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APPLICATION NUMBER:

215515Orig1s000

CLINICAL PHARMACOLOGY REVIEW(S)

Office of Clinical Pharmacology Review

NDA Number	215515
Link to EDR	\\CDSESUB1\evsprod\nda215515\0003\
Submission Date	04/14/2021
Submission Type	505(b)(1), NME NDA (Standard review)
Brand Name	AMVUTTRA
Generic Name	Vutrisiran
Dosage Form and Strength	Injection: 25 mg/0.5 mL in a single-dose prefilled syringe
Dosing Regimen and Route of Administration	25 mg administered by subcutaneous injection once every 3 months
Proposed Indication	Treatment of polyneuropathy of hereditary transthyretin (TTR)-mediated amyloidosis (hATTR amyloidosis) in adults
Applicant	Alnylam Pharmaceuticals Inc.
Associated IND	139086
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Table of Contents

1. EXECUTIVE SUMMARY 3
1.1 Recommendations 3
1.2 Post-Marketing Requirements and Commitments 4
2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT 4
2.1 Pharmacology and Clinical Pharmacokinetics 4
2.2 Dosing and Therapeutic Individualization 5
2.2.1 General dosing 5
2.2.2 Therapeutic individualization 5
2.3 Outstanding Issues 6
2.4 Summary of Labeling Recommendations 6
3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW 6
3.1 Overview of the Product and Regulatory Background 6
3.2 General Pharmacology and Pharmacokinetic Characteristics 7
3.3 Clinical Pharmacology Review Questions 8
3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness? 8
3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought? 9
3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors? 11
3.3.4 Are there clinically relevant effect from extrinsic factors and what is the appropriate management strategy? 12
4. APPENDICES 14
4.1 Summary of Bioanalytical Method Validation and Performance 14
4.1.1 LC/MS-HRAM Assays for Measurement of Vutrisiran in Plasma and Urine 14
4.1.2 ELISA Method for Measurement of Serum TTR 16
4.2 Clinical PK and PD Assessments 16
4.3 Pharmacometrics Review 21
4.4 Effect of Immunogenicity on PK, PD, and Efficacy 34
4.5 Pharmacogenomics 36
4.6 List of Clinical Studies in the Development Program 37

1. EXECUTIVE SUMMARY

Alnylam Pharmaceuticals, Inc submitted a New Drug Application (NDA) for AMVUTTRA (vutrisiran) for the treatment of polyneuropathy of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) in adults.

In hATTR amyloidosis, inherited variants in the transthyretin (TTR) gene lead to deposition of liver-derived TTR amyloid fibrils in multiple tissues including the peripheral nervous system. Vutrisiran is a N-acetylgalactosamine (GalNAc)-conjugated, double-stranded, small-interfering ribonucleic acid (siRNA) that selectively distributes in the liver and reduces the synthesis of variant and wild-type TTR protein, to reduce the deposition of amyloid deposits and halt the disease progression. The proposed dosing regimen is 25 mg administered once every three months (Q3M) by subcutaneous (SC) injection.

The clinical development program consists of two clinical studies. A Phase 1 single-ascending dose (SAD) study (Study 001) evaluated the safety, tolerability, PK, and PD of vutrisiran in healthy subjects. Based on TTR reduction observed in the Phase 1 study, PK/PD analysis was performed to support Phase 3 dose selection. The pivotal Phase 3 study (HELIOS-A) was a randomized, open-label study that evaluated the efficacy, safety, PK, and PD of vutrisiran in adult patients with hATTR amyloidosis with polyneuropathy. The efficacy was evaluated using modified neuropathy impairment score +7 (mNIS+7) change from baseline at Month 9 as the primary endpoint, and Norfolk Quality of Life–Diabetic Neuropathy (Norfolk QoL-DN) total score change from baseline at Month 9 as key secondary endpoint. The applicant utilized the placebo group from the phase 3 study (APOLLO) for their drug product ONPATTRO (patisiran) approved in 2018 for the same indication as external control.

In the HELIOS-A study, SC administration of 25 mg vutrisiran Q3M resulted in significant improvements compared to the placebo arm of Study APOLLO in the primary endpoint (mNIS+7) and the two secondary endpoints (Norfolk QoL-DN and 10-MWT) assessed at Month 9. These data demonstrated the effectiveness of vutrisiran in patients with hATTR amyloidosis with polyneuropathy.

This review is mainly focused on the evaluation of the following aspects: (1) the adequacy of proposed dosing in general population; (2) the effect of intrinsic and extrinsic factors on PK, PD, and efficacy; and (3) the adequacy of PK bridging between the vial with syringe presentation and the prefilled syringe presentation.

1.1 Recommendations

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

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Primary evidence of effectiveness was established from a single pivotal randomized, open-label Phase 3 study (HELIOS-A) in adult patients with hATTR amyloidosis with polyneuropathy, using change from baseline in mNIS+7 at Month 9 as the primary endpoint and change from baseline in Norfolk QoL-DN total score at Month 9 as key secondary endpoint, compared with an external placebo control from the Sponsor's patisiran phase 3 study (APOLLO). Treatment with vutrisiran from HELIOS-A resulted in significant improvement in both the primary and key secondary endpoints compared to the external control.
General dosing instructions	The recommended dosing regimen is 25 mg administered by SC injection Q3M.
Dosing in patient subgroups (intrinsic and extrinsic factors)	No dose adjustment is recommended for patients based on age, sex, race, body weight, TTR genotype (V30M or non-V30M), mild and moderate renal impairment (eGFR≥30 to <90 mL/min/1.73 m^2) or mild hepatic impairment (total bilirubin ≤1 x ULN and AST >1 x ULN, or total bilirubin >1.0 to 1.5 x ULN and any AST). The effect of severe renal impairment and moderate/severe hepatic impairment on the PK and PD of vutrisiran has not been studied. Drug interaction liability with vutrisiran is considered low. No significant impact on efficacy is expected with different vutrisiran presentations (PFS-S vs. vial) or different sites of injection (abdomen, upper arm, or thigh).
Labeling	The labeling concepts proposed by the Applicant are generally acceptable.
Bridge between the to-be-marketed and clinical trial presentations	The vial with syringe presentation was used in the Phase 1 clinical study and pivotal efficacy study. The to-be-marketed presentation PFS-S was introduced to the Phase 3 trial to replace the "vial with syringe" after the month 9 efficacy assessment. Bridging between the vial and PFS-S was established by pop-PK and similar median TTR percent reductions from baseline. Refer to Section 3.3.4.

1.2 Post-Marketing Requirements and Commitments

None from OCP review team.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Mechanism of Action

Vutrisiran is a double-stranded siRNA-GalNAc conjugate that causes degradation of variant and wild-type TTR messenger RNA (mRNA) through RNA interference, which results in a reduction of serum TTR protein and TTR protein deposits in tissues.

Pharmacokinetics

Following a single subcutaneous administration, vutrisiran C_{max} increased proportionally with dose while AUC was slightly more than dose proportional over the dosage range of 5 to 300 mg (0.2 to 12 times of the recommended dosage). No accumulation of vutrisiran was observed in plasma after repeated every 3 months dosing.

Absorption: In humans, vutrisiran was rapidly absorbed from the SC injection site into plasma. The median (range) T_{max} of vutrisiran is 4 (0.17, 12.0) hours.

Distribution: Human plasma binding of vutrisiran was concentration-dependent, ranging from 77.9% at 0.5 µg/mL to 19.0% at 50 µg/mL. Plasma protein binding of vutrisiran is expected to be approximately 80% at therapeutic dose, based on mean C_{max} of approximately 0.1 µg/mL. Vutrisiran distributes primarily to the liver after subcutaneous administration. The population estimate for the apparent central compartment volume of distribution of vutrisiran in humans was 10.1 L.

Metabolism: Vutrisiran is metabolized by endo- and exo- nucleases to short nucleotide fragments of varying sizes within the liver. Metabolite profiling in human plasma samples indicates that there are no detectable circulating metabolites of vutrisiran.

Excretion: Following administration of vutrisiran at a single dose of 25 mg, the median (range) apparent clearance is 21.4 (19.8, 30) L/hour, and the median (range) elimination half-life of vutrisiran is 5.2 (2.2, 6.4) hours. The mean fraction of unchanged vutrisiran eliminated in urine was approximately 19.4 %, and the mean renal clearance ranged from 4.5 to 5.7 L/hour.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The recommended dose of AMVUTTRA is 25 mg administered by SC injection once every 3 months (Q3M). This dosing regimen is identical to that evaluated in pivotal efficacy study HELIOS-A.

2.2.2 Therapeutic individualization

No therapeutic individualization is required for vutrisiran based on intrinsic or extrinsic factors.

No dedicated clinical studies were conducted in subjects with renal or hepatic impairment. According to pop-PK modeling, mild/moderate renal impairment or mild hepatic impairment did not significantly impact the PK of vutrisiran (See Section 3.3.3). Therefore, no dose adjustment is warranted in patients with mild/moderate renal impairment or mild hepatic impairment. The effect of severe renal impairment and moderate/severe hepatic impairment on the PK and PD of vutrisiran has not been studied.

AMVUTTRA is administered by SC route; thus, food is not anticipated to affect the exposure of AMVUTTRA. In addition, the drug-drug interaction liability is considered low (See Section 3.3.4). The PK, PD and efficacy of vutrisiran are not expected to be significantly affected by intrinsic factors such as body weight, age, race, sex, TTR genotype (V30M or non-V30M), as well as extrinsic factors such as vutrisiran presentation (PFS-S vs. vial with syringe), and injection site (See Section 3.3.4).

2.3 Outstanding Issues

None.

2.4 Summary of Labeling Recommendations

The labeling concepts proposed by the Applicant are generally acceptable.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

In hATTR amyloidosis, inherited variants in the TTR gene lead to destabilization of the tetrameric protein and disassociation of the TTR subunits into dimers and individual variant and wild-type monomers, which subsequently misfold. These misfolded TTR monomers can then self-assemble into oligomers and form amyloid fibrils and plaques in the extracellular space of various tissues. The continued deposition of liver-derived TTR amyloid fibrils in multiple tissues, including the peripheral nervous system, gastrointestinal tract, and heart, results in debilitating polyneuropathy and/or cardiomyopathy and is ultimately fatal.

Vutrisiran is an RNAi therapeutic comprised of a synthetic, chemically modified, double-stranded siRNA that specifically targets variant and wild-type TTR mRNA. Reduction of TTR production in the liver, which are the fundamental pathogenic proteins causing hATTR amyloidosis, will reduce ongoing deposition of amyloid deposits, thus halting disease progression.

Vutrisiran is a second-generation siRNA-GalNAc conjugate and comprises a double stranded oligonucleotide covalently linked to a ligand containing three GalNAc residues to enhance specific delivery to hepatocytes. All nucleosides are 2'-O-methyl or 2'-fluro modified and are connected through 3'-5' phosphodiester linkages. The antisense strand (A-131359) contains four phosphorothioate linkages, and the sense strand (A-131354) contains two phosphorothioate linkages. These physiochemical characteristics lead to enhanced metabolic stability and prolonged liver residence time, allowing for infrequent dosing.

Vutrisiran injection for subcutaneous use contains 50 mg/mL vutrisiran (b) (4) . The clinical dose is a fixed dose of 25 mg administered as a subcutaneous (SC) injection once every 3 months (Q3M).

There are currently two approved therapies available in the United States for the treatment of the polyneuropathy of hATTR amyloidosis in adults: ONPATTRO® (patisiran) and TEGSEDI® (inotersen). Both treatments act by targeting the mutant and wild-type TTR mRNA and reducing the TTR synthesis. The

proposed dosing interval of vutrisiran is 3-months, which is longer compared to the approved products ONPATTRO (once every three weeks by IV infusion) and TEGSEDI (once weekly by SC injection).

Vutrisiran has been granted Orphan Drug Designation for the treatment of ATTR amyloidosis on May 25, 2018, and granted Fast Track Designation for the treatment of polyneuropathy of hATTR amyloidosis in adults on April 3, 2020.

Two clinical studies were submitted as part of the vutrisiran NDA. Study HELIOS-A (ALN-TTRSC02-002) is a randomized, open-label, pivotal Phase 3 Study in patients with hATTR amyloidosis with polyneuropathy, using external placebo control from study APOLLO. Study 001 (ALN-TTRSC02-001) is a Phase 1 SAD study evaluating PK, PD, and safety of vutrisiran in healthy subjects. These studies are summarized in Appendix 4.6 (**Table 10**).

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	Vutrisiran is a double-stranded siRNA-GalNAc conjugate that causes degradation of variant and wild-type TTR mRNA through RNA interference, which results in a reduction of serum TTR protein and TTR protein deposits in tissues
QT Prolongation	The QT prolongation effect of vutrisiran was evaluated in Study ALN-TTRSC02-001 using exposure-response analysis. At 9-times the recommended dose of 25 mg once every three months, Amvuttra does not prolong the QT interval to any clinically relevant extent. A dedicated thorough QT study has not been conducted with vutrisiran. Please refer to the QT-IRT review submitted to DARRTS on July 6, 2021.
General Information	
Bioanalysis	The PK of vutrisiran was evaluated by monitoring the antisense strand of vutrisiran in plasma and urine (Study 001) and in plasma (HELIOS-A) using validated liquid chromatography/tandem mass spectrometry-high resolution accurate mass (LC/MS-HRAM) assays. Pharmacodynamics was evaluated by measuring serum TTR using a sandwich enzyme-linked immunosorbent assay (ELISA) method. Details are described in section 4.1.
Dose Proportionality	Following single subcutaneous doses ranging from 5 to 300 mg, vutrisiran C_{max} was shown to be dose proportional, while the increase of AUC_{inf} and AUC_{last} was slightly more than dose proportional.
Accumulation	No accumulation of vutrisiran was observed in plasma after 25 mg Q3M dosing
Immunogenicity	Across Studies 001 and HELIOS-A, a total of 4 (2.2%) vutrisiran-treated subjects developed treatment-emergent ADA. No clinically significant differences in the safety, efficacy, PK, or PD profiles of vutrisiran were observed in patients who tested positive for ADA.
Absorption	
C_{max} and AUC	At steady-state, the mean (CV%) C_{max} of vutrisiran is 0.12 (64%) µg/mL, and mean (CV%) AUC_{0-24} of vutrisiran is 0.80 (35%) µg·h/mL

T_{max}	Following SC administration, the median (range) T_{max} of vutrisiran is 4 (0.17, 12.0) hours
Distribution	
Volume of Distribution	The population estimate for the apparent central compartment volume of distribution (Vc) of vutrisiran in humans was 10.1 L
Plasma Protein Binding	Plasma protein binding is expected to be around 80% based on C_{max} at the recommended dose 25 mg. In vitro protein binding was concentration-dependent and decreased with increasing vutrisiran concentrations (78% at 0.5 µg/mL to 19% at 50 µg/mL).
Organ Distribution	Vutrisiran distributes primarily to the liver after subcutaneous dosing
Transporter-based DDI	Transporter substrate/inhibition studies have not been performed with vutrisiran. In vitro DDI data from similar GalNAc-siRNA conjugate molecules suggest a low potential for DDI with transporters.
Elimination	
Terminal Elimination half-life	The elimination half-life and apparent clearance (Median (Range)) of vutrisiran is 5.2 (2.2, 6.4) hours and 21.4 (19.8, 30) L/hour, respectively.
Metabolism	
Primary Metabolic Pathway(s)	No major circulating metabolites were identified in human. Vutrisiran is primarily metabolized by endo- and exo- nucleases to short nucleotide fragments of varying sizes within the liver.
Metabolism-based DDI	In vitro studies suggest that vutrisiran is not a substrate of major CYP enzymes, and not an inhibitor of major CYP enzymes at concentrations up to 612 µM (10,000 µg/mL). Vutrisiran was not tested as an inducer of CYP enzymes in vitro. In vitro DDI data from similar GalNAc-siRNA conjugate molecules suggest a low potential of DDI to induce CYP enzymes.
Excretion	
Primary Excretion Pathways	After a single subcutaneous injection at the recommended dose of 25 mg in healthy subjects, the mean fraction of unchanged vutrisiran eliminated in urine was approximately 19.4 %. The mean renal clearance of vutrisiran ranged from 4.5 to 5.7 L/hour across the dose levels tested (25 to 300 mg).

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 effectiveness for this application is based on the interim analysis of clinical efficacy endpoints at Month 9 in study HELIOS-A (ALN-TTRSC02-002), which showed statistically significant improvement in primary and key secondary endpoints for patients treated with vutrisiran compared to the external control. HELIOS-A is an ongoing Phase 3, randomized, open-label study to evaluate the efficacy, safety, PK, and PD of vutrisiran, using patisiran as a reference comparator within the study and using the placebo treatment in APOLLO (Study ALNTTR02-004) as the external control; APOLLO is a completed phase 3, placebo-controlled, randomized, double-blind study of patisiran. Both studies were conducted in adult patients with hATTR amyloidosis with polyneuropathy.

In HELIOS-A, a total of 164 patients were randomized (3:1) to receive either vutrisiran 25 mg SC injection administered Q3M or patisiran 0.3 mg/kg IV infusion administered once every three weeks (Q3W). The study includes 2 parts: an 18-month Treatment Period, with the primary efficacy analysis at Month 9; and an 18-month Treatment Extension Period, in which all the patients receive vutrisiran. The primary endpoint is mNIS+7 change from baseline at Month 9, and the key secondary endpoint is Norfolk QoL-DN total score change from baseline at Month 9. Vutrisiran demonstrated statistically significant improvement compared to APOLLO placebo on both the primary endpoint (LS mean change from baseline was -2.2 for AMVUTTRA and 14.8 for placebo; mean difference = -17.0, $p<0.0001$) and key secondary efficacy endpoint (LS mean change from baseline was -3.3 for AMVUTTRA and 12.9 for placebo; mean difference = -16.2, $p<0.0001$). Improvement was also observed on other secondary and exploratory measurements such as ambulatory function (10-MWT) and nutritional status (mBMI). Please refer to clinical and statistical reviews for additional details regarding the efficacy assessments.

Inclusion of patisiran reference comparator arm in HELIOS-A allowed for a descriptive comparison of PD and efficacy between vutrisiran and patisiran treatments from HELIOS-A, as well as the comparison between the patisiran treatment groups from HELIOS-A and APOLLO. Overall, the effect of TTR reduction, neuropathy (mNIS+7) and quality of life (Norfolk QoL-DN) are numerically similar among the vutrisiran and patisiran treatment groups in both studies. Refer to clinical pharmacology review of NDA 210922 ONPATTRO by Dr. Venkateswaran Chithambarampillai for additional details on Study APOLLO.

The magnitude of serum TTR reduction in Study HELIOS-A was similar with those observed from patisiran arms either within the study or from Study APOLLO. The median steady state peak (Day 211), trough (Day 253), and time averaged (Month 6 to Month 9) percent TTR reductions from baseline with vutrisiran 25mg SC Q3M were 86.5%, 84.8%, and 83.6%, respectively, which were comparable to the reduction observed with patisiran in the within-study patisiran arm (86.5, 79.7%, and 80.1%, respectively) and the patisiran arm of Study APOLLO (median trough TTR reduction of 81% at Month 9 and 18). This magnitude of TTR reduction was associated with demonstrated effectiveness by patisiran treatment in Study APOLLO, thus provided supportive evidence for the effectiveness of vutrisiran at the recommended dose.

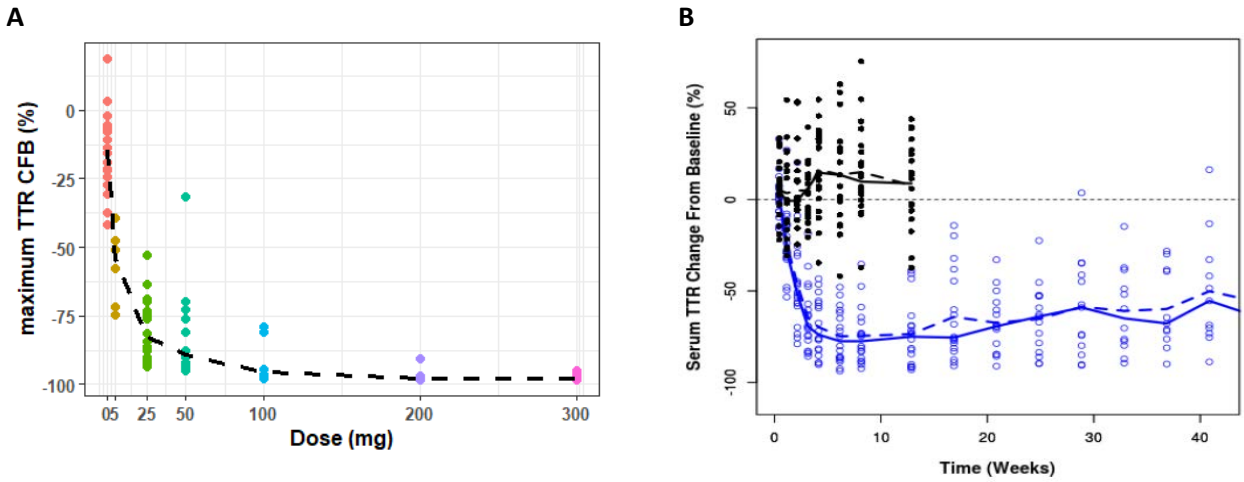
3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes, the proposed dosing regimen of 25 mg Q3M is appropriate for the treatment of the polyneuropathy of hATTR in adults.

The proposed dosing regimen is the same as the regimen used in Phase 3 registrational trial (HELIOS-A), which was selected based on TTR reduction from the Phase 1 SAD study (Study 001). Study 001 evaluated the PK and PD effects with a wide dose range of vutrisiran, in which the median TTR reductions from baseline were 15%, 55%, 83%, 89%, 95%, 98%, 98% with placebo, 5 mg, 25 mg, 50 mg, 100 mg, 200 mg, and 300 mg, respectively (**Figure 1A**). The TTR reduction following a single dose of vutrisiran ≥25 mg was comparable to the median trough TTR reductions of 81% observed with patisiran in Study APOLLO, where clinically meaningful improvements in mNIS+7 was observed. In addition, with a single dose of 25 mg vutrisiran, the maximum TTR reduction was achieved by 6 weeks and maintained

for up to 90 days post dose with a slow recovery thereafter (**Figure 1B**), which supported the selection of every 3 months (Q3M) dosing interval.

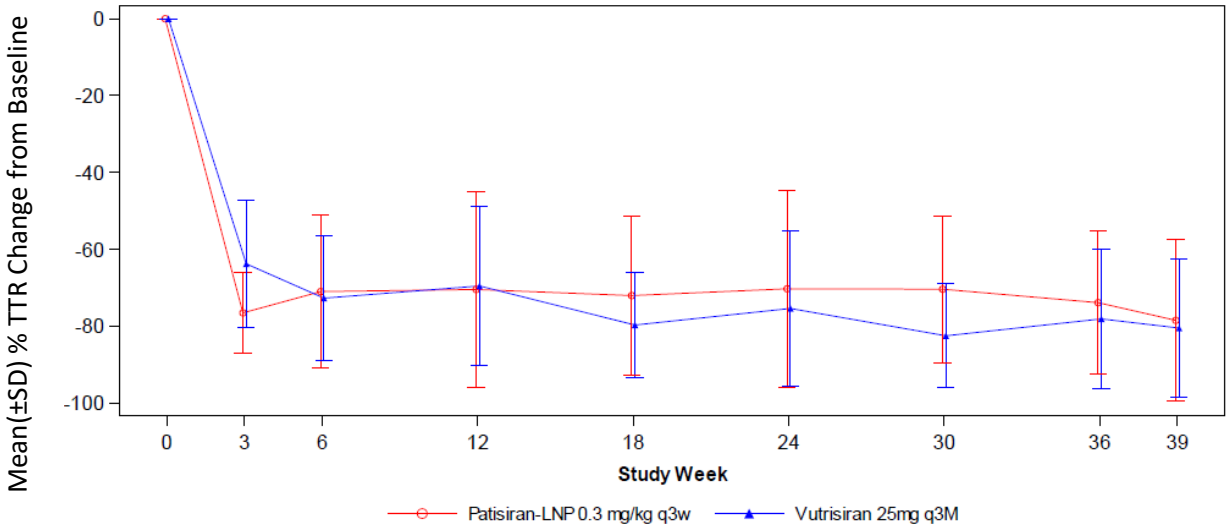
Figure 1 Observed TTR Reduction in Study 001. (A) Relationship between vutrisiran dose and maximum percentage of TTR change from baseline (CFB) after a single dose of vutrisiran (dashed line indicates median); (B) Observed serum TTR reduction over time after a single dose of placebo (black color) or 25 mg vutrisiran (blue color; solid and dashed lines indicate medians and means, respectively)



***Source**: (A) Reviewer's analysis; (B) Applicant's Summary of Clinical Pharmacology Figure 47, Page 106*

The adequacy of vutrisiran dosing regimen 25 mg Q3M was further confirmed in Study HELIOS-A. At Week 6, vutrisiran 25 mg achieved a TTR reduction of 74.2% which was near steady-state **(Figure 2)**. The TTR reduction effect sustained over the dosing interval with median steady-state trough reduction of 84.8%, comparable to the patisiran group in Study HELIOS-A **(Figure 2)** and the patisiran group in Study APOLLO. In addition, the proposed dosing regimen showed significant improvement in primary efficacy endpoint and key secondary endpoint, with acceptable safety profile. Please refer to clinical review for additional details regarding safety assessments.

Figure 2: HELIOS-A: Mean (SD) TTR Percent Change from Baseline Over 9 Months in Patients after Administration of Vutrisiran 25 mg Q3M or Patisiran 0.3 mg/kg Q3W



***Source**: Applicant's Summary of Clinical Pharmacology Figure 50, Page 110.*

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

No alternative dosing regimen of vutrisiran is required based on intrinsic factors for the treatment of the polyneuropathy of hATTR in adults.

In addition to the Studies 001 and HELIOS-A, the applicant has performed pop-PK modeling and evaluated the impact of various intrinsic factors on the PK, PD (i.e., TTR reduction from baseline), and efficacy (mNIS+7 change from baseline). The analysis suggested that no dose adjustment is required based on body weight, age, race, sex, and TTR genotype (V30M or non-V30M). Please refer to the Pharmacometrics review (Appendix 4.3) and Pharmacogenomics review (Appendix 4.5) for details. Additional justifications of dosing recommendation for renal impairment and hepatic impairment are discussed below.

Renal Impairment

A dedicated renal impairment study was not performed. However, the PK population of clinical studies included 43 subjects with mild renal impairment (34 from Study HELIOS-A and 9 from Study 001) and 10 subjects with moderate renal impairment (from Study HELIOS-A). Based on pop-PK analysis, no clinically meaningful differences in vutrisiran PK were observed in patients with mild renal impairment (estimated glomerular filtration rate [eGFR] ≥60 to <90 mL/min/1.73m^2) or moderate renal impairment (eGFR ≥30 to <60 mL/min/1.73m^2) compared to patients with normal renal function. Vutrisiran has not been studied in patients with severe renal impairment.

The renal route is a minor elimination pathway for vutrisiran based on the results of Study 001, which showed that only 15% to 25% of the administered dose was recovered in the urine. Renal impairment is therefore not expected to impact the PD, safety, or efficacy of vutrisiran.

The clinical pharmacology review team recommends that no dose adjustment in patients with mild or moderate renal impairment (eGFR ≥30 to <90 mL/min/1.73m^2), and to state in the labeling that AMVUTTRA has not been studied in patients with severe renal impairment or end-stage renal disease.

Hepatic Impairment

A dedicated hepatic impairment study was not conducted. However, based on the NCI-ODWG criteria, 8 subjects enrolled in Study HELIOS-A had mild hepatic impairment (total bilirubin ≤ upper limit of normal [ULN] and AST > ULN, or total bilirubin >1.0 to 1.5 x ULN and any AST), while all the other subjects had normal hepatic function (total bilirubin ≤ ULN and AST ≤ULN). According to pop-PK analysis, no clinically meaningful differences on PK of vutrisiran was observed in patients with mild hepatic impairment compared to patients with normal hepatic function. Vutrisiran has not been studied in patients with moderate or severe hepatic impairment.

Vutrisiran is primarily metabolized by endonucleases and exonucleases and is not a substrate of cytochrome P450 (CYP) enzymes. Therefore, the change of CYP enzyme activity due to hepatic impairment is not expected to influence vutrisiran metabolism. In addition, vutrisiran is not expected to accumulate in plasma even in patients with hepatic impairment, because of the short plasma half-life and the long interval between doses. However, the effect of moderate and severe hepatic impairment on PK, PD, and efficacy, and safety of vutrisiran cannot be ruled out because of potential change in asialoglycoprotein receptor (ASGPR) expression and ASGPR-mediated uptake of GalNAc-siRNA conjugates into the livers of hepatically-impaired patients.

The clinical pharmacology review team recommends no dose adjustment for patients with mild hepatic impairment. For patients with moderate or severe hepatic impairment, the team recommends stating that AMVUTTRA has not been studied in this population.

3.3.4 Are there clinically relevant effect from extrinsic factors and what is the appropriate management strategy?

Food Effect

AMVUTTRA is administered by SC route; thus, food is not anticipated to affect the exposure of AMVUTTRA.

Drug-drug Interaction

No clinical drug-drug interaction (DDI) studies have been performed. Vutrisiran is not expected to cause clinical DDI or to be affected by inhibitors or inducers of CYP enzymes or transporters.

In vitro assays suggested that vutrisiran was neither a substrate nor an inhibitor of any major CYP enzyme. Vutrisiran was not a substrate of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 as suggested by results of incubation with recombinant cytochrome P450 enzymes. Incubation with pooled human liver microsomes suggest that vutrisiran is not a direct or time-dependent inhibitor of these CYP isoforms at concentrations up to 612 µM (10,000 µg/mL), which is much higher than the C_{max} of vutrisiran after 25 mg SC injection (0.12 µg/mL).

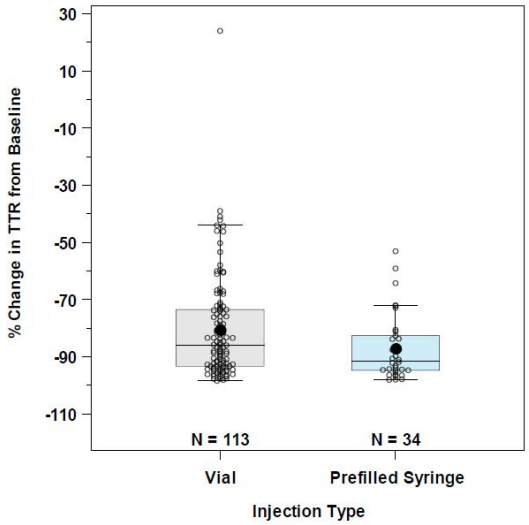
No in vitro study was performed to evaluate the potential of vutrisiran as CYP inducer or as substrate/inhibitor of drug transporters. However, in vitro DDI data from similar GalNAc-siRNA conjugate molecules that share similar physicochemical properties with vutrisiran indicated that they are not substrates, inhibitors, or inducers of major CYPs and are not substrates and inhibitors of drug transporters. Therefore, a low potential for such interactions is expected for vutrisiran. The review team decided it is reasonable to state in the labeling that vutrisiran is not expected to cause clinical DDI or to be affected by inhibitors or inducers of CYP enzymes or transporters.

The interpretation of in vitro CYP induction/inhibition results has taken into account the impact of higher concentration of GalNAc-siRNA conjugates in liver than in plasma. Since GalNAc-siRNAs are efficiently delivered to the hepatocytes via ASGPR, the vutrisiran concentration in liver is anticipated to be much higher than that in plasma. For the evaluation of CYP enzyme induction potential, the enrichment in hepatocytes can be represented by the in vitro testing conditions for CYP induction. To evaluate the CYP inhibition potential, the Applicant used the estimated total liver concentration in human as the worst-case scenario, and concluded that clinical DDI evaluation is not needed. Taken together, the in vitro studies suggested low potential of clinical DDI for vutrisiran to inhibit or induce CYP enzymes at clinically relevant concentrations.

Impact of Vutrisiran Presentation (Vial vs. PFS-S) on PK and PD

In HELIOS-A, subjects received various combinations of vial with syringe and PFS-S for vutrisiran administration. According to pop-PK analysis, administration with PFS-S was associated with 35% higher C_{max} but similar AUC_{0-24} compared to vial with syringe (Section 4.3 Pharmacometrics review, **Figure 8**). This increase in vutrisiran exposure (C_{max}) with the use of PFS-S compared to vial with syringe is not expected to reduce the efficacy of vutrisiran. Similar median TTR percent reductions from baseline were observed with vial with syringe and PFS-S (**Figure 3**). In addition, no clinically meaningful difference was observed on safety between the two presentations of vutrisiran (refer to clinical review).

Figure 3: HELIOS-A: Vutrisiran Month 9 Percent Change in TTR From Baseline, Vial vs. PFS-S



***Source**: Applicant's Summary of Clinical Pharmacology Figure 46, Page 104.*

Effect of Injection Site on Vutrisiran Exposure

The Sponsor evaluated the impact of SC injection site (abdomen, upper arm, or thigh) on PK, PD, efficacy, and safety using data from Study HELIOS-A. At the time of the primary analysis at Month 9, 107/122 (88%) patients had all subcutaneous (SC) injections administered in the abdomen and 15/122 (12%) patients had at least one SC injection site in the arm or thigh, with only three patients receiving an injection in the thigh.

Sponsor's descriptive analysis showed that the median plasma concentration of vutrisiran at 3 hours postdose following SC injection in the arm was similar to injection in the abdomen with overlapping concentration ranges. Considering the PK/PD profile of vutrisiran, the review team concluded that the injection site is not expected to significantly affect the PD and efficacy even if there is slight difference in plasma exposure. Thus, the proposed administration instruction of "Choose an injection site from the following areas: the abdomen, thighs, or upper arms" in labeling is considered acceptable.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

4.1.1 LC/MS-HRAM Assays for Measurement of Vutrisiran in Plasma and Urine

The PK of vutrisiran was evaluated by monitoring the antisense strand of vutrisiran in plasma and urine (Study 001) and in plasma (HELIOS-A) using validated liquid chromatography/tandem mass spectrometry-high resolution accurate mass (LC/MS-HRAM) assays. Plasma and urine samples were processed by liquid/liquid and supported liquid phase extractions, respectively, and analyzed by LC/MS-HRAM. QC concentrations were 10.0, 30.0, 400 and 800 ng/mL. The methods met the acceptance criteria for bioanalytical methods according to the Bioanalytical Method Validation Guidance for Industry. Performance characteristics and validation attributes for each bioanalytical method are presented in **Table 1**. Additionally:

- In humans, no major circulating metabolites were identified after administration of a single 50 mg dose of vutrisiran to healthy volunteers. Therefore, metabolites were not quantitated in HELIOS-A.
- In all runs, analyte carryover was observed in control blanks following a ULOQ standard at a level >20% of the mean acceptable LLOQ response. The occurrence of carryover with this method could not be eliminated entirely; therefore, study samples were analyzed in a systematic order to minimize the impact of carryover. No impact is expected on the quality of the data or the integrity of the study.

Table 1 Bioanalytical method validation results for vutrisiran in human plasma and urine

Parameter	Plasma	Urine
Analyte	ALN-TTRSC02	ALN-TTRSC02

Internal Standard (IS)	(b) (4)	(b) (4)
Lower Limit of Quantitation (ng/mL)	10.0 ng/mL	10.0 ng/mL
Extraction Recovery of Drug at low/medium/high QC levels (%)	66.2 %, 65.1%, and 62.1% for antisense 69.4 %, 63.7%, and 60.7% for sense	30.2%, 38.5%, and 40.2% for antisense; 28.8%, 41.7%, and 45.4% for sense
Extraction Recovery of IS at low/medium/high QC levels (%)	58.5%, 57.7%, and 52.4% for antisense; 58.3 %, 63.4%, and 56.9% for sense	35.2%, 38.4%, and 41.3% for antisense; 37.0%, 37.8%, and 40.1% for sense
Standard Curve Concentrations (ng/mL) and linearity R^2	10.0, 20.0, 50.0, 150, 350, 700, 900 and 1,000 ng/mL Mean R-squared is 0.9955 (antisense) and 0.9966 (sense)	10.0, 20.0, 50.0, 150, 350, 700, 900 and 1,000 ng/mL Mean R-squared is 0.9954 (antisense) and 0.9962 (sense)
LLOQ QC Intraday Precision (%)	Antisense: 6.9% to 10.2%; Sense: 8.2% to 12.0%	Antisense: 4.1% to 9.7%; Sense: -2.7% to 17.0%
LLOQ QC Intraday Accuracy (%)	Antisense: -8.1% to 11.0%; Sense: 1.0% to 12.0%	Antisense: 0.0% to 15.0%; Sense: 7.3% to 13.6%
Low, Medium and High QC Intraday Precision (%)	Antisense: 2.8% to 10.2%; Sense: 1.1% to 6.8%	Antisense: 2.0% to 10.2%; Sense: 2.0% to 5.9%
Low, Medium and High QC Intraday Accuracy (%)	Antisense: -7.0% to 4.0%; Sense: -8.7% to 2.3%	Antisense: -9.8% to -0.3%; Sense: -9.0% to 9.8%
QC Interday Precision (%CV)	Antisense: 3.8% to 11.3%; Sense: 1.9% to 10.4%	Antisense: 4.6% to 6.7%; Sense: 5.6% to 6.5%
QC Interday Accuracy (%Diff)	Antisense: -4.0% to 1.0%; Sense: -6.7% to 6.0%	Antisense: -5.1% to -3.7%; Sense: -4.4% to -4.3%
Long-Term Stability in Matrix (Days)	64 days at -20 °C and 69 days at -70 °C	21 days at -20 °C 314 days (0.1% CHAPS) at -70 °C 320 days (1.0% CHAPS) at -70 °C
Extract Stability	121 hours at 5 °C	255 hours at 5 °C
Bench-Top Stability in Matrix	6 hours at 5 °C and 24 hours at room temperature	6 hours at 5 °C
Carryover of Analyte and Internal Standard	Carryover greater than acceptable limits detected for both sense and antisense.	Carryover greater than acceptable limits detected for both sense and antisense.
Freeze-Thaw Stability	4 cycles; frozen at -20 °C and -70 °C and thawed at 5 °C	4 cycles; frozen at -20 °C and -70 °C and thawed at 5 °C; 10 cycles; frozen at -70 °C and thawed at 5 °C
Dilution (concentration tested/dilution factor)	4,000 ng/mL (DF=10)	4,000 ng/mL (DF=10)
Matrix Selectivity	6 Lots of Blank Matrix	6 Lots of Blank Matrix
Matrix Effect Blanks	No significant interference found at the retention times of interest for Analyte or IS.	No significant interference found at the retention times of interest for Analyte or IS.

4.1.2 ELISA Method for Measurement of Serum TTR

A sandwich ELISA was developed and validated for the quantitation of TTR in human serum. The microtiter plate was coated with a rabbit polyclonal antibody which bound specifically to human TTR. A capture antibody (sheep polyclonal anti-TTR antibody) followed by alkaline phosphatase conjugated donkey anti-sheep antibody were used for detection. The validation procedures (bioanalysis validation report #302417) was previously reviewed and considered acceptable (refer to clinical pharmacology review of NDA210922 ONPATTRO by Dr. Venkateswaran Chithambarampillai). The method performance for quantitation of serum TTR protein in Study 001 (ALN-TTRSC02-001) and Study HELIOS-A (ALN-TTRSC02-002) were also adequate to support clinical sample analysis and the use of TTR as a PD marker. The method validation for serum TTR measurement is summarized in **Table 2**.

Table 2. ELISA for serum TTR measurement

Method validation	302417
Method description	Enzyme-linked immunosorbent assay
Clinical studies supported	001 and HELIOS-A
Biomarker (PD marker)	Transthyretin
Biological matrix	Human serum
Calibration range	1.13-69.44 ng/mL
Lower limit of quantification	1.13 ng/mL
QC concentrations	1.13, 3.00, 11.00, 28.00, and 69.44 ng/mL
Parallelism (dilution linearity)[a]	From 4000- to 32,000-fold
Stability in biological matrix	Long-term: stable at -80°C for 733 days
Intra-assay precision (%CV)	0.7% to 16.8%
Intra-assay accuracy (theoretical %)	74.8% to 118.3%
Inter-assay precision (%CV)	8.2% to 17.7%
Inter-assay accuracy (theoretical %)	94.6% to 104.9%
Additional validation parameters	Benchtop stability, stability at 4°C, freeze/thaw stability, interference and selectivity, and prozone effect.

***Source**: Applicant's Summary of Biopharmaceutic Studies and Analytical Methods, Figure 13, Page 39.*

4.2 Clinical PK and PD Assessments

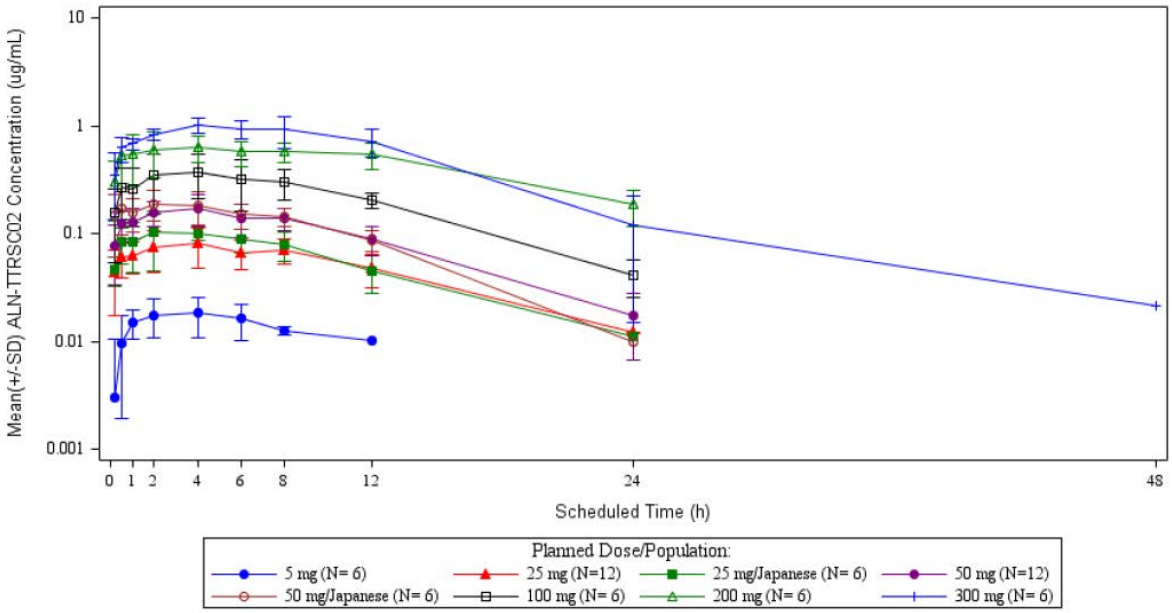
Pharmacokinetics

The PK of vutrisiran was characterized by non-compartment analysis (NCA) based on PK data from Study 001 in 60 healthy subjects and Study HELIOS-A in 120 patients with hATTR amyloidosis. The single doses

evaluated in study 001 include 5, 25, 50, 100, 200, and 300 mg, followed by 2 additional cohorts of subjects of Japanese descent (25 and 50 mg). Each cohort consisted of 8 subjects (6 vutrisiran : 2 placebo). Study drug was administered using vials with syringes.

Average plasma concentration-time profiles and PK parameters of vutrisiran was summarized in **Figure 4** and **Table 3**, respectively. Following a single SC dose of 5 to 300 mg vutrisiran in healthy subjects, the median T_{max} ranged from 3 to 5 hours postdose and the mean t½ ranged from 4 to 7.5 hours (**Table 3**). After reaching C_{max}, vutrisiran concentrations declined rapidly to the LLOQ within 24 to 48 hours (**Figure 4**). Among all patients who received 25 mg vutrisiran (N=18), the median (range) apparent volume of distribution (Vz/F) was 181 L (70.9 to 190 L), t½ was 5.23 hours (2.24 to 6.36 hours), and the median (range) apparent total body clearance (CL/F) was 21.4 L/h (19.8 to 30.0 L/h).

Figure 4: Study 001: Mean (±SD) Plasma Vutrisiran Concentration-time Profile by Dose Group



***Source**: Study 001 CSR Figure 14.2.3.1*

Table 3: Study 001: Summary of Single-dose Plasma and Urine PK Parameters for Vutrisiran

PK Parameter (Unit)	Statistic	Vutrisiran							
		Non-Japanese Subjects						Subjects of Japanese Descent	
		5 mg (N=6)	25 mg (N=12)	50 mg (N=12)	100 mg (N=6)	200 mg (N=6)	300 mg (N=6)	25 mg (N=6)	50 mg (N=6)
Plasma									
C_{max} (µg/mL)	Mean (SD)	0.0196 (0.00713)	0.0875 (0.0312)	0.178 (0.0517)	0.396 (0.188)	0.727 (0.190)	1.09 (0.218)	0.120 (0.0488)	0.218 (0.0690)
t_{max} (h)	Median (min, max)	4.00 (2.00, 8.00)	4.01 (0.17, 12.0)	4.00 (0.50, 12.0)	5.00 (2.00, 12.0)	4.03 (2.00, 12.0)	5.00 (2.00, 8.17)	4.00 (2.00, 6.00)	3.00 (0.50, 4.02)
$t_{½}$ (h)	Mean (SD)	NC (NC)	5.23 (NC)	4.15 (1.05)	6.04 (NC)	7.53 (NC)	5.53 (1.77)	4.30 (NC)	4.61 (NC)
AUC_{last} (h*µg/mL)	Mean (SD)	0.131 (0.0379)	0.854 (0.217)	1.85 (0.296)	4.48 (0.937)	11.3 (2.38)	15.9 (2.87)	1.04 (0.148)	1.86 (0.247)
AUC_{inf} (h*µg/mL)	Mean (SD)	NC (NC)	1.02 (NC)	2.18 (0.385)	5.20 (NC)	12.7 (NC)	15.5 (2.48)	1.20 (NC)	1.61 (NC)
CL/F (L/h)	Mean (SD)	NC (NC)	25.4 (NC)	23.5 (3.93)	19.3 (NC)	17.8 (NC)	19.8 (2.86)	20.9 (NC)	31.0 (NC)
Urine									
Fe_{0-24h} (%)	Mean (SD)	15.4 (3.34)	19.4 (6.15)	20.1 (4.83)	23.2 (4.30)	25.0 (9.92)	25.4 (5.43)	20.3 (6.23)	22.4 (11.1)
CL_R (L/h)	Mean (SD)	NC (NC)	5.34 (NC)	5.24 (1.81)	5.74 (1.13)	4.45 (1.78)	5.01 (0.62)	4.51 (NC)	5.41 (NC)

Abbreviations: Fe_{0-24}=fraction of unchanged drug eliminated in urine over 24 hours; NC=not calculated.
***Source**: Applicant's Summary of Clinical Pharmacology Table 5 Page 38*

The fraction of unchanged drug eliminated in urine over 24 hours (Fe_{0-24}) ranged from 15.4% to 25.4% and the mean CL_R of vutrisiran ranged from 4.45 to 5.74 L/h across the dose levels tested (25 to 300 mg) (**Table 3**). These data indicate that renal clearance is not a major route of elimination of vutrisiran; consequently, changes in renal function are not expected to meaningfully influence the PK or PD of vutrisiran.

In Study HELIOS-A, following SC administration, vutrisiran is rapidly absorbed with individual T_{max} values ranging from 2 to 6.6 hours (**Table 4**). Vutrisiran plasma PK profile, C_{max} and AUC_{0-24} were similar on Days 1 and at steady state (Day 253), indicating an absence of accumulation of vutrisiran in plasma after Q3M dosing of 25 mg.

Table 4: HELIOS-A: Summary of Plasma Pharmacokinetic Parameters for Vutrisiran

Parameter	Statistic	Day 1 (first dose 25 mg)	Day 253 (after 25 mg q3M dosing)
C_{max} (µg/mL)	N	120	108
	Mean (SD)	0.11 (0.09)	0.12 (0.07)
	Median (min, max)	0.09 (0.0, 0.6)	0.09 (0.0, 0.4)
	CV (%)	82.2	64.3
t_{max} (h)	N	120	108
	Median (min, max)	3.12 (2.0, 6.6)	3.00 (2.0, 6.5)
AUC_{0-24} (h·µg/mL)	N	20	19
	Mean (SD)	0.79 (0.31)	0.80 (0.28)
	Median (min, max)	0.73 (0.4, 1.4)	0.78 (0.4, 1.5)
	CV (%)	38.9	35.0
C_{max} R_{AC}[a]	Mean (SD)	-	1.15 (0.52)
	CV (%)	-	45.3
AUC_{0-24} R_{AC}[b]	Mean (SD)	-	0.99 (0.21)
	CV (%)	-	21.7

***Source**: Study 002 CSR Table 14.4.1.2. RAC: accumulation ratio.*

Dose Proportionality

The natural log-transformed PK parameters C_{max}, AUC_{last}, and AUC_{inf} were plotted against natural log-transformed vutrisiran dose. The power model is described as ln(PK)=α+β*ln(dose). Dose proportionality was based on whether the 90% CI for the proportionality constant (β) contained 1.

The estimated β and associated 90% CIs for C_{max}, AUC_{last}, and AUC_{inf} were 0.99 (0.92 to 1.05), 1.18 (1.14 to 1.22), and 1.10 (1.01 to 1.20), respectively, indicating a dose proportional increase in C_{max} and a slightly greater than dose-proportional increase in AUC_{last} and AUC_{inf} across the dose range studied.

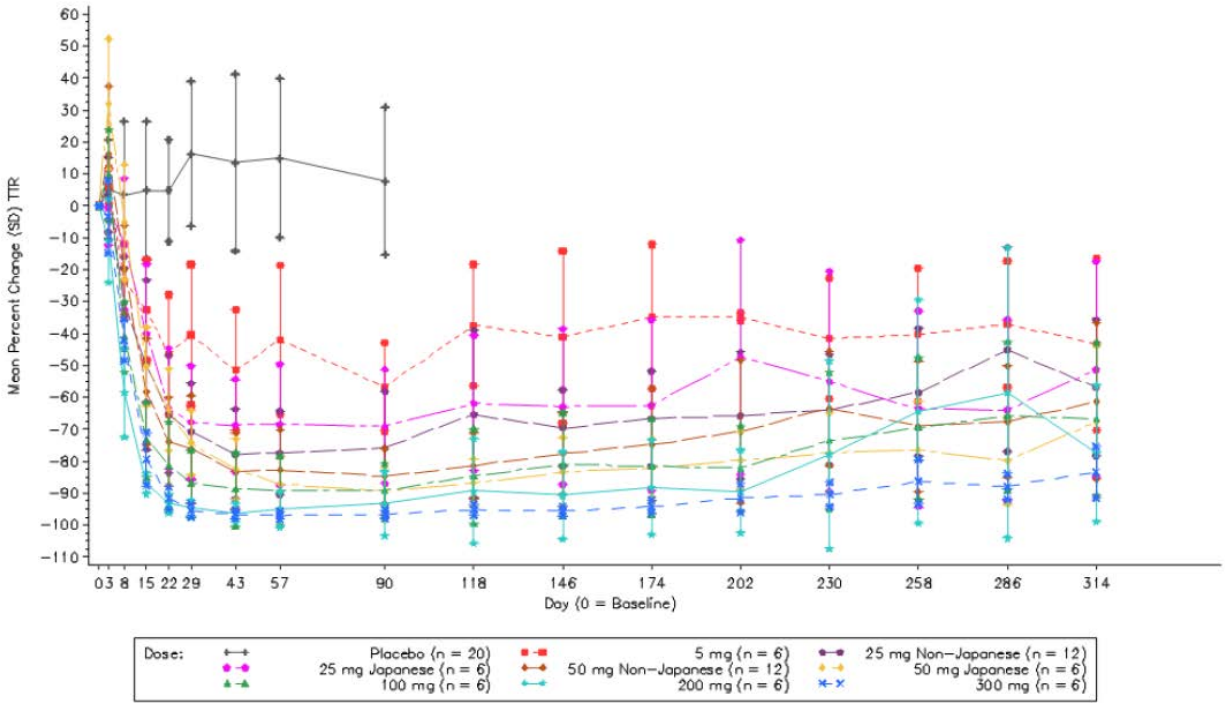
Pharmacodynamics

The magnitude of TTR reduction increased in a dose-dependent manner from 5 to 100 mg and plateaued at the 2 highest doses of 200 and 300 mg (**Figure 5**). The median maximum percent reductions were similar in subjects treated with 25 and 50 mg (85.3 and 87.8%, respectively, **Table 5**), while a greater reduction was observed at the 100 mg dose (95.3%). The TTR reduction appeared to plateau at the 2 highest doses, 200 mg and 300 mg (97.8 and 97.6%, respectively).

The duration of TTR reduction was also dose dependent. For all vutrisiran dose groups, the nadir in TTR levels was achieved by 6 weeks and maintained for approximately 90 days (**Figure 5**). After Day 90, TTR levels began to recover in all dose groups.

TTR serves as a carrier of vitamin A, which was measured to assess secondary effects of TTR reduction by vutrisiran. Vutrisiran showed a dose-dependent reduction in serum vitamin A level in parallel with TTR reduction.

Figure 5: Study 001: Mean (±SD) Percent Change from Baseline in TTR by Time Point and Dose Cohort



***Source**: Study 001 CSR Figure 14.4.3.3.*

Table 5: Study 001: Percent Change from Baseline in TTR - Maximum and on Day 90, by Dose Cohort

Timepoint Statistic	Placebo (N=20)	Vutrisiran								
		Non-Japanese Subjects						Subjects of Japanese Descent		All Vutrisiran (N=60)
		5 mg (N=6)	25 mg (N=12)	50 mg (N=12)	100 mg (N=6)	200 mg (N=6)	300 mg (N=6)	25 mg (N=6)	50 mg (N=6)	
Maximum Percent Change										
Mean (SD)	-15.54 (15.23)	-57.09 (13.91)	-82.91 (9.02)	-81.42 (17.77)	-90.74 (8.59)	-96.61 (2.90)	-97.14 (1.24)	-74.93 (15.37)	-89.85 (7.28)	-83.50 (15.73)
Median (min, max)	-14.68 (-41.9, 18.7)	-54.49 (-74.7, -39.4)	-85.33 (-93.7, -68.8)	-87.82 (-94.6, -31.5)	-95.33 (-97.6, -78.8)	-97.79 (-98.3, -90.8)	-97.64 (-98.4, -95.0)	-74.92 (-93.0, -52.9)	-92.89 (-94.9, -76.0)	-90.16 (-98.4, -31.5)
Percent Change from Baseline to Day 90										
Mean (SD)	7.75 (23.15)	-56.71 (13.78)	-75.83 (17.75)	-84.69 (8.56)	-89.11 (8.71)	-93.37 (9.92)	-96.69 (1.34)	-69.09 (17.86)	-89.35 (7.51)	-81.48 (16.59)
Median (min, max)	8.71 (-37.3, 43.9)	-54.0 (-74.7, -39.4)	-81.75 (-91.8, -40.6)	-84.65 (-94.6, -69.6)	-92.17 (-97.1, -76.7)	-97.34 (-97.8, -73.1)	-97.08 (-98.2, -94.9)	-71.44 (-93.0, -38.8)	-92.09 (-94.7, -75.1)	-87.75 (-98.2, -38.8)

***Source**: Study 001 CSR Table 14.4.3.1*

4.3 Pharmacometrics Review

EXECUTIVE SUMMARY

This document is a review of the sponsor's population pharmacokinetic (PK) analysis which support labeling statements.

SPONSOR'S POPULATION PK ANALYSIS

Objectives: To describe plasma concentration time profiles of vutrisiran following single and multiple dose SC administration in healthy subjects and patients with hereditary ATTR (hATTR) amyloidosis

Data: PK data of 182 subjects from Studies ALN-TTRSC02-001 (or Study 001) and ALN-TTRSC02-002 (or HELIOS-A) were used to develop population PK models for vutrisiran. The covariate characteristics of the data is provided in **Table 6**.

Table 6: Summary statistics of the 182 subjects included in the PK dataset

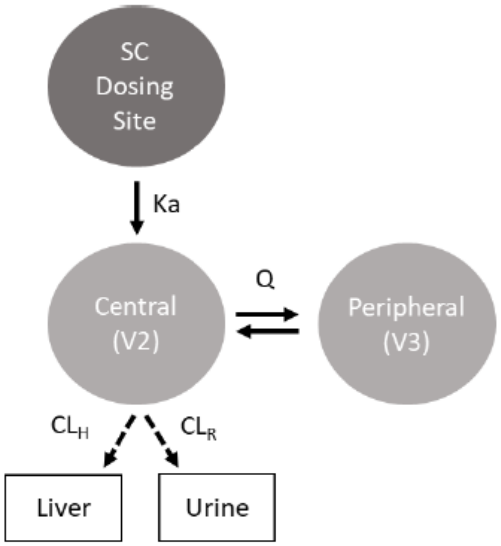
Covariates	
Demographics Median (Min, Max)	
Age (years)	48 (19, 85)
Body weight (Kg)	69 (38, 130)
eGFR (mL/min/1.73m2)	96 (34, 241)
Male N (%)	106 (58)
Race N (%)	
White	116 (64)
Asian	40 (22)
Black	12 (7)
Others	14 (8)

Disease Status *N (%)*	
Healthy	60 (33)
Hereditary ATTR amyloidosis	122 (67)
Hepatic Impairment (Mild)	8 (4)
Renal Impairment (Mild/Moderate)	59 (32) / 10 (6)
Others *N (%)*	
Prefilled syringe ≥1	65 (36)
ADA positive	6 (3)

Method: Nonlinear mixed effect PK modeling was conducted using NONMEM version 7.4.1. The base structural model was first developed to describe vutrisiran PK profiles. Covariate modeling was done using full covariate model approach. Continuous covariates were included into the model as a power relationship, while categorical covariates were implemented as a difference to a reference group. The final model was evaluated using goodness-of-fit plots, individual-predicted PK profiles, bootstrap methods, and visual predictive checks.

Results: The PK of vutrisiran was described by a two compartments model with first-order absorption. The plasma clearance was the sum of hepatic and renal clearance, in which renal clearance was fixed to the individual patient's baseline renal function (eGFR*BSA/1.73) value (**Figure 6**). Body weight was added on clearance and volume of distribution (central and peripheral) using allometric exponents; lower body weight and pre-filled syringe (PFS-S) were associated with faster rate of absorption from the SC compartment. The parameter estimates of the final pop PK model for vutrisiran are shown in **Table 7**.

Figure 6: Schematic representation of the PK model for vutrisiran



Abbreviations: CL_H=hepatic uptake clearance; CL_R=renal clearance; Ka=first-order absorption rate constant; PK=pharmacokinetic; Q=inter-compartmental clearance between central and peripheral compartment; SC=subcutaneous; V_2=volume of distribution in central compartment; V_3=volume of distribution in peripheral compartment.

Source: *Applicant's population PK report: Figure 7, Page 37.*

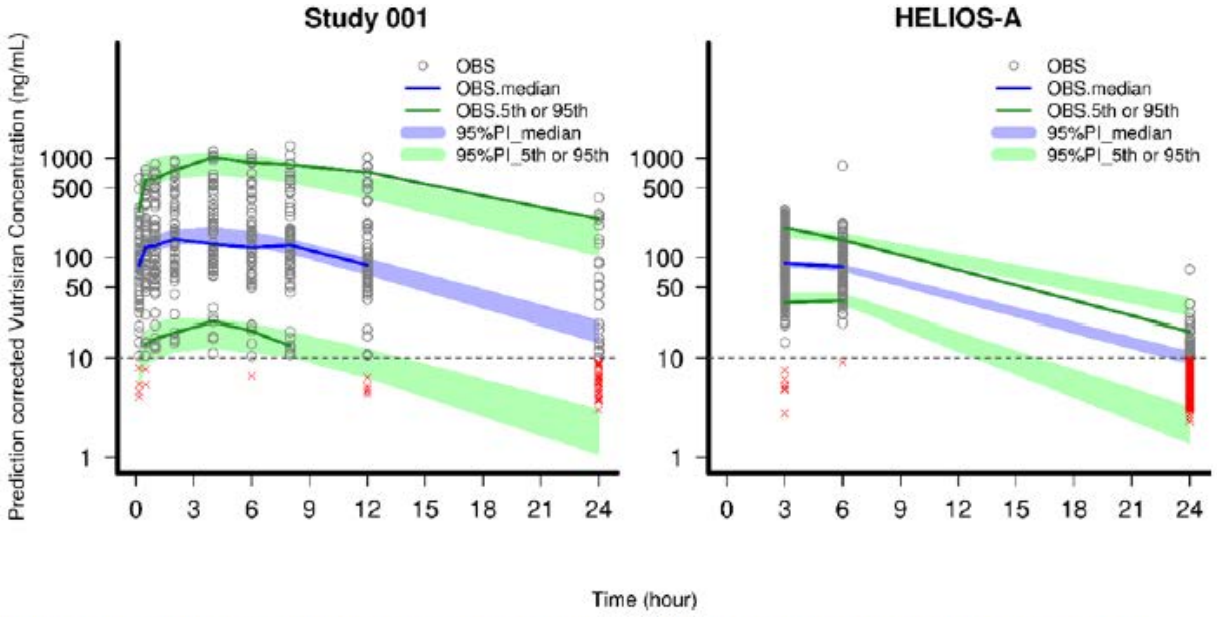
Table 7: Parameter estimates of sponsor's final population PK model for vutrisiran

PK Parameters	Population Estimates	IIV (%)	RSE (%)	95% CI		Shrinkage (%)
				Lower	Upper	
Ka_{vial} (h^{-1})	$0.1423*(weight/70)^{-1.46}$	39.7	4.37	0.120	0.168	13.9
Ka_{PFS} (h^{-1})	$0.2346*(weight/70)^{-1.46}$		8.24	0.185	0.296	
CL_H (L/h)	$14.59*(weight/70)^{0.75}$	33.5	1.44	13.5	15.8	26.4
CL_R (L/h)	eGFR*BSA/1.73	NA	NA	NA	NA	NA
Q (L/h)	$41.26*(weight/70)^{0.75}$	NA	4.49	29.7	57.0	NA
V_2 (L)	10.07*(weight/70)	NA	5.76	7.74	13.0	NA
V_3 (L)	52.46*(weight/70)	NA	3.67	39.3	69.5	NA
Residual error for healthy subjects (%)	23.8	NA	8.53	19.8	27.8	NA
Residual error for hATTR amyloidosis patients (%)	33.7	NA	6.48	29.4	38.0	NA

Source: *Applicant's population PK report: Table 10, Page 39*

The population PK model for vutrisiran was assessed with diagnostics plots including goodness-of-fit and visual predictive checks (**Figure 7**). Overall, the PK model adequately describes the PK of vutrisiran. The impact of significant covariates was evaluated on C_{max} and AUC_{0-24} using forest plots (**Figure 8**). Patients weighing <64 kg were predicted to be 25% higher in AUC and 51% higher in C_{max}, while the patients weighing ≥75 kg were 22% lower in AUC and 44% lower in C_{max} when compared to patients weighing 64 to <75-kg. In terms of vutrisiran presentation (PFS-S vs. vial with syringe), PFS-S presentation resulted in 35% higher C_{max} compared to vial with syringe with no effect on AUC_{0-24}. To evaluate the impact of renal function, typical eGFR values of 90, 60, 30, and 15 mL/min/1.73 m^2 were used to represent patients with normal renal function, mild, moderate, and severe renal impairment, respectively. The magnitude of effect on AUC and Cmax was within ≤20% except in the case of severe renal impairment where 27% higher AUC was estimated. It should be noted that these are only model predictions for severe renal impairment because there were no patients with severe renal impairment in Study 001 or HELIOS-A.

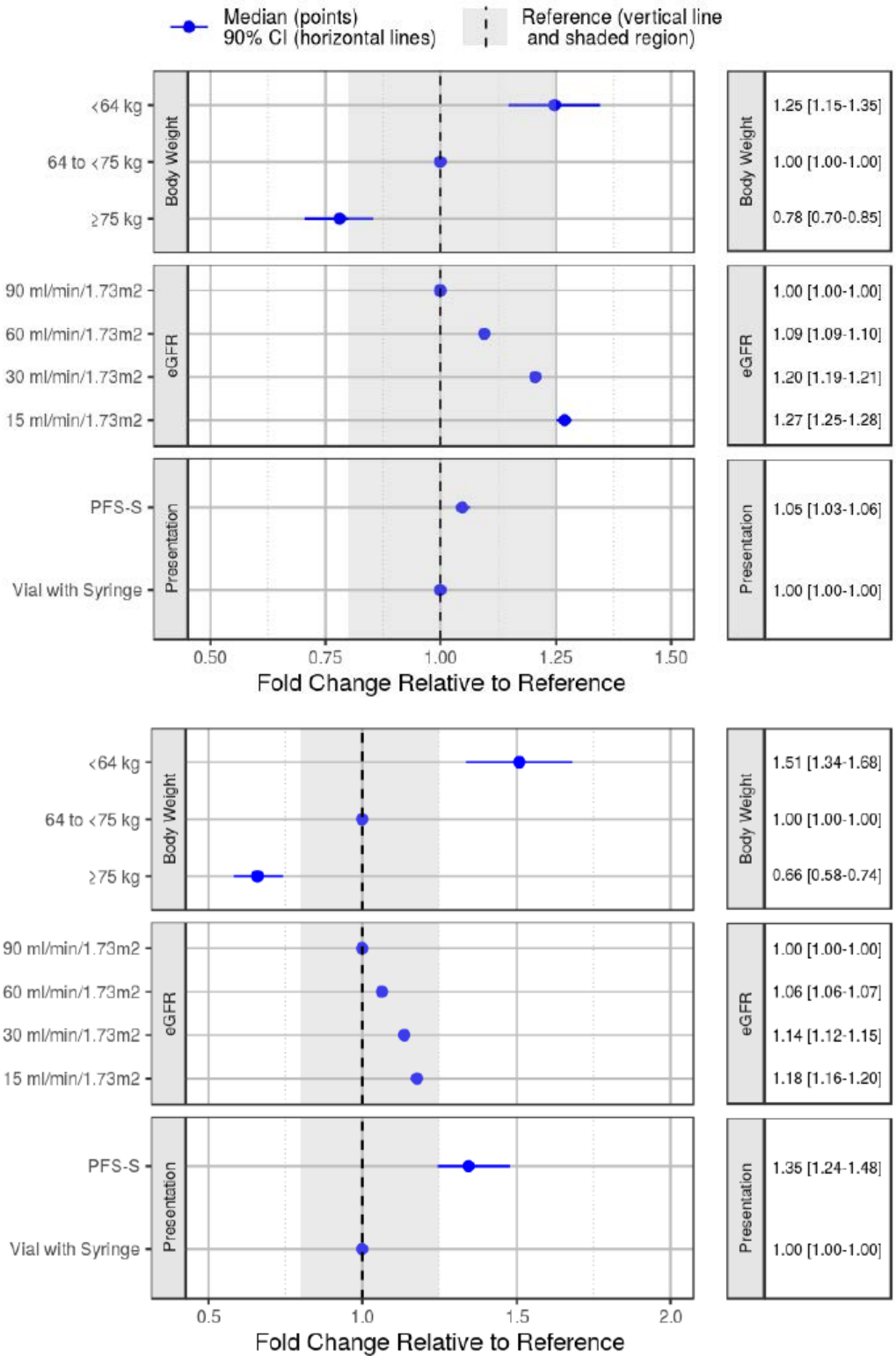
Figure 7: Prediction-corrected visual predictive checks for vutrisiran stratified by study.



Abbreviations: BLQ=below the lower limit of quantitation; LLOQ=lower limit of quantitation; PI=prediction interval; PK=pharmacokinetic; VPC=visual predictive check; OBS=observations.
Notes: Open circles represent the prediction-corrected observed data. Solid green lines are 5th and 95th percentiles of observed data. The solid blue line is the observed median. The shaded areas are 95% PIs of predicted data. Dotted line represents LLOQ of vutrisiran (10 ng/mL). Red crosses indicate postdose samples reported as BLQ.

Source: *Applicant's population PK report: Figure 10, Page 42.*

Figure 8: Covariate effects on AUC_{0-24} (top) and C_{max} (bottom) at vutrisiran 25 mg Q3M



Source: *Applicant's population PK report: Figure 11-12, Page 44-45.*

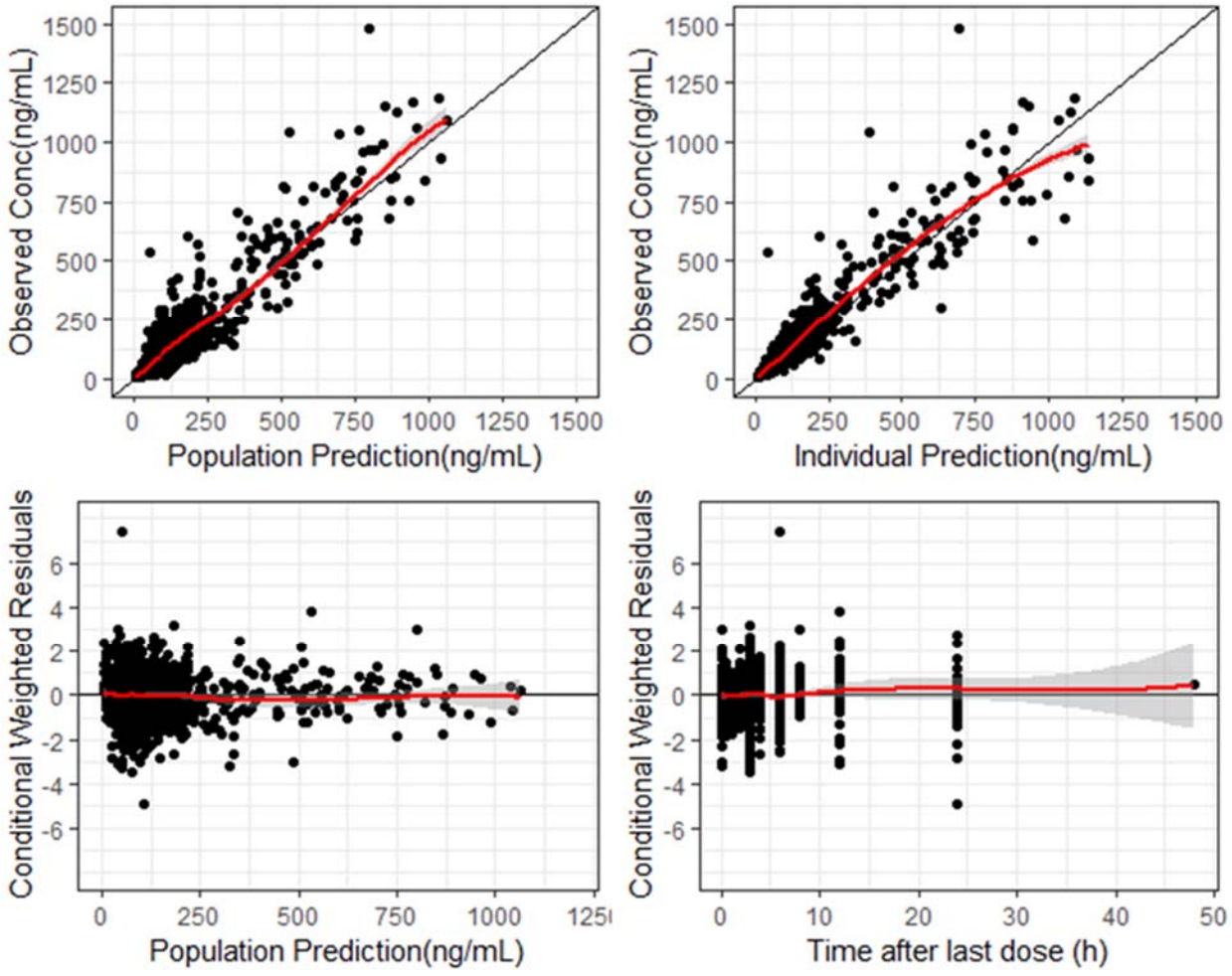
REVIEWER'S ANALYSIS

Sponsor's population PK model evaluation

The reviewer was able to run the sponsor's final PK model and obtained similar results. Model diagnostics for vutrisiran are shown in **Figure 9** and **Figure 10**, respectively. Overall, the PK model adequately describes the PK of vutrisiran.

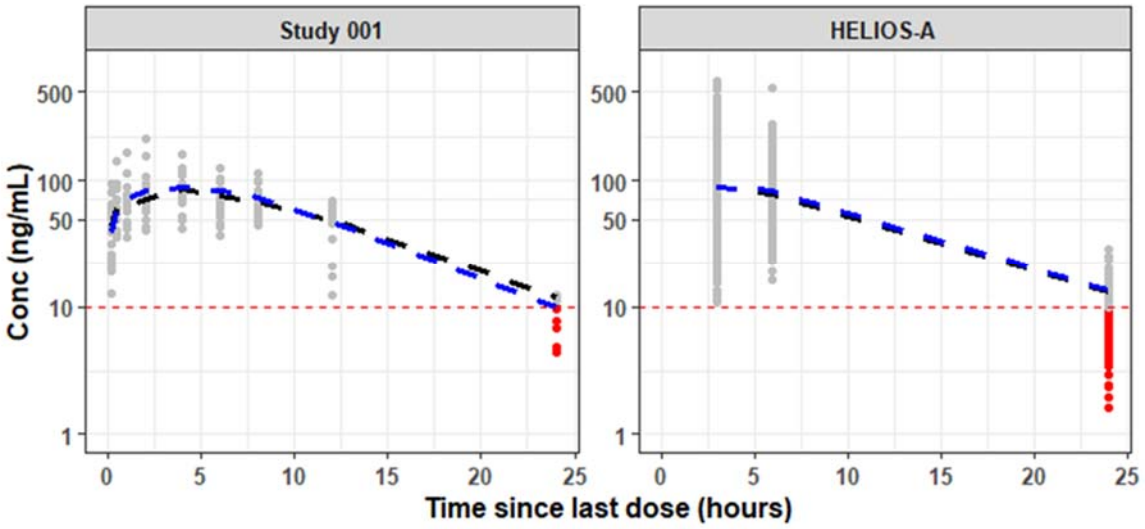
The clinically relevant impact of the covariates on the PK of vutrisiran was evaluated using the final PK model. From the final PK model, individual post-hoc PK parameters (empirical Bayes Estimates) were used to simulate individual PK profiles from each patient, and then compared at group-level across the covariate-of-interest.

Figure 9: Goodness-of-fit plots of sponsor's population PK model for vutrisiran



***Source**: Reviewer's analysis*

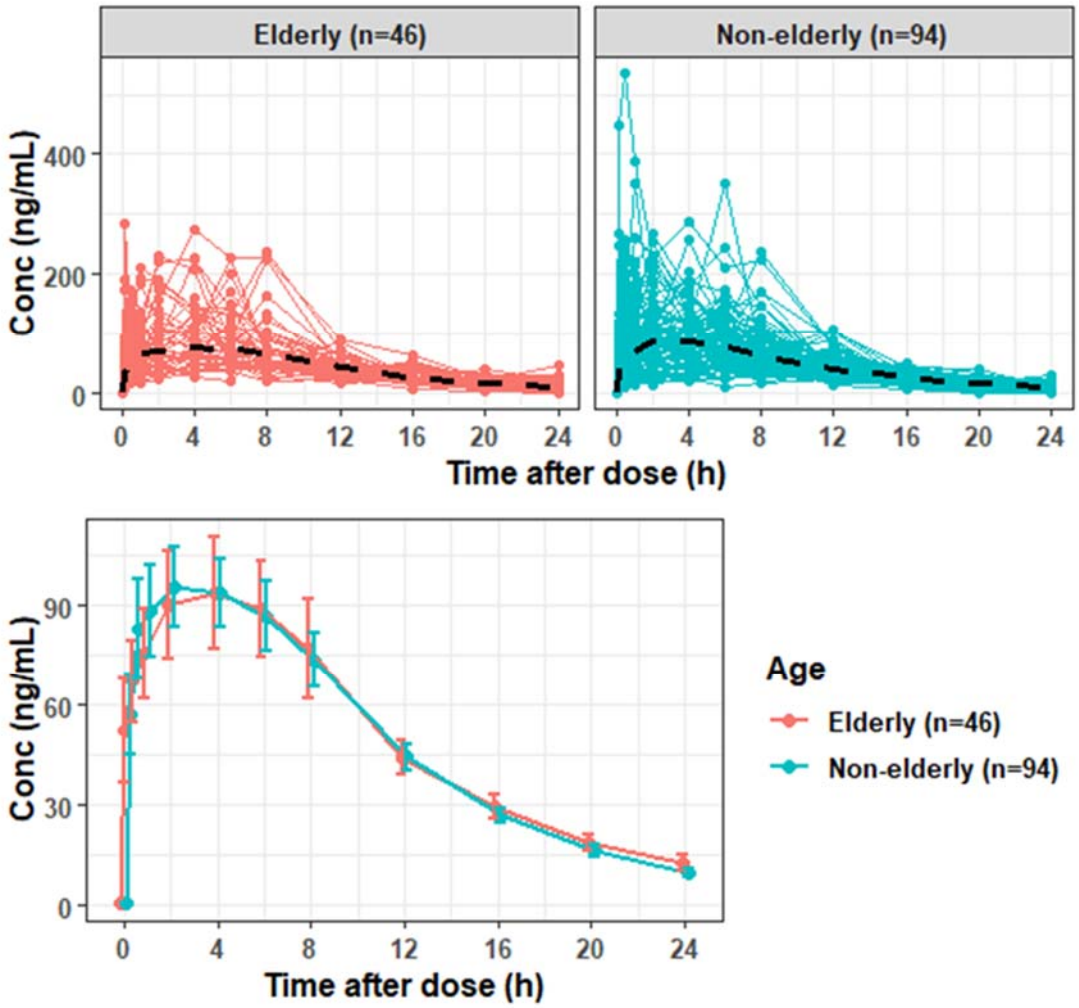
Figure 10: Comparison of the model-predicted median PK profiles with observed data stratified by study. Blue and black lines represented model-predicted and observed median of the data.



Grey and red solid circle represents observations above limit of quantification (LOQ, shown as red dashed line) and model predicted BLQ observations, respectively.
***Source**: Reviewer's analysis*

Age effect: The PK profiles of vutrisiran were divided into elderly (≥65 years, n=46) and non-elderly (<65 years, n=94) based on their age and plotted along with the corresponding median population predictions (**Figure 11**). Overall, overlap of 95% confidence intervals of the PK profiles by age quartiles suggested lack of clinically relevant impact of age on the PK of vutrisiran. Additionally, the pop PK model developed from the data of 182 subjects (range: 18-85 years) did not identify age as a significant covariate affecting vutrisiran PK.

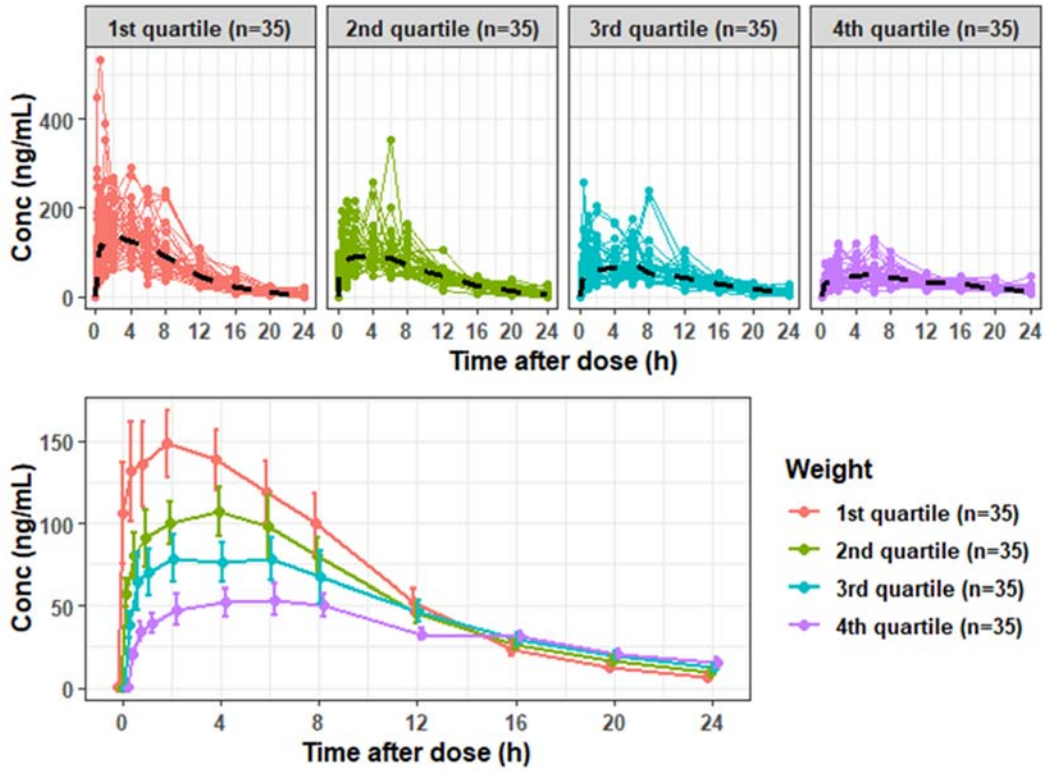
Figure 11: Top row: Individual PK profiles of vutrisiran by age quartiles. Black dashed line indicates median population predictions from the final PK model; Bottom row: Mean (95% CI) PK profiles of vutrisiran by age quartiles.



Source: *Reviewer's analysis*

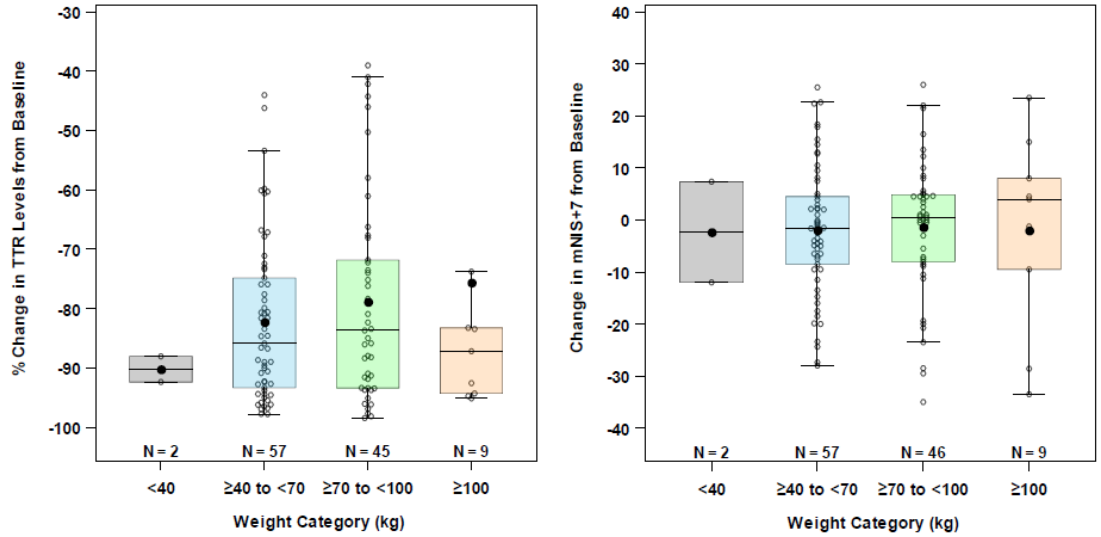
Body weight effect: The PK data of vutrisiran were distributed into quartiles (i.e., 1st quartile: n=35, 38-59; 2nd quartile: n=35, 59-69; third quartile: n=35, 69-80; and 4th quartile: n=35, 80-130) based on their weight distribution and plotted along with the corresponding median population predictions (**Figure 12**). Overall, increase in body weight resulted in lower AUC and C_{max} of vutrisiran. However, similar TTR levels and mNIS+7 changes at Month 9 were observed across bodyweight groups in hATTR patients (**Figure 13**). Therefore, the changes in PK exposures do not suggest clinically relevance impact of body weight on vutrisiran PK.

Figure 12: Top row: Individual PK profiles of vutrisiran by body weight quartiles. Black dashed line indicates median population predictions from the final PK model; Bottom row: Mean (95% CI) PK profiles of vutrisiran by body weight quartiles.



Source: *Reviewer's analysis*

Figure 13: Changes from baseline in TTR and mNIS+7 by bodyweights from HELIOS-A

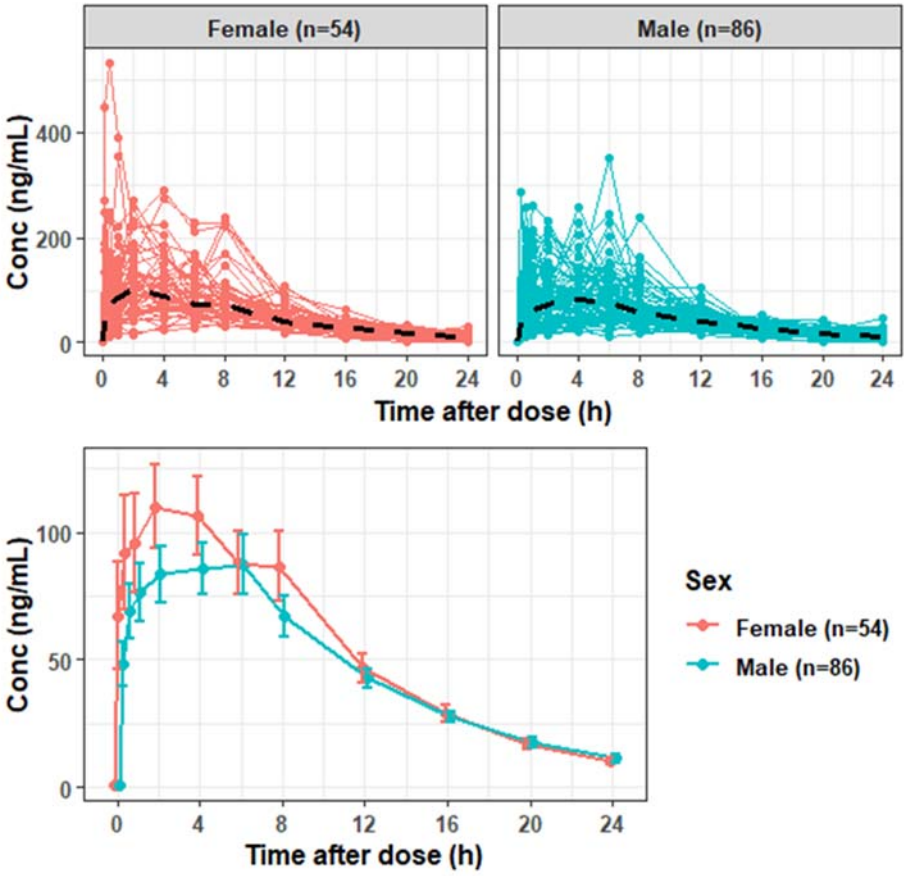


Abbreviations: mITT=modified intent-to-treat; mNIS+7=modified neuropathy impairment score+7; TTR=transthyretin

Source: *Applicant's Summary of Clinical Pharmacology report: Figure 36, Page 93.*

Sex effect: The PK profiles of 86 males and 54 females were compared and plotted along with the corresponding population predictions (**Figure 14**). Overall, the median PK profiles of male subjects has shown a decrease of 20.9% in C_{max} and 14% in AUC_{0-24}. Also, the pop PK model did not identify sex as a significant covariate of vutrisiran PK after accounting for bodyweight and vutrisiran presentations in the PK model. This suggested lack of clinically relevant impact of sex on the PK of vutrisiran.

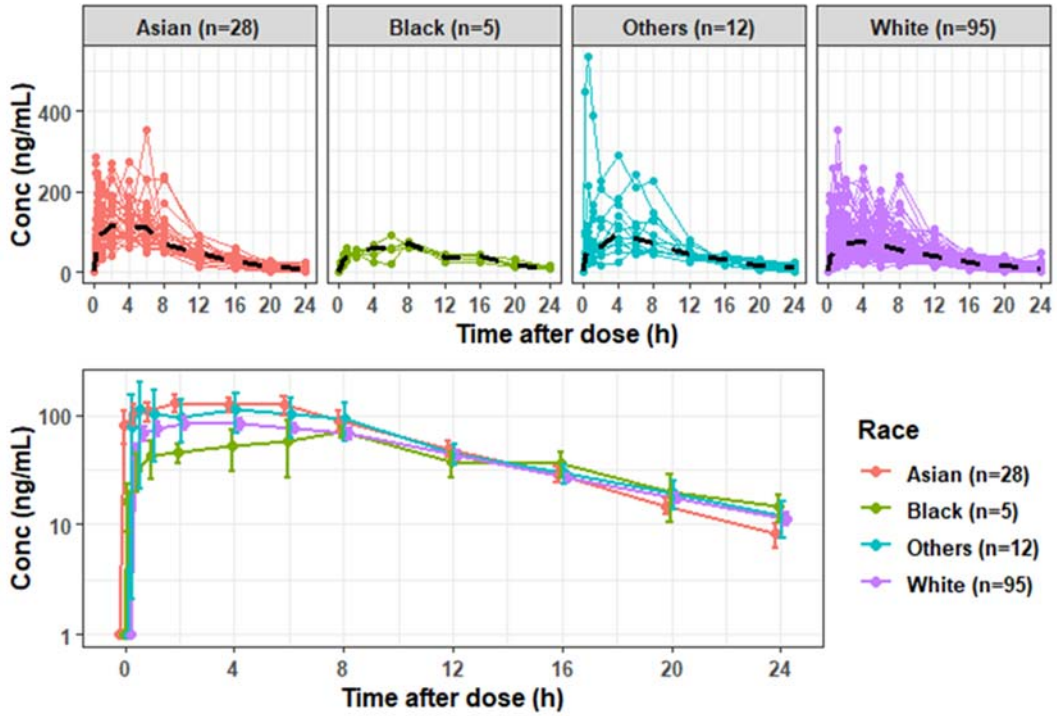
Figure 14: Top row: Individual PK profiles of vutrisiran by sex. Black dashed line indicates median population predictions from the final PK model; Bottom row: Mean (95% CI) PK profiles of vutrisiran by sex.



Source: *Reviewer's analysis*

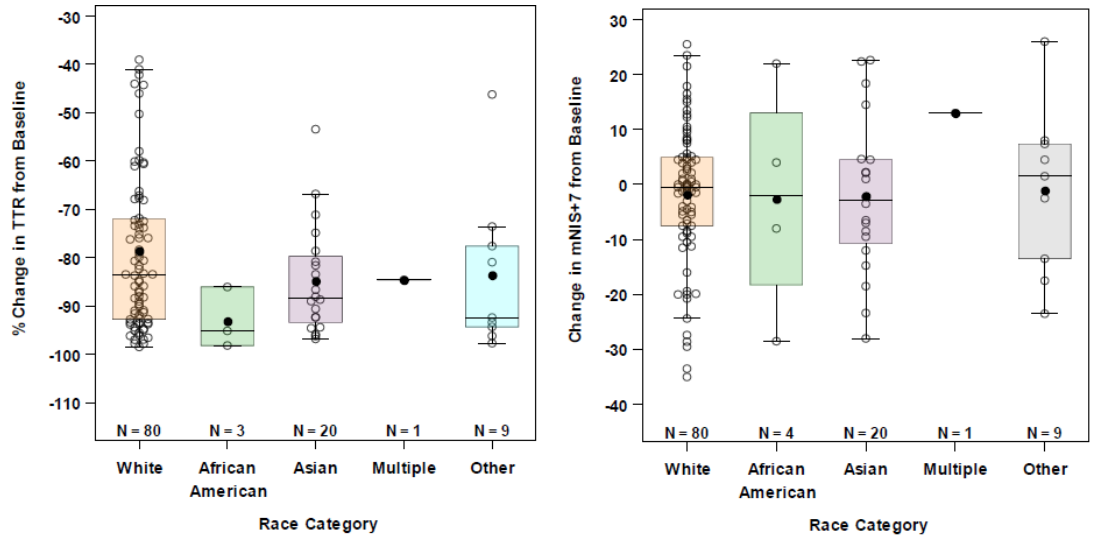
Race effect: The PK data of 95 White, 28 Asian, five Black and 12 other subjects were compared and plotted along with the corresponding median population predictions (**Figure 15**). The data was limited for Black and other categories. The median PK profiles of White subjects have shown 34.8% lower Cmax and 25.3% lower AUC_{0-24} when compared to Asian subjects. However, the pop PK model did not identify race as a significant covariate of vutrisiran PK after accounting for bodyweight and vutrisiran presentations in the PK model. Also, similar TTR levels and mNIS+7 changes at Month 9 were observed across races in hATTR patients (**Figure 16**). Therefore, the changes in PK exposures do not suggest clinically relevance impact of race on vutrisiran PK.

Figure 15: Top row: Individual PK profiles of vutrisiran by race. Black dashed line indicates median population predictions from the final PK model; Bottom row: Mean (95%CI) PK profiles of vutrisiran by race.



Source: *Reviewer's analysis*

Figure 16: Changes from baseline in TTR and mNIS+7 by race from HELIOS-A

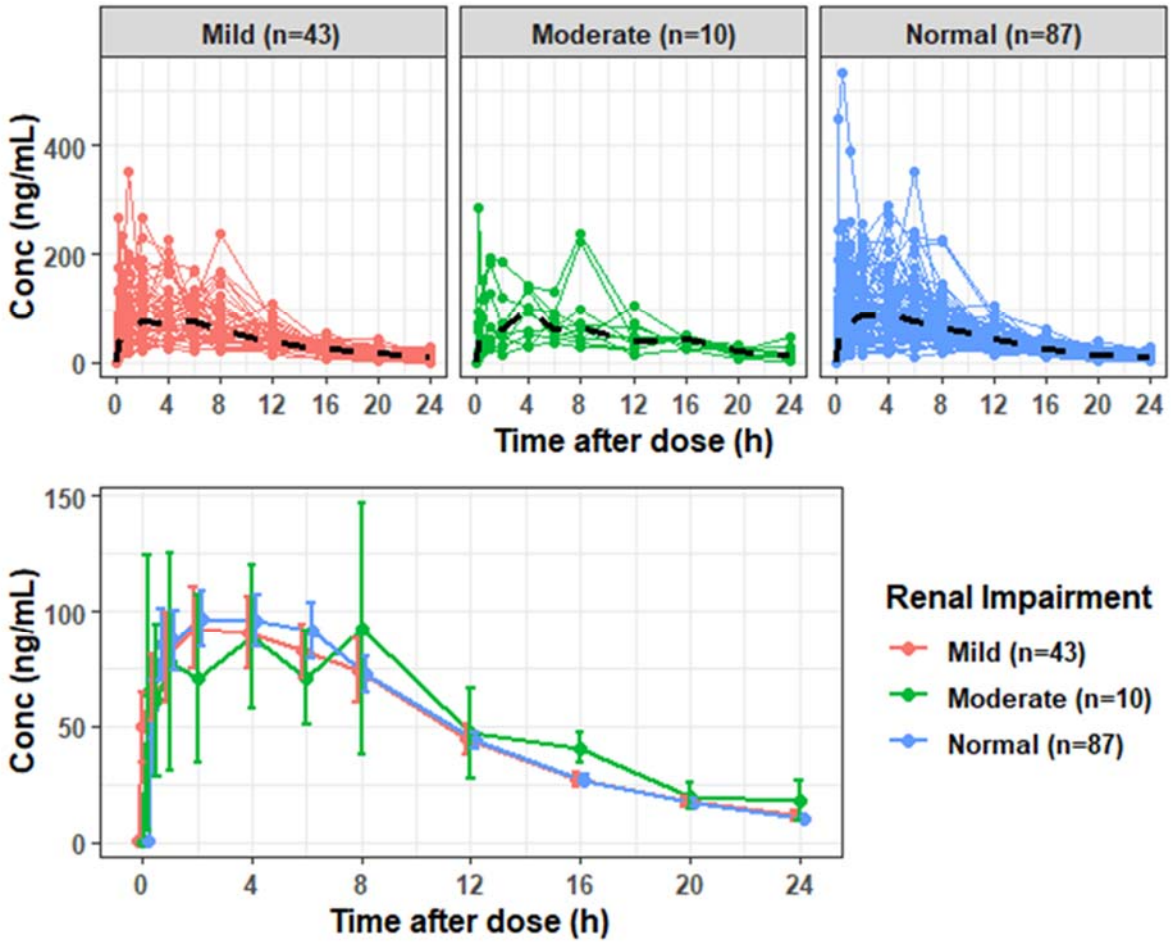


Abbreviations: mITT=modified intent-to-treat; mNIS+7=modified neuropathy impairment score+7; TTR=transthyretin

Source: *Applicant's Summary of Clinical Pharmacology report: Figure 31, Page 89.*

Renal impairment effect: The PK data of 87 normal, 43 mild renal impaired, and 10 moderate renal impaired subjects were compared and plotted along with the corresponding median population predictions (**Figure 17**). Overall, overlap of 95% confidence intervals of the PK profiles by renal status suggested lack of clinically relevant impact of renal impairment on the PK of vutrisiran. Limited data for subjects with moderate renal impairment resulted in large confidence interval. Of note, while the data was not available for the subjects with severe renal impairment, the population PK analysis suggested no dose adjustment for subjects with severe renal impairment (**Figure 8)**.

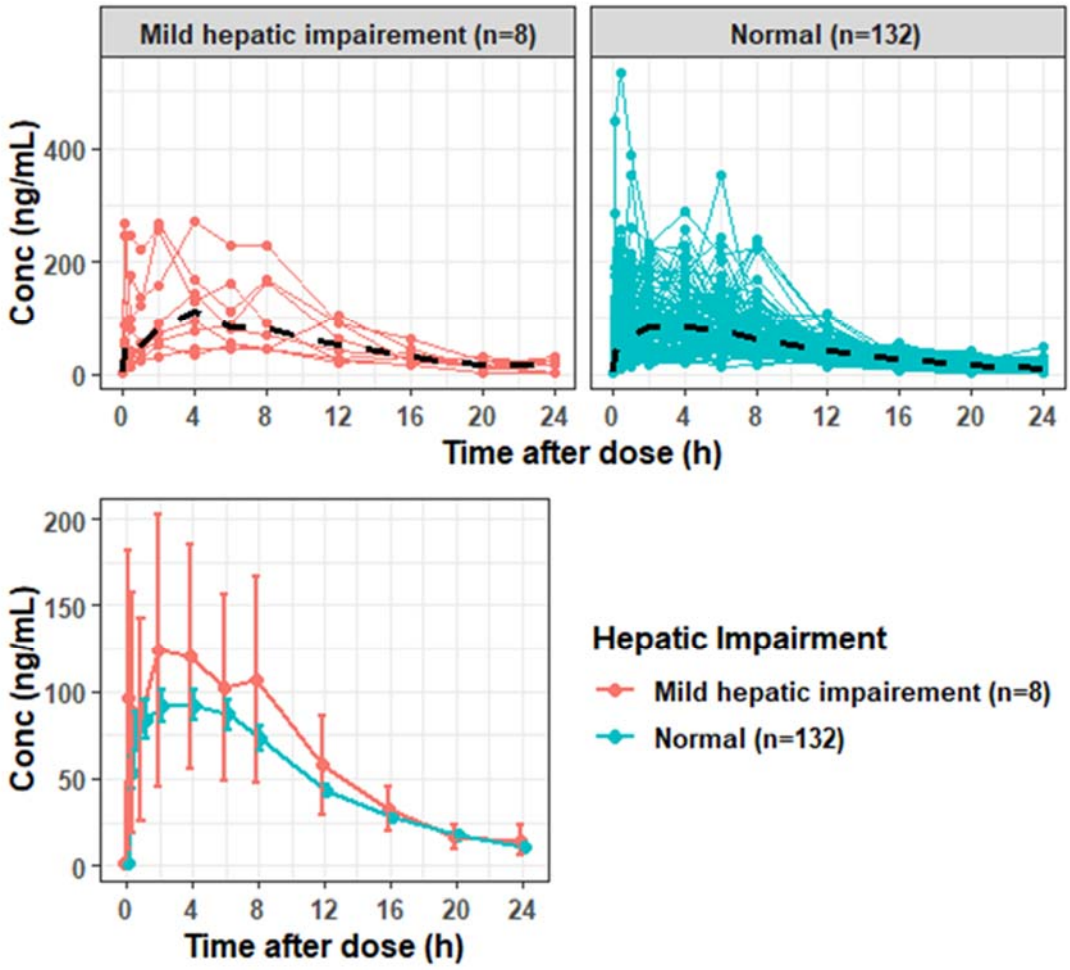
Figure 17: Top row: Individual PK profiles of vutrisiran by renal status. Black dashed line indicates median population predictions from the final PK model; Bottom row: Mean (95%CI) PK profiles of vutrisiran by renal status.



Source: *Reviewer's analysis*

Hepatic impairment effect: The PK data of 132 normal, 8 mild hepatic impaired subjects were compared and plotted along with the corresponding median population predictions (**Figure 18**). Overall, overlap of 95% confidence intervals of the PK profiles by hepatic status suggested lack of clinically relevant impact of mild hepatic impairment on the PK of vutrisiran. Of note, the data was not available for the subjects with moderate and severe hepatic impairment.

Figure 18: Top row: Individual PK profiles of vutrisiran by hepatic status. Black dashed line indicates median population predictions from the final PK model; Bottom row: Mean (95%CI) PK profiles of vutrisiran by hepatic status.



Source: *Reviewer's analysis*

REFERENCES

1. Alnylam's population PK report: Population pharmacokinetic analysis of vutrisiran in healthy subjects and patients with hereditary transthyretin-mediated amyloidosis, 23 March 2021.

2. Alnylam's Summary of clinical pharmacology studies report

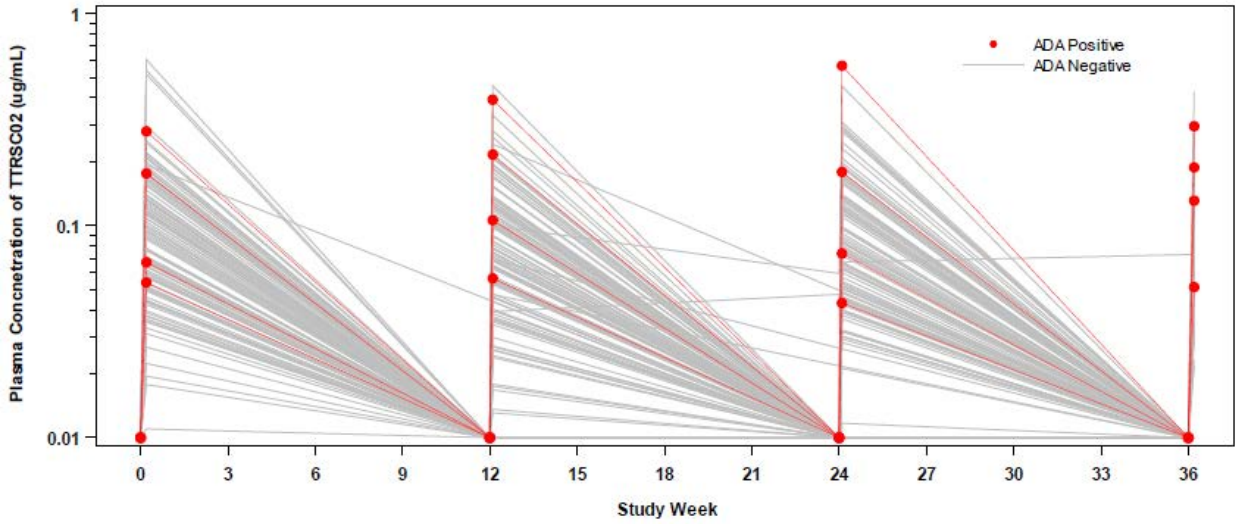
4.4 Effect of Immunogenicity on PK, PD, and Efficacy

In study 001, the incidence of ADA after single SC doses of vutrisiran was low (1.7%, 1/60). A non-Japanese subject who received 25 mg of vutrisiran had a low ADA titer (50) on Day 29, with all samples after Day 29 tested negative.

In the vutrisiran group of HELIOS-A Study, the incidence of treatment-induced ADA (defined as confirmed ADA positive postdose without preexisting baseline ADA) was 2.5% (3/120). The titers were low (50), with patients testing negative at a subsequent visit. One subject was ADA positive at baseline and on at least one post-baseline sample. There was no subject with treatment-boosted ADA (defined as baseline ADA titers increased > 4 × baseline postdose).

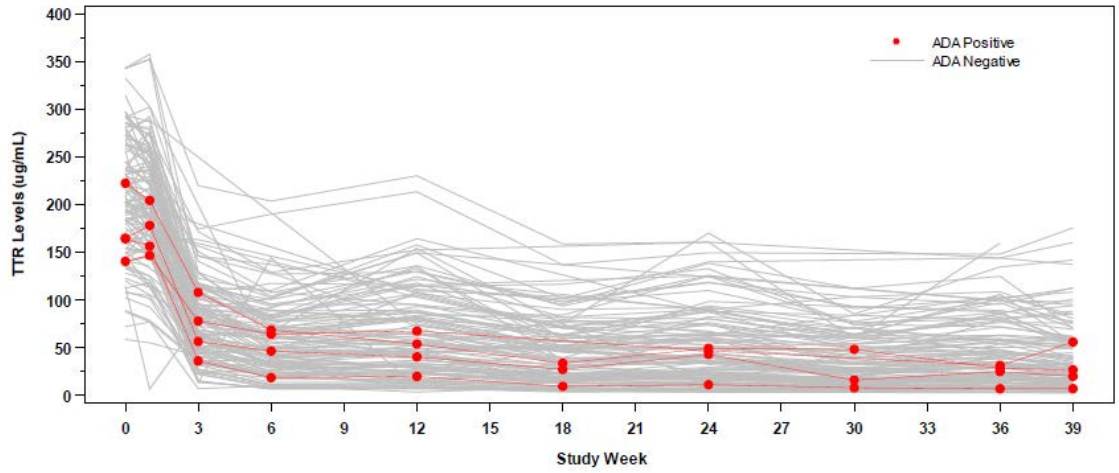
No significant difference was observed on vutrisiran PK, PD, and efficacy between ADA-positive patients and ADA-negative patients. Vutrisiran plasma concentration in ADA-positive patients were comparable to that in ADA-negative patients (**Figure 19**). Individual serum TTR reduction profiles in vutrisiran patients who tested ADA positive were comparable to those who tested ADA negative (**Table 8, Figure 20**). Change from baseline in mNIS+7 at Month 9 was similar in ADA-positive and ADA-negative patients treated with vutrisiran (**Table 9**). A neutralizing assay for ADA against vutrisiran was not developed; however, because of the low incidence and low impact of ADA as described above, the neutralizing activity of ADA is not expected to affect the conclusions on immunogenicity assessments. Refer to the immunogenicity assay review by the Office of Biotechnology Products for details regarding the immunogenicity assay.

Figure 19: HELIOS-A: Individual Vutrisiran Plasma Concentration-Time Profiles in ADA-Positive and ADA-Negative Patients Over 9 Months



***Source**: Study 002 CSR1 Figure 14.4.7.1*

Figure 20: HELIOS-A: Individual TTR Concentration-Time Profiles in ADA-positive and ADA-negative Vutrisiran-treated Patients Over 9 Months



***Source**: Study 002 CSR1 Figure 14.4.8.1*

Table 8: Impact of immunogenicity on PD of vutrisiran in Study HELIOS-A

Statistic	ADA Positive	ADA Negative
N	4	117
Mean (±SD) [min-max]	76.61 (13.14) [59.3, 91.2]	74.56 (15.99) [1.2, 96.9]

Note: TTR Percent Change from Baseline at Month 9 by ADA Status in Vutrisiran-treated Patients. ADA positive group includes 3 patients with treatment-emergent ADA plus 1 patient who was positive at baseline and on at least one post-baseline sample.

***Source**: Study 002 CSR1 Figure 14.2.5.17*

Table 9: Impact of immunogenicity on efficacy of vutrisiran in Study HELIOS-A

Statistic	ADA Positive	ADA Negative
N	4	110
Mean (±SD) [min-max]	-4.22 (6.49) [-12.0, 2.1]	-1.61 (13.40) [-35.0, 26.0]

Note: Change from Baseline in mNIS+7 at Month 9 by ADA status in vutrisiran-treated patients. ADA positive group includes 3 patients with treatment-emergent ADA plus 1 patient who was positive at baseline and on at least one post-baseline sample.

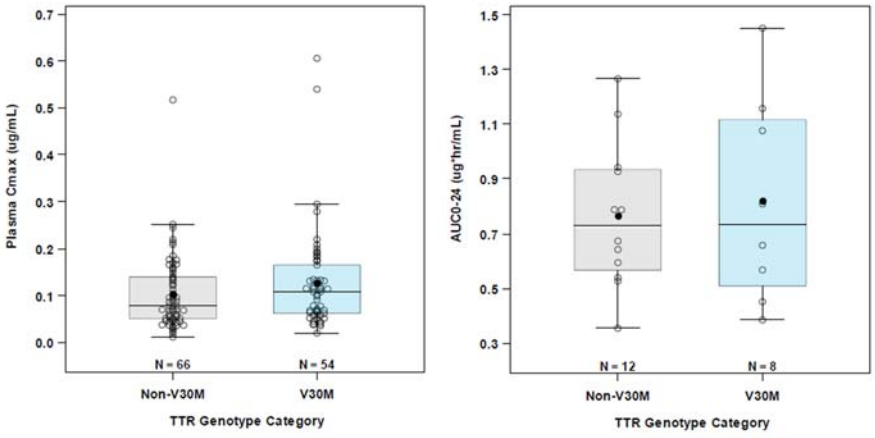
***Source**: Study 002 CSR1 Table 14.4.6*

Reviewer's comment: Overall, the incidence of treatment-emergent ADA in vutrisiran-treated patients was low (2.2%, 4/120) across the two clinical studies, and there was no treatment-boosted ADA. Treatment-emergent ADA titers were low (50) and transient. No time pattern was observed in the emergence of ADA, which occurred as early as Day 29 and as late as Day 260. Presence of ADA did not affect the PK, PD, or safety profile of vutrisiran across both studies, and in HELIOS-A no impact of ADA was observed on efficacy. Although these data do not demonstrate an impact of anti-drug antibody development on the efficacy or safety of AMVUTTRA in these patients, the available data are too limited to make definitive conclusions.

4.5 Pharmacogenomics

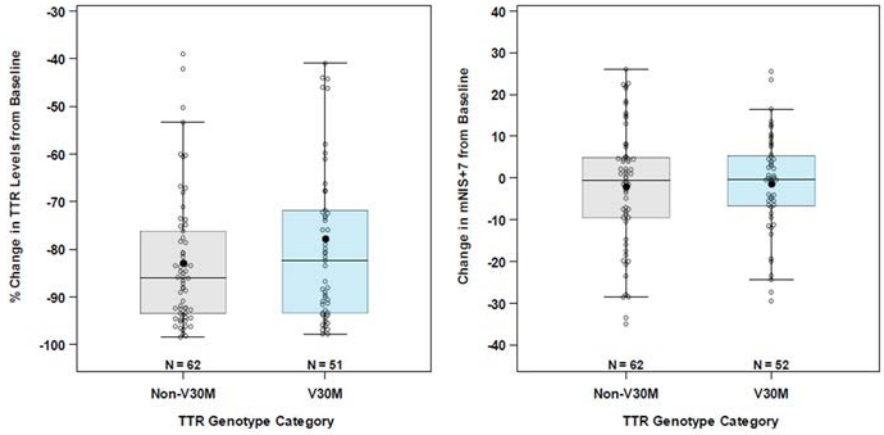
Overall, the plasma PK, PD, and efficacy of vutrisiran was comparable between hATTR amyloidosis patients with V30M and non-V30M TTR genotypes (**Figures 21-23**).

Figure 21. Vutrisiran C_{max} and AUC_{0-24} by TTR Genotype



***Source**: Applicant's Summary of Clin Pharm page 94*

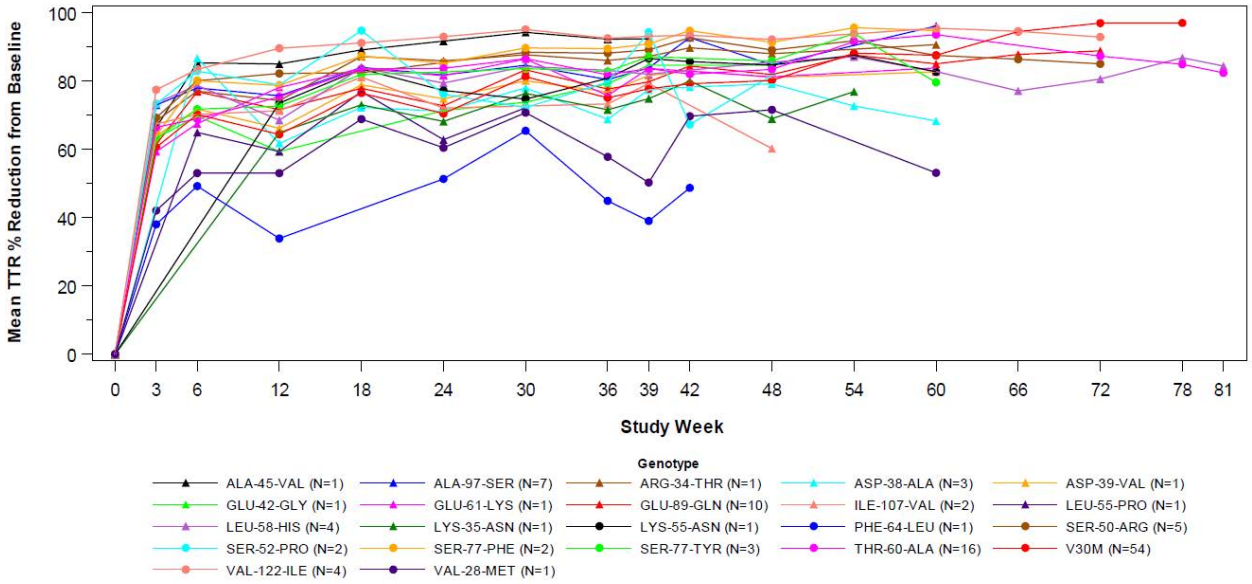
Figure 22. Percent Change in TTR and mNIS+7 from Baseline at Month 9 by *TTR* Genotype



***Source**: Applicant's Summary of Clin Pharm page 95*

Similar mean percent TTR reductions were observed regardless of TTR variant from baseline to month 9 in the prespecified TTR genotype subgroups of V30M compared to non-V30M (71.3±18.5 vs. 77.3±12.9, respectively). Moreover, the applicant enrolled 22 different TTR variants in their pivotal study. Given the limited numbers of patients in these subgroups, comparison by individual TTR variant is unlikely to yield any actionable results. In addition, the binding site (nucleotides 615 to 637 in exon 4 of the TTR mRNA) of vutrisiran is not in an area that contains any common single-nucleotide polymorphisms likely to impact Watson-Crick binding and subsequent silencing of any of the TTR variants eligible for treatment.

Figure 23. Mean Percentage Reduction from Baseline over Time by Genotype



***Source**: Applicant's ALN-TTRSC02-002 CSR Body Section 14 Figure 14.2.5.2, page 689*

4.6 List of Clinical Studies in the Development Program

Table 10 Summary of Clinical Studies

Study Identifier	Study Population	Study Design	Treatment
ALNTTRSC02-001	Healthy adults (n=80)	Phase 1 randomized (3:1), single-blind, placebo-controlled, single-ascending dose study	Vutrisiran; Single dose 5, 25, 50, 100, 200, or 300 mg; SC injection
ALNTTRSC02-002 (HELIOS-A)	Adult patients with hATTR amyloidosis with polyneuropathy (n=164)	Phase 3 randomized (3:1), open-label study with reference comparator arm and external placebo control Part 1: 18-month Treatment Period (vutrisiran or patisiran); primary efficacy analysis at Month 9	Part 1: Vutrisiran 25 mg Q3M by SC injection or Patisiran 0.3 mg/kg (up to 30 mg for patients ≥100 kg) Q3W; IV infusion

		Part 2: 18-month Treatment Extension Period, all patients receive vutrisiran	
ALN-TTR02-004 (APOLLO, the external control for HELIOS-A)	Adult patients with hATTR amyloidosis with polyneuropathy	Phase 3 randomized (2:1), double-blind, placebo-controlled study, with primary analysis at Month 18 The placebo arm serves as an external control for HELIOS-A	Patisiran 0.3 mg/kg (up to 30 mg for patients ≥100 kg) Q3W; IV infusion

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