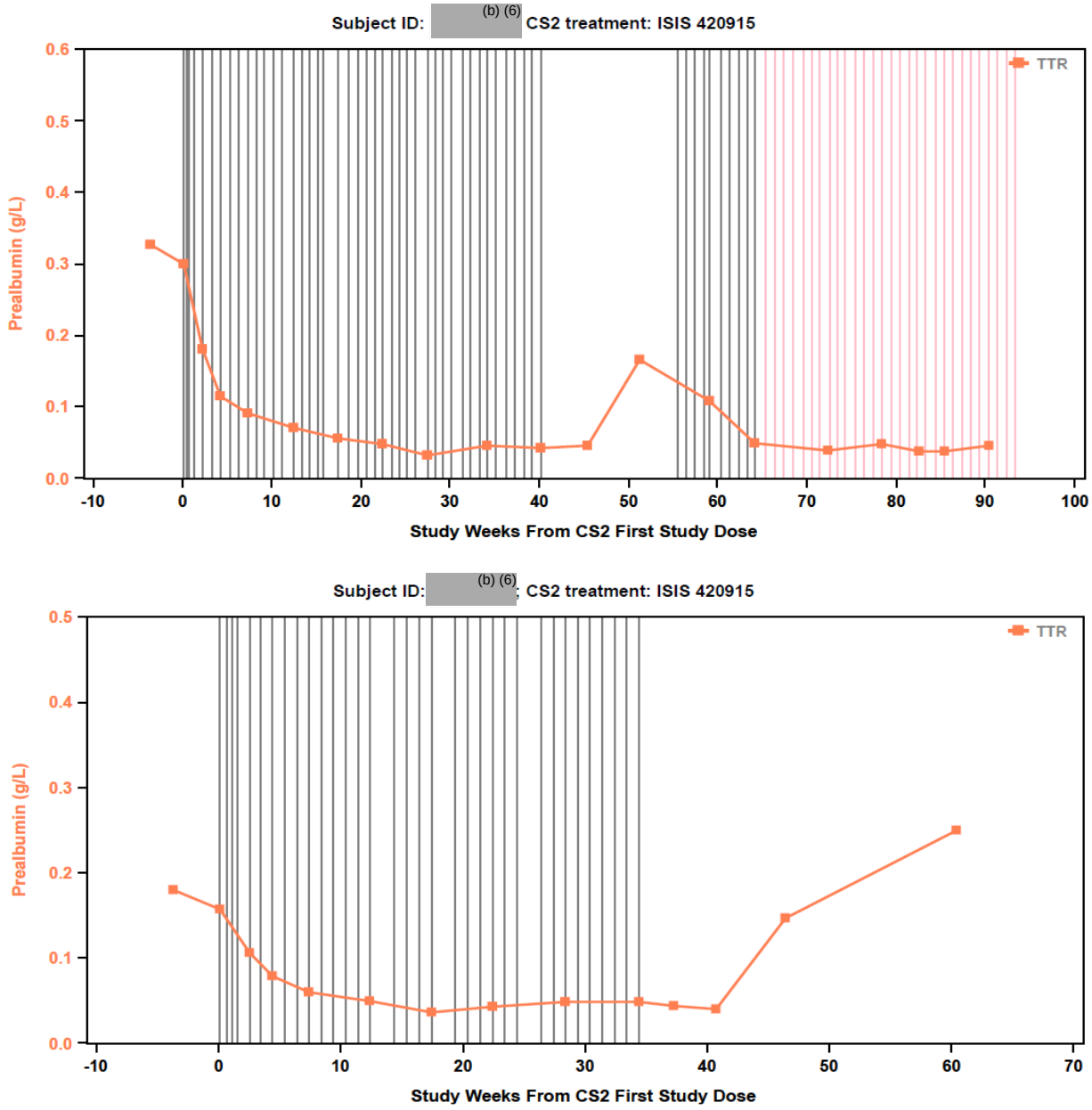
Figure 17: Scatter Plot of Transthyretin (TTR) Level (% Change from Baseline) versus Inotersen Plasma Concentrations at Day 449 (Week 65).

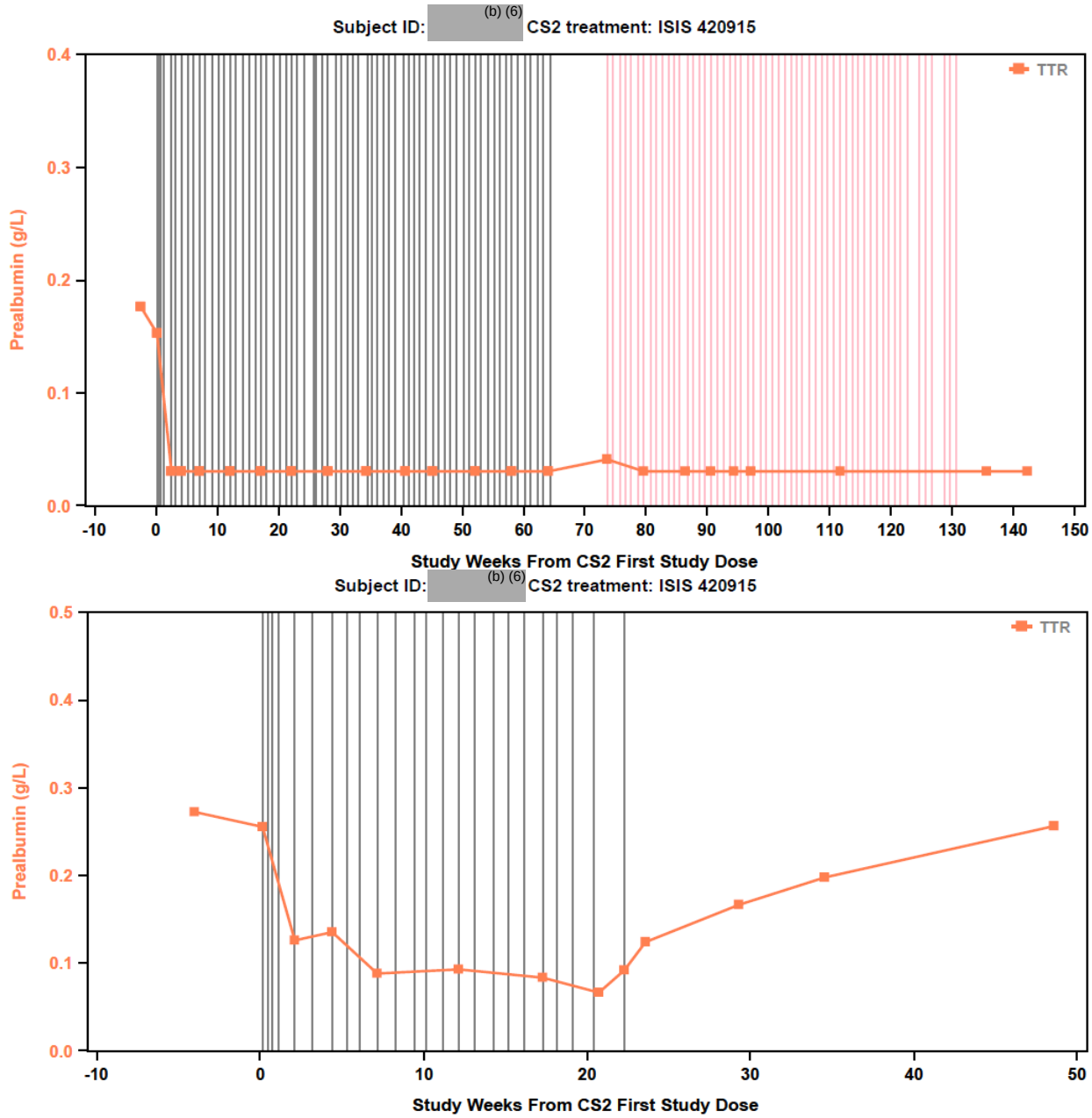


Source: Figure 3.19, CS2 Study report, Page 1880, Module 5.3.5.1.

In Studies CS2 and CS3, only one dosing regimen was tested (300 mg once weekly). We specifically looked at subjects who received less frequent doses in CS2 for any reason. Generally, these subjects showed sustained reduction in TTR levels after inotersen interruption/ discontinuation for at least 1 week. Figure 18 shows representative subjects.

Figure 18: TTR Levels over Time for Representative Subjects with inotersen Dose Interruption/ Discontinuation.





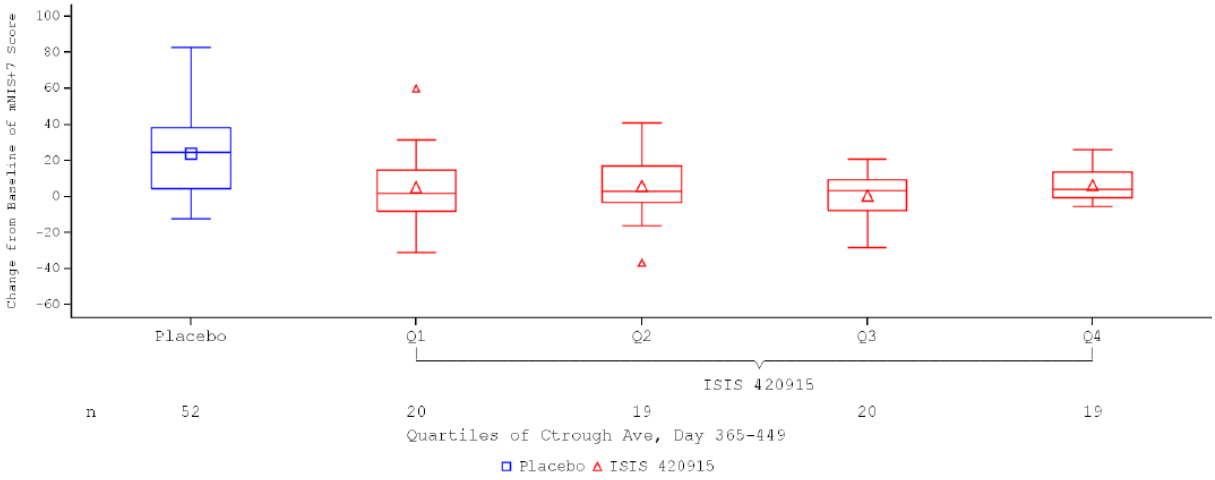
Results like "< 3.0" and ">75" are represented as "3.0" and "75".
Grey and pink lines represent the dosing records from CS2 study and CS3 study respectively. Solid and dash lines represent the dosing records for ISIS 420915 and placebo respectively. When the dose amount was less than the full dose, the length of the line was proportional the amount administered.

Source: Figure 6, Integrated Summary of Efficacy, Module 5.3.5.3.

Efficacy Endpoints: mNIS+7 Composite Scores and Norfolk QoL-DN Total Scores

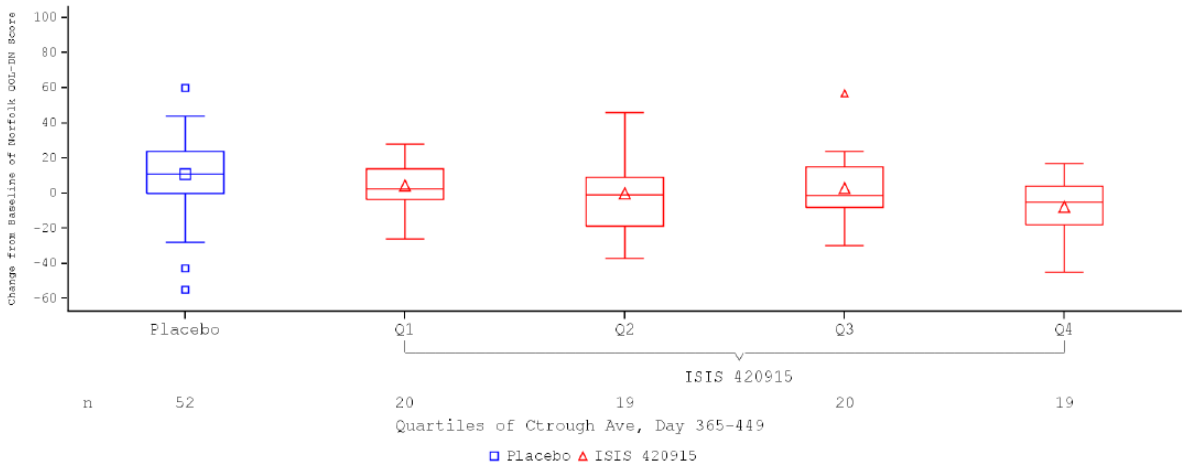
The relationship between change from baseline in mNIS+7 composite score (Figure 19) and change from baseline in Norfolk QoL-DN total score (Figure 20) at Week 65 versus C_{trough} average levels at Day 365 to Day 449 are shown below. No apparent relationship is observed, although the ability of the data to detect a relationship is limited because the applicant evaluated single dose level (300 mg once weekly) in CS2 study.

Figure 19: Box Plot of mNIS+7 score (Change from Baseline) on Week 66 by Quartiles of C_{trough} Ave, Day 365-449.



Source: Figure 3.23, CS2 Study report, Page 1890, module 5.3.5.1.

Figure 20: Box Plot of Norfolk QoL-DN score (Change from Baseline) on Week 66 by Quartiles of C_{trough} Ave, Day 365-449.

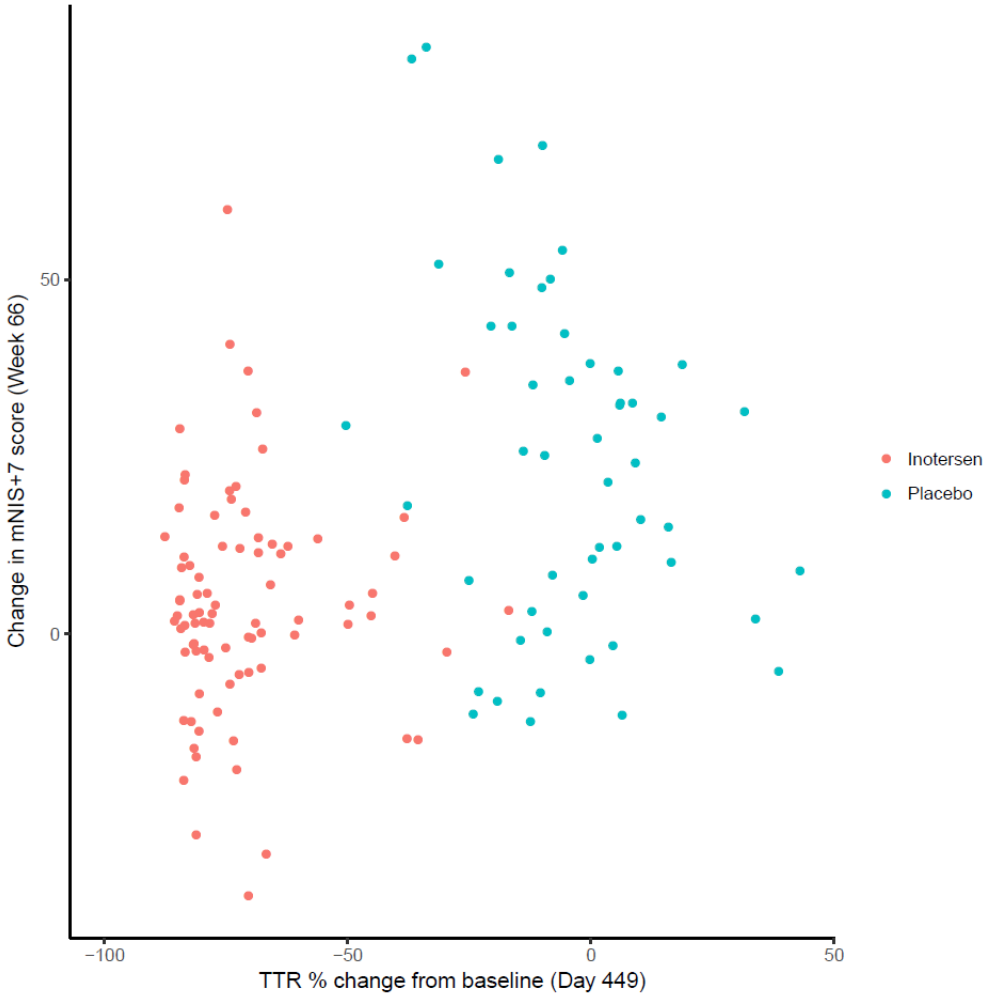


Source: Figure 3.27, CS2 Study report, Page 1898, module 5.3.5.1.

Correlation between TTR Levels and Efficacy Endpoints

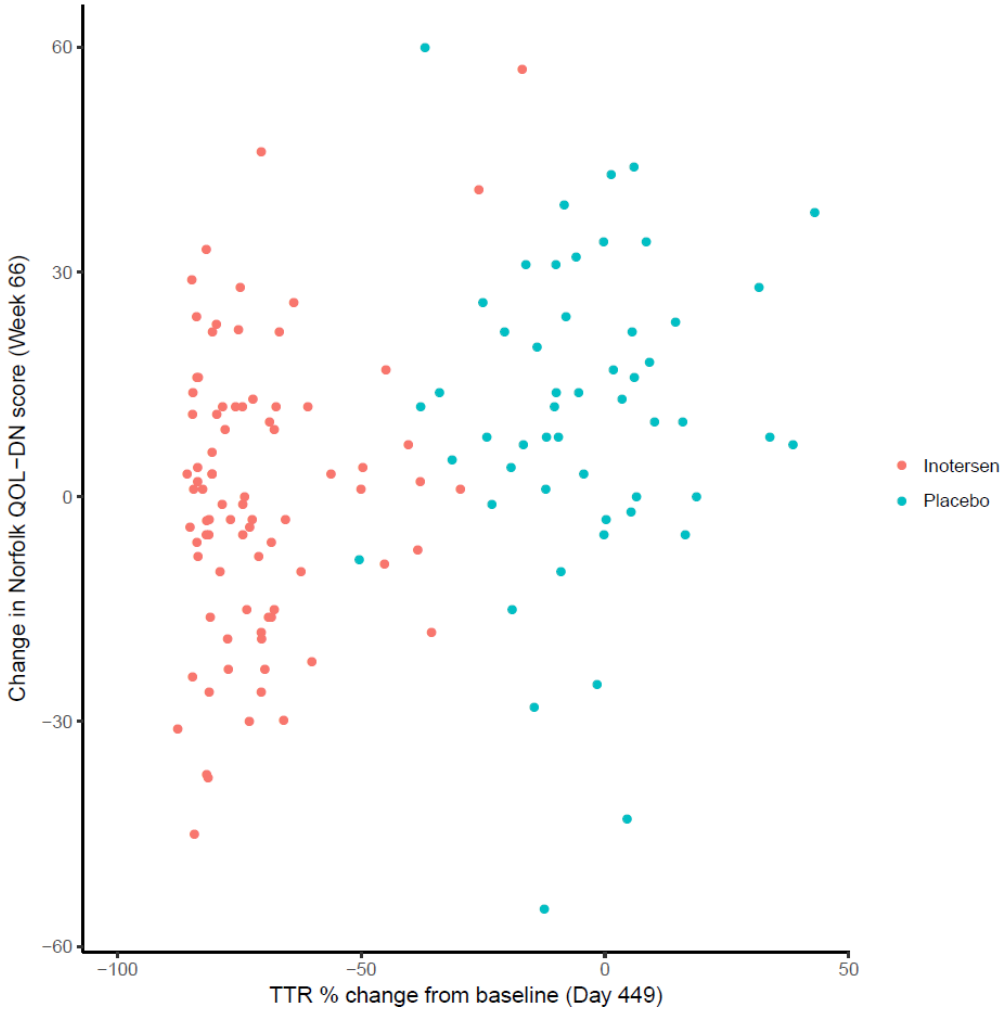
The associations between efficacy measures (mNIS+7 and Norfolk QOL-DN score) and TTR levels were explored. Figure 21 and Figure 22 show the association between Day 449 percent change of baseline TTR level and Week 66 mNIS+7 and Norfolk QoL-DN change from baseline.

Figure 21: Association between Day 449 percent of baseline TTR Level and Week 66 mNIS+7 Change from Baseline.



Source: FDA analysis

Figure 22: Association between Day 449 percent of baseline TTR Level and Week 66 Norfolk QOL-DN Score Change from Baseline.



Source: FDA analysis

Reviewer Comment

It is not known how change from baseline in TTR are associated with clinically meaningful effects on mNIS+7 composite score and Norfolk QoL-DN total score.

Effect of Inotersen on Platelet Count

Reduction in platelet count was observed in CS2 study, and thrombocytopenia was identified as a treatment emergent adverse event with inotersen. Based on confirmed central and local laboratory values, platelet abnormalities below the LLN (<140 x 10^9/L) at any time while on study occurred at a higher incidence in the inotersen group (~54%) compared with the placebo group (~13%) in CS2. A higher proportion of subjects in the inotersen group also had a confirmed decrease in platelets ≥30% or ≥50% from baseline compared with subjects in the placebo group (Table 10). Three inotersen-treated subjects in Study CS2 developed confirmed Grade 4 thrombocytopenia prior to implementation of the proposed monitoring rules of platelet count. Grade 4 thrombocytopenia was effectively managed with corticosteroid treatment in addition to discontinuation of inotersen in 2 of these cases. However, the third subject presented with an intracranial hemorrhage and died before treatment could be administered.

Table 10: Subjects with Platelet Abnormalities (Central and Local Laboratory Values).

	CS2 (On-Study, Safety Set)		CS3 (On-Study, Safety Set)		Inotersen Integrated Set
	Placebo (N=60)	**Inotersen 300 mg (N=112)**	**Placebo-Inotersen (N=40)**	**Inotersen-Inotersen (N=74)**	**Inotersen 300 mg (N=152)**
Any value <140 x 10^9/L, n (%)	10 (16.7)	62 (55.4)	21 (52.5)	47 (63.5)	93 (61.2)
Confirmed Value, n (%)					
≥30% Decrease from Baseline/ Inotersen Baseline	3 (5.0)	79 (70.5)	25 (62.5)	53 (71.6)	111 (73.0)
≥50% Decrease from Baseline/ Inotersen Baseline	1 (1.7)	20 (17.9)	10 (25.0)	21 (28.4)	44 (28.9)
<140 x 10^9/L	8 (13.3)	60 (53.6)	19 (47.5)	43 (58.1)	86 (56.6)
<100 x 10^9/L	1 (1.7)	26 (23.2)	13 (32.5)	23 (31.1)	48 (31.6)
<75 x 10^9/L	0	12 (10.7)	3 (7.5)	8 (10.8)	20 (13.2)
<50 x 10^9/L	0	3 (2.7)	0	1 (1.4)	4 (2.6)
<25 x 10^9/L	0	3 (2.7)	0	0	3 (2.0)
Source: Table 69, Clinical Safety Summary, Page 132-133, Module 2.7.4.					

Note: an initial laboratory value was confirmed by the next available laboratory result performed on a different day and within 7 days of the initial value. If there was no retest within 7 days, then the initial value was presumed confirmed. Central laboratory and local laboratory values were included in this summary.

Effect of Dose Pausing/Dose Reduction on Platelet Count

Forty-eight subjects had confirmed platelet count of <100 x 10^9/L. In about 11.2% of subjects overall (9.8% in CS2 and 8.8% in CS3), the platelet count fell below 75×10^9/L (the dose pause threshold), and dosing of inotersen was paused until the platelet count had recovered to >100×10^9/L. Inotersen was reinitiated following dose pause in the majority of these subjects either at a full dose or reduced dose (see Table 11 and Table 12). In a total of 9 subjects, dose was reduced at certain time point to maintain platelet counts at above 75×109/L.

Table 11: CS2 Subjects Who Paused Dosing Due to Platelet Count <75×10^9/L.

Subject Number[a]	Number of Dose Pauses	Week Number for Dose Pauses	Length of Pause (Weeks)	Dosing Reinitiated (Yes/No)/ Dose on Reinitiation[b]
(b) (6)	1	18-29	12	No
	1	19-21	3	Yes/Full Dose
	4	31-35 37-40 56-58 61-63	5 4 3 3	Yes/Full Dose
	2	28-31 35-41	4 7	Yes/Reduced Dose
	2	22-28 55-64	7 10	Yes/Reduced Dose
	2	48-54 62-65	7 4	Yes/Full Dose
	1	17-19	3	Yes/Full Dose
	1	43-46	4	Yes/Full Dose
	1	58-62	5	Yes/Full Dose
	3	30-31 36 44	2 1 1	Yes/Full Dose
	1	60	1	Yes/Full Dose

a. The 3 subjects who permanently discontinued study drug due to CTCAE Grade 4 thrombocytopenia were not included. b. Reduced dose was 150 mg/week.
Source: Table 70, Clinical Safety Summary, Page 134, Module 2.7.4.

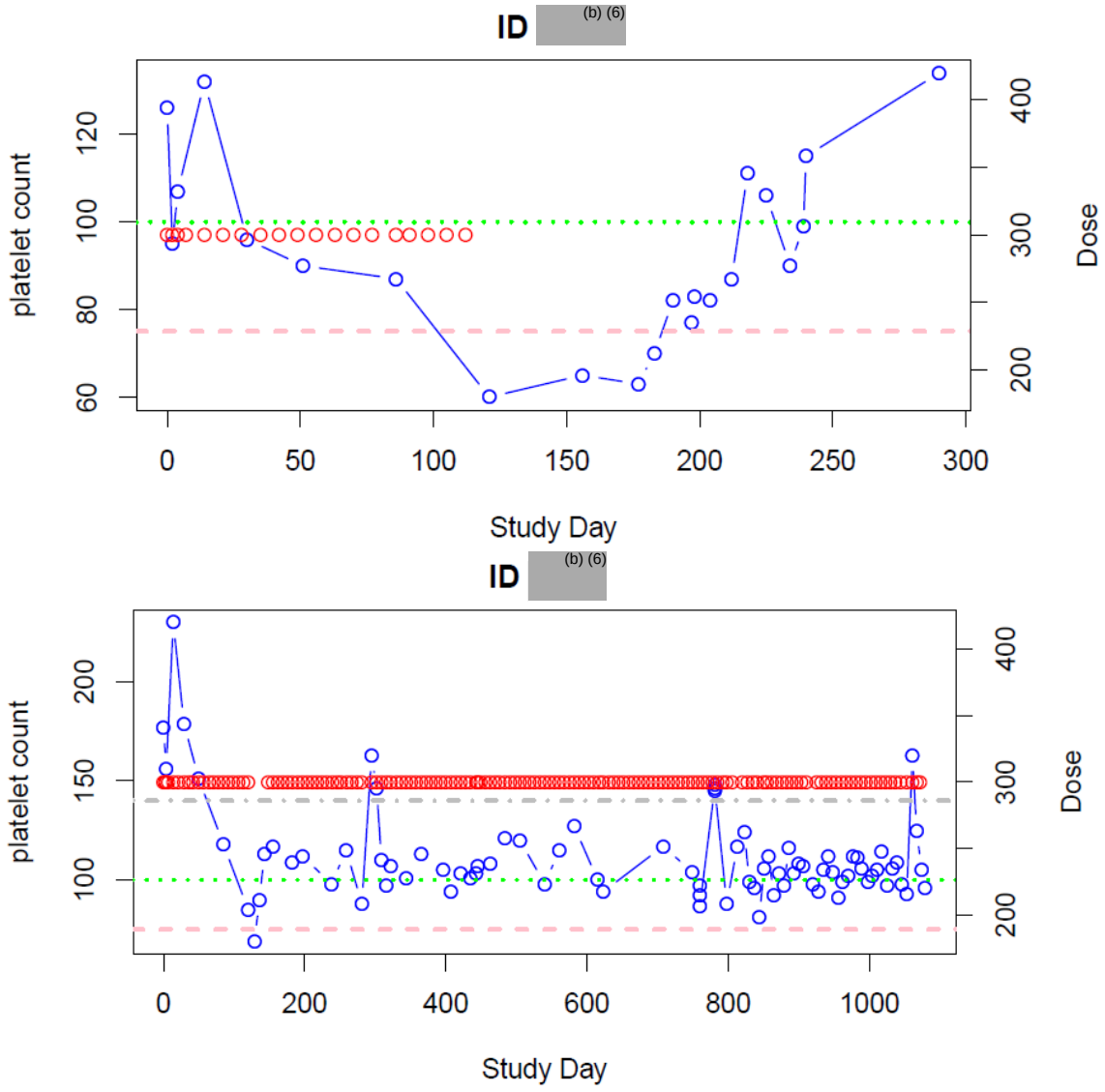
Table 12: CS3 Subjects Who Paused Dosing Due to Platelet Count<75×10^9/L.

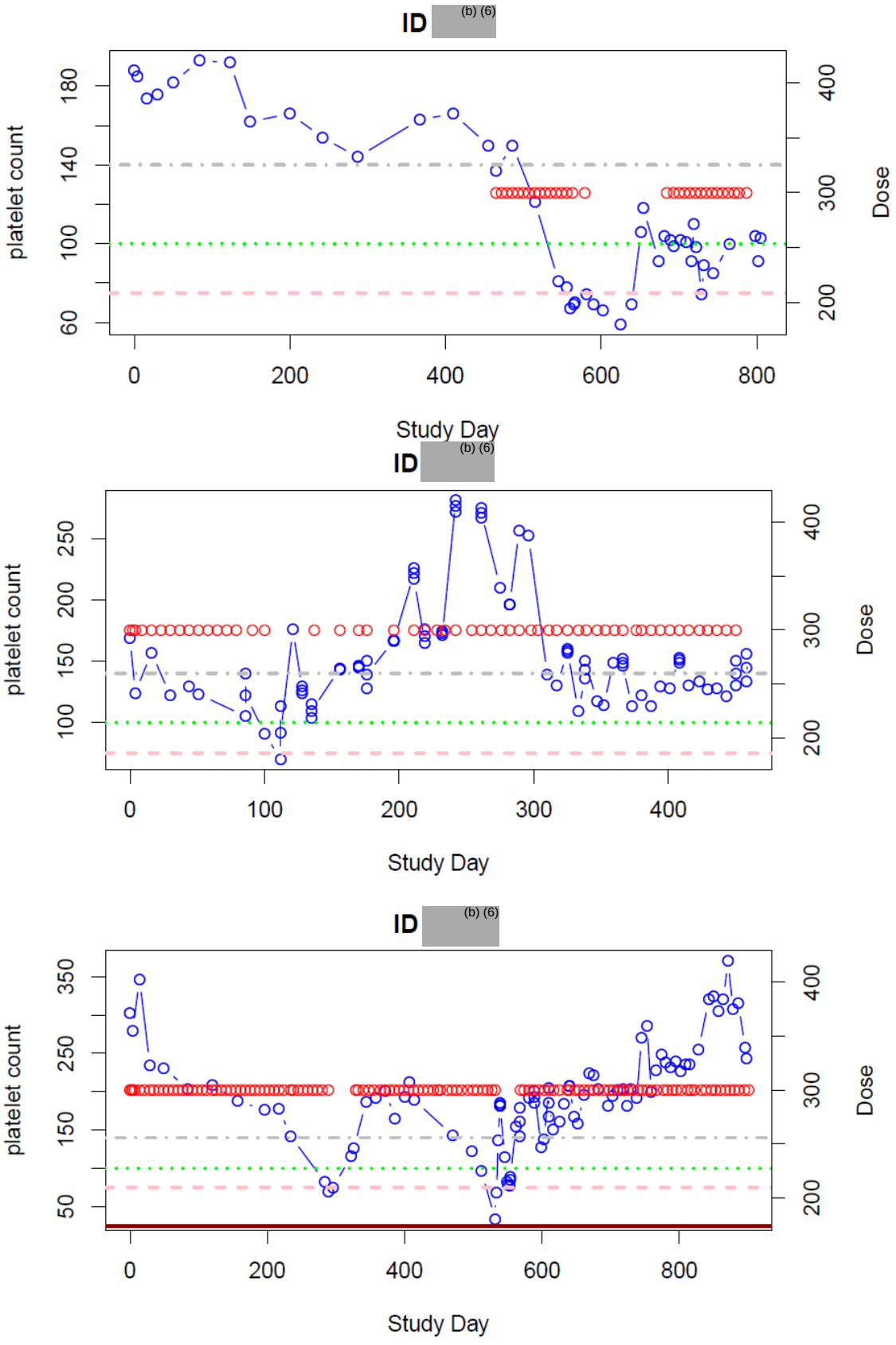
Subject Number	Number of Dose Pauses	Week Number for Dose Pauses	Length of Pause (Weeks)	Dosing Reinitiated (Yes/No)/ Dose on Reinitiation[a]
(b) (6)	1	46-52	7	No
	1	31-44	14	Yes/Reduced Dose
	3	17 86-87 105	1 2 1	Yes/Reduced Dose
	3	7-16 20-24 44-57	10 5 14	Yes/Reduced Dose
	2	16 18-27	1 10	Yes/Full Dose
	3	48-51 55-58 101-119	4 4 19	Yes/Reduced Dose
	1	13-16	4	Yes/Full Dose
	1	29-37	9	Yes/Full Dose
	2	51-68 87-103	18 17	Yes/Reduced Dose
	6	50-52 54-63 74-81 93-100 105-109 129-133	3 10 8 8 5 5	Yes/Reduced Dose

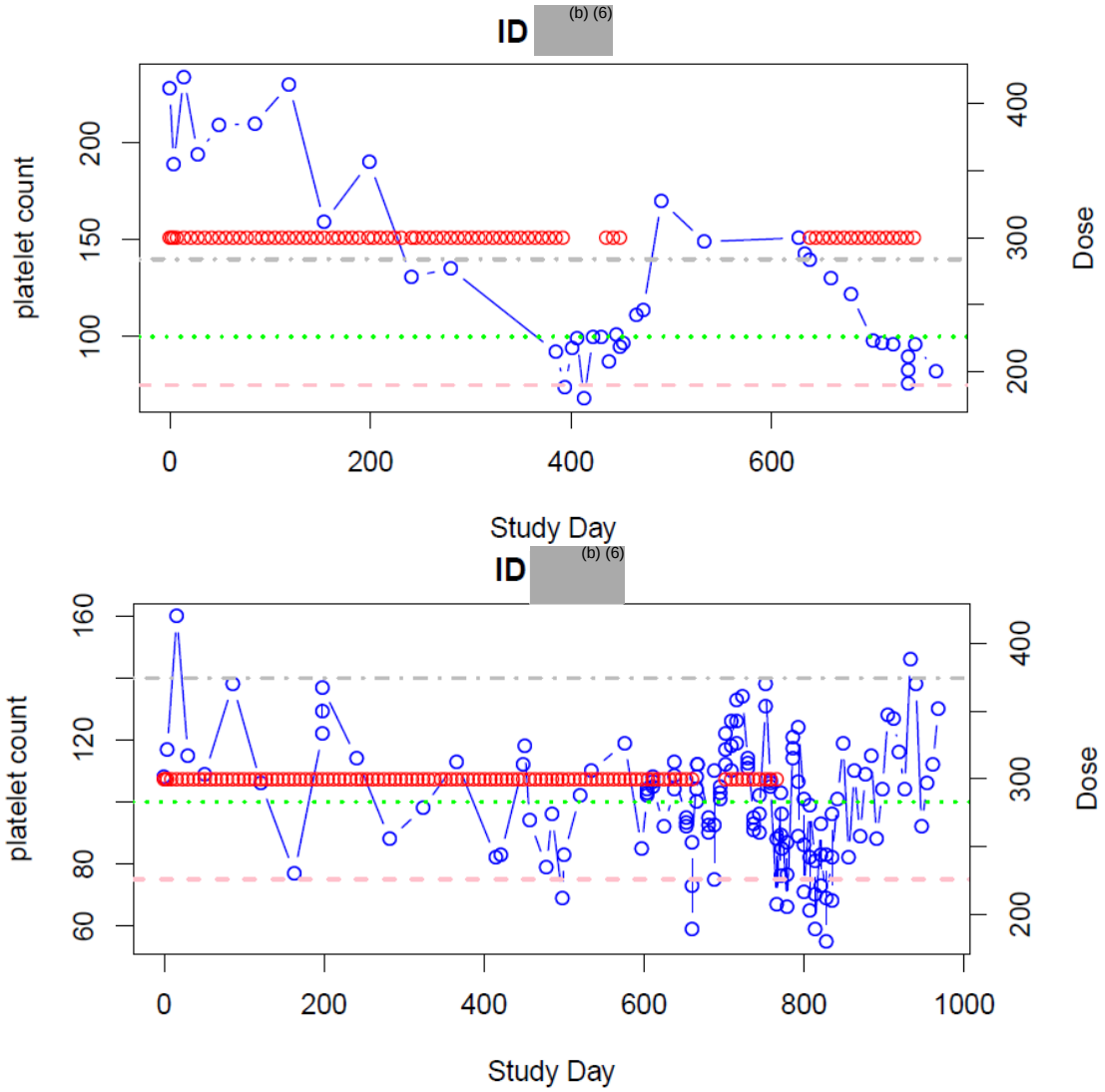
Source: Table 71, Clinical Safety Summary, Page 135, Module 2.7.4,
a. Reduced dose was 150 mg/week (Subject (b) (6) received 150 mg every 2 weeks). b. Subject was also dose paused for platelet count <75 x 109/L in CS2

In 7 subjects, thrombocytopenia was managed through dose pausing only. These are included in Figure 23 below. Figure 24 shows platelet count over time during CS2 and CS3 in relation to inotersen dosing in selected subjects who had both dose pause and dose reduction because of thrombocytopenia.

Figure 23: Platelet Count Over Time in Subjects with Dose Pause.

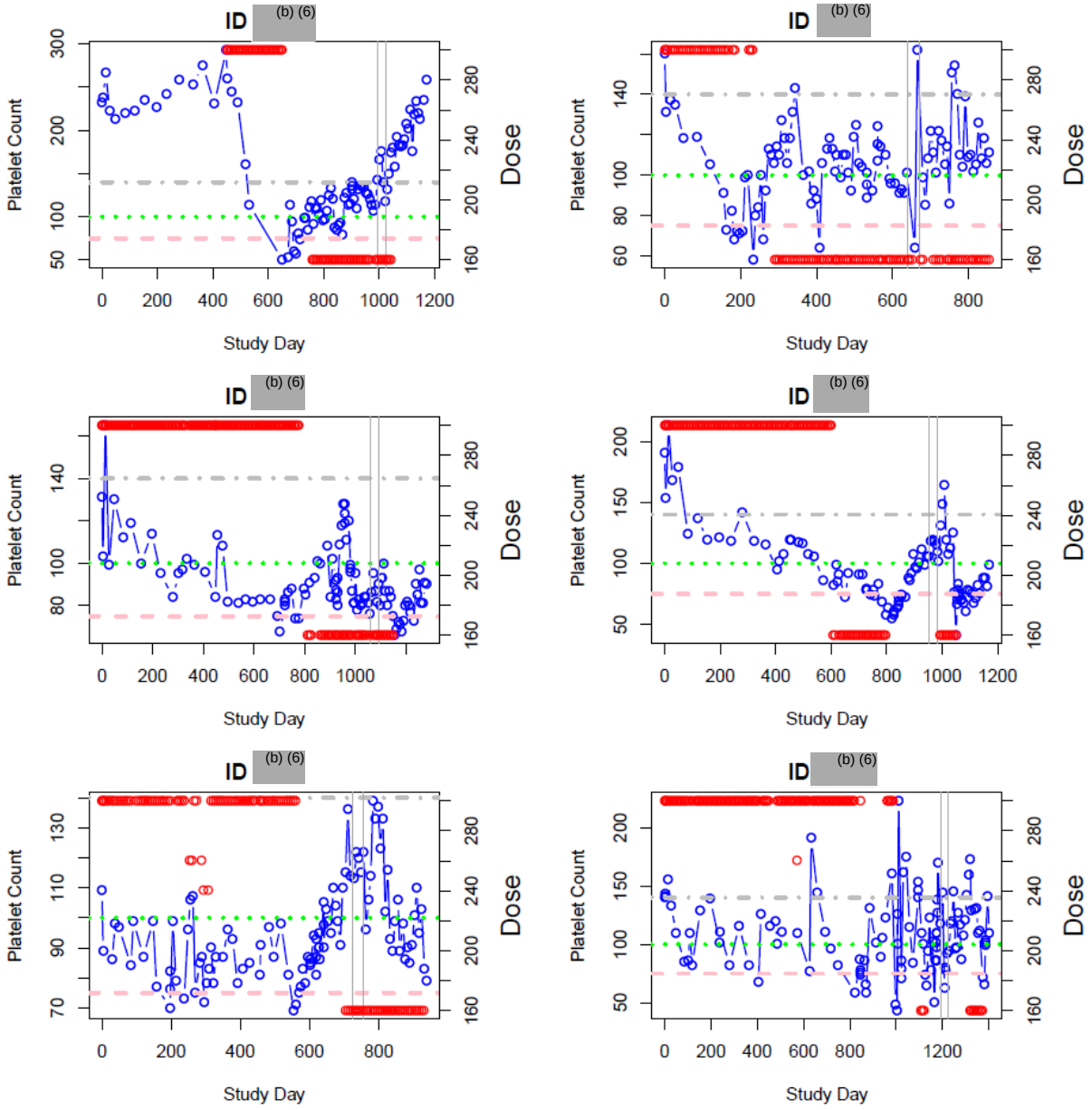


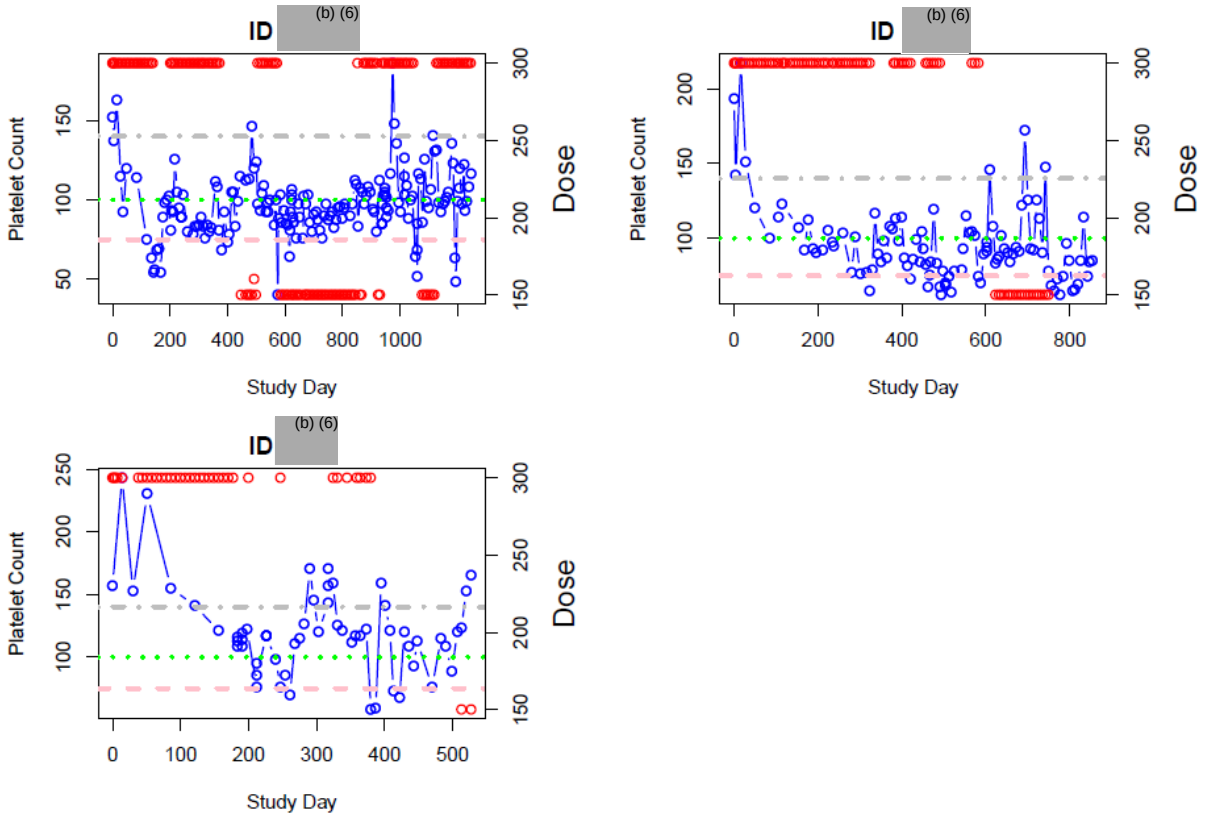




Note: Blue is platelet count (x10^9/L), red is the inotersen dose. Horizontal lines are 140, 100, and 75 (x10^9/L) of platelet count.

Figure 24: Platelet Count Over Time in Subjects with Dose Reduction.



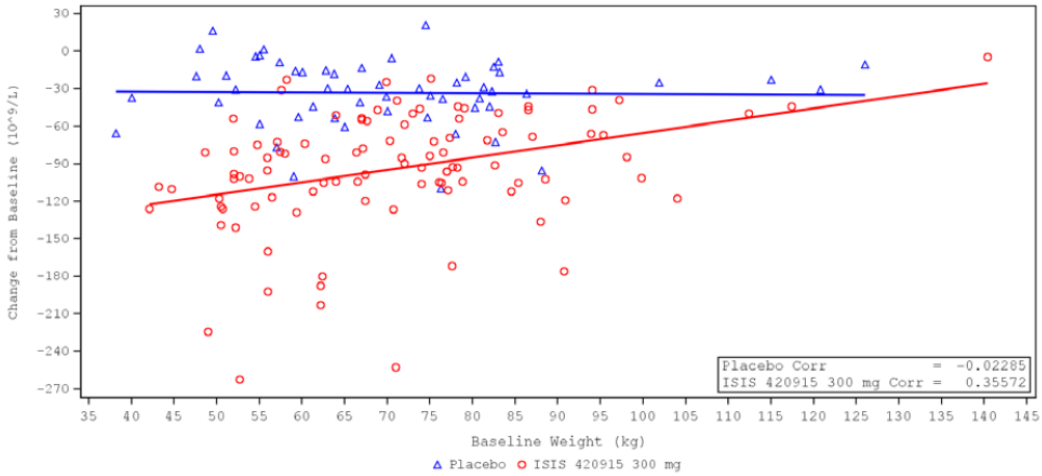


Note: Blue is platelet count (x10^9/L), red is the inotersen dose, grey vertical lines indicates the start and end of corticosteroid treatment. In 6 out of these 9 subjects, corticosteroids were administered. Horizontal lines are 140, 100, and 75 (x10^9/L) of platelet count.

Effects of Subject's Baseline Characteristics on Magnitude of Platelet Count Reduction

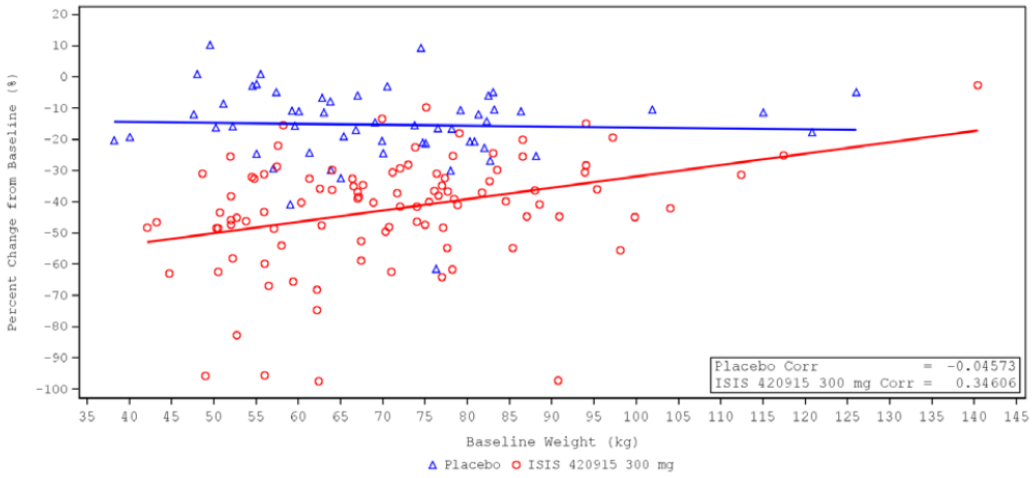
The applicant investigated the effect of subject's baseline characteristics such as age, sex and weight on magnitude of platelet count decline. Most patients' characteristics did not influence the magnitude of platelet count reduction. Analysis of the effect of body weight shows that overall, lower body weight is associated with larger absolute and percent reduction in platelet count (Figure 25 and Figure 26). Severe thrombocytopenia occurred in 3 subjects. These subjects had baseline body weight evenly distributed from 49 to 90 kg.

Figure 25: Scatter Plot for Maximum Change of Platelet from Baseline vs. Baseline Body Weight (On-Treatment).



Source: Figure 5, 420915-CR01: Report on Investigation into Platelet Count Reductions in the Inotersen CS2 Clinical Program, Page: 26.

Figure 26: Scatter Plot for Maximum Percent Change of Platelet from Baseline vs. Baseline Body Weight (On-Treatment).



Source: Figure 6, 420915-CR01: Report on Investigation into Platelet Count Reductions in the Inotersen CS2 Clinical Program, Page 26.

Possible Underlying Mechanism of Platelet Count Reduction

The applicant's investigations failed to find a single cause or risk factor that could be used for risk stratification purposes. Several causes for thrombocytopenia (including a classical heparin-induced thrombocytopenia-type mechanism, bone marrow dysfunction, consumptive coagulopathies such as disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, and thrombotic microangiopathy and sepsis, platelet activation, and systemic complement activation) were excluded based on the applicant's analyses.

Applicant's analyses indicated that an immune-based mechanism seems likely in the Grade 4 thrombocytopenia cases, but an immune mechanism is less clear for the less severe decline in platelet counts.

The applicant evaluated Ionis Integrated Safety Database of clinical trial data from subjects treated with 2'-MOE ASOs. Results show that severe reductions in platelet count are likely dose and sequence dependent[1]. Per that analysis, the percentage of subjects who experienced decreases in platelet counts >30% from BSLN was 1.7% in placebo and averaged 8.7% in ASO-treated subjects and increased with increasing 2'-MOE ASO dose (Table 13). For more details, please refer to Crooke ST et al., 2017.

[1] Crooke, Stanley T., Brenda F. Baker, Joseph L. Witztum, T. Jesse Kwoh, Nguyen C. Pham, Nelson Salgado, Bradley W. McEvoy et al. "The Effects of 2'-O-Methoxyethyl Containing Antisense Oligonucleotides on Platelets in Human Clinical Trials." nucleic acid therapeutics 27, no. 3 (2017): 121-129.

Table 13: Incidence of Confirmed Platelet Reductions Across All Trials, Phase 1 to Phase 3 (Crooke et al., 2017).

Post-BSLN platelet count Confirmed, n (%)			2'-MOE ASO dose, mg/week					
	Placebo	ASO total	>0–75	>75–175	>175–275	>275–375	>375–475	>475
(A) n	749	2,249	158	344	1,231	257	219	40
LLN-100 $x10^9$/L	13 (1.7)	109 (4.8)	1 (0.6)	6 (1.7)	68 (5.5)	17 (6.6)	14 (6.4)	3 (7.5)
<100–75 $x10^9$/L	0 (0)	6 (0.3)	0 (0)	0 (0)	4 (0.3)	1 (0.4)	1 (0.5)	0 (0)
<75–50 $x10^9$/L	1 (0.1)	1 (0.04)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.5)	0 (0)
<50–25 $x10^9$/L	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<25 $x10^9$/L	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
(B) n	777	2,363	161	361	1,300	267	233	41
<0.7· BSLN	13 (1.7)	205 (8.7)	2 (1.2)	10 (2.8)	121 (9.3)	37 (13.9)	31 (13.3)	4 (9.8)
<0.5· BSLN	2 (0.3)	20 (0.8)	0 (0)	0 (0)	10 (0.8)	1 (0.4)	9 (3.9)	0 (0)
n	777	2,368	161	362	1,303	267	234	41
<75 $x10^9$/L	1 (0.1)	4 (0.2)	0 (0)	0 (0)	1 (0.1)	1 (0.4)	1 (0.4)	1 (2.4)
<50 $x10^9$/L	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Note: Table presenting lowest platelet level achieved versus 2'-MOE ASO dose administered. In panel (A), all subjects had BSLN platelet levels within the normal range (LLN-ULN), while Panel (B) includes an additional 142 subjects whose BSLN levels were outside the range of normal. BSLN, baseline; LLN, lower limit of normal; ULN, upper limit of normal.

Source: Table 3, Crooke ST et al., 2017.

Reviewer Comment

Based on literature, there are 2 types of thrombocytopenia associated with ASOs: a common dose-dependent with gradual and slow decline in platelet count that might be asymptomatic or could result in mild or moderate bleeding, and a rare idiosyncratic rapid onset severe thrombocytopenia accompanied with catastrophic fatal bleeding[2].

[2] Chi, Xuan, Philip Gatti, and Thomas Papoian. "Safety of antisense oligonucleotide and siRNA-based therapeutics." Drug discovery today 22, no. 5 (2017): 823-833.

Reviewer Summary and Conclusion Based on Exposure-Response Analyses

As illustrated, TTR levels appear to be maintained for 1 week or more after inotersen dose interruption/discontinuation. TTR levels seem to slowly return to baseline within 12 weeks of inotersen dose interruption. On the other hand, previous experience with ASOs suggests that thrombocytopenia might be dose-dependent. While appropriate strategies could mitigate the risk, lower doses or less frequent regimen may be associated with better safety profile and similar efficacy.

4.4 Immunogenicity Effect on PK, PD, Efficacy, and Safety

The method to measure binding ADAs can only detect IgG isotypes; therefore, the overall incidence can be underestimated. Refer to the immunogenicity assay review by the Office of Biotechnology Products for details regarding the immunogenicity assay review.

Nonetheless, stratification by peak ADAs titer quartiles, ADAs onset quartiles, ADAs duration quartiles, and ADAs longevity showed that ADAs do not appear to influence TTR percent change from baseline, mNIS+7 composite score, Norfolk QoL-DN total score or platelet count reduction. However, as mentioned previously, the ADA assay can only detect IgG. Mis-classification of subjects with other ADA isotypes could contribute to inability to detect any differences between groups.

The effect of IgG ADA on inotersen PK could not be interpreted as the PK assay did not differentiate between free and ADA-bound inotersen.

4.5 Pharmacogenomics

Approximately, 100 mutations in the *TTR* gene are known to cause hATTR-PN. The V30M mutation is the most common mutation to cause hATTR-PN. In the U.S., approximately 40% of subjects with hATTR-PN have the V30M mutation. The applicant enrolled a total of 27 different *TTR* mutations in the pivotal study (CS2). In addition, the applicant stratified randomization for the V30M mutation. Patients with the V30M mutation encompassed approximately 51% of subjects enrolled in CS2. The efficacy findings were not significantly different based on the presence or absence of the V30M mutation.

Reviewer Comment

Inotersen binds to nucleotide positions 618-637 in the 3' UTR of the TTR mRNA. A search of dpSNP (www.ncbi.nlm.nih.gov/SNP/) reveals no common SNPs (allele frequencies > 0.05) occur within this binding site and only 4 rare SNPs (mean allele frequency < 0.00005) were identified in this region. Moreover, almost all reported *TTR* mutations occur in the coding region. Inotersen also silences wild-type TTR mRNA as well as the known variants. Hence, any pharmacogenomic variation in target sequence is unlikely to impact the safety or efficacy of inotersen.

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