

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

215842Orig1s000

OTHER REVIEW(S)

MEMORANDUM
REVIEW OF REVISED LABELING
Division of Medication Error Prevention and Analysis 2 (DMEPA 2)
Office of Medication Error Prevention and Risk Management (OMEPRM)
Office of Surveillance and Epidemiology (OSE)
Center for Drug Evaluation and Research (CDER)

Date of This Memorandum:	September 1, 2023
Requesting Office or Division:	Division of Cardiology and Nephrology (DCN)
Application Type and Number:	NDA 215842
Product Name, Dosage Form, and Strength:	Rivfloza (nedosiran) injection, Vial: 80 mg/0.5 mL Prefilled syringe: 128 mg/0.8 mL and 160 mg/mL
Applicant Name:	Novo Nordisk Inc.
TTT ID #:	2022-2123-2
DMEPA 2 Safety Evaluator:	Ebony Whaley, PharmD, BCPPPS
DMEPA 2 Associate Director for Human Factors:	Lolita Sterrett, PharmD
DMEPA 2 Deputy Director:	Chi-ming (Alice) Tu, PharmD, BCPS

1 PURPOSE OF MEMORANDUM

The Applicant submitted revised Prescribing Information (PI) on August 28, 2023 for Rivfloza. The Division of Cardiology and Nephrology (DCN) requested that we review the revised PI for Rivfloza (Appendix A) to determine if it is acceptable from a medication error perspective.

2 DISCUSSION

The Applicant previously proposed [REDACTED] (b) (4)
[REDACTED]
[REDACTED]. However, in the revised PI submitted on August 28, 2023, the Applicant proposes the following the recommended dosing:

- Pediatric patients aged 9 to 11 years old who weigh ≥ 50 kg: 160 mg once monthly using the 160 mg/mL prefilled syringe (PFS)

- Pediatric patients aged 9 to 11 years old who weigh < 50 kg: 3.3 mg/kg once monthly (not to exceed 128 mg) using the vial presentation

We note the Applicant previously submitted HFVS results to support the use of the PFS in the following intended users: adult patients, pediatric patients aged 12 to 17 years old, and adult caregivers. However, the HFVS did not include simulated use testing of pediatric patients aged 9 to 11 years old (b) (4). Because no HFVS data was submitted to (b) (4) -

(b) (4) pediatric patients aged 9 to 11 years old, (b) (4). However, we acknowledge that HFVS data was submitted to support caregiver administration of the PFS, and we previously determined the caregiver HFVS data was acceptable.^a As such, we expect that caregivers will be able to administer the PFS to pediatric patients aged 9 to 11 years old.

Additionally, at the time of the June 5, 2023 Late Cycle Meeting^b, the vial presentation was intended for use in pediatric patients aged under 12 years old, and we note the Applicant indicated they anticipate the vial presentation would be used by a small patient population (i.e., approximately 30 patients over the 3-year timeframe from 2024 to 2026). As such, we find it reasonable to presume there would similarly be a smaller subset of the patient population aged 9 to 11 years old who weigh ≥ 50 kg and who would use the 160 mg/mL PFS.

If the Review Team finds the revised dosing for patients aged 9 to 11 years old who weigh ≥ 50 kg acceptable, then we recommend that administration of the PFS be limited to healthcare providers and caregivers only for patients aged 9 to 11 years old. (b) (4)

(b) (4), we recommend revisions to Section 2.2 Administration Instructions of the Prescribing Information (PI) and the PFS Instructions for Use (IFU) to include a statement to indicate that healthcare providers and caregivers should administer the PFS to pediatric patients aged 9 to 11 years old who weigh ≥ 50 kg.

3 CONCLUSION AND RECOMMENDATIONS

From a medication error perspective, we recommend the PI and IFU be revised to indicate that caregivers should administer the 160 mg/mL PFS to pediatric patients aged 9 to 11 years old

^a Whaley, E. Human Factors Study Results and Label and Labeling Review for Rivfloza (NDA 215842). Silver Spring (MD): FDA, CDER, OSE, DMEPA 2 (US); 2023 JUL 12. TTT ID No.: 2022-2123 and 2022-2125.

^b Proctor, B on behalf of Aliza Thompson. Late Cycle Meeting Minutes for Rivfloza (NDA 215842). Silver Spring (MD): FDA, CDER, OND, OCHEN, DCN (US); 2023 JUL 5.

who weigh ≥ 50 kg. Below we have provided recommendations for the Division and the Applicant.

3.1 RECOMMENDATIONS FOR THE DIVISION OF CARDIOLOGY AND NEPHROLOGY

The Applicant did not submit human factors validation study (HFVS) data for the Rivfloza (nedosiran) injection prefilled syringe (PFS) to (b) (4) pediatric patients aged 9 to 11 years old who weigh ≥ 50 kg to include use of the 160 mg/mL PFS. (b) (4)

(b) (4)

(b) (4)

If the Review Team finds the revised dosing for patients aged 9 to 11 years old who weigh ≥ 50 kg acceptable, then we recommend that administration of the PFS be limited to healthcare providers and caregivers only for patients aged 9 to 11 years old. Additionally, we recommend including the following statements in Section 2.2 Administration Instructions of the Prescribing Information and in the PFS Instructions for Use:

- “Prefilled syringe: A healthcare provider, caregiver, or patient 12 years of age and older may inject RIVFLOZA using the pre-filled syringe. In pediatric patients 9 to 11 years of age who weigh ≥ 50 kg, a healthcare (b) (4) or caregiver may inject RIVFLOZA using the pre-filled syringe.”

If the Review Team agrees with our recommendation above, then we would like to communicate our recommendation in 3.2 below to Novo Nordisk Inc.

3.2 RECOMMENDATIONS FOR NOVO NORDISK INC

For Rivfloza (nedosiran) injection NDA 215842, we note you intend to revise your label to recommended pediatric patients aged 9 to 11 years old who weigh ≥ 50 kg are indicated to receive a dose of 160 mg once monthly using the 160 mg/mL prefilled syringe (PFS).

Because you did not submit human factors validation study (HFVS) data for the PFS to (b) (4) pediatric patients aged 9 to 11 years old, (b) (4)

(b) (4)

As such, we find the labeling for Rivfloza (nedosiran) injection should indicate that for pediatric patients aged 9 to 11 years old who weigh ≥ 50 kg, administration of the 160 mg/mL PFS should be limited to healthcare providers and caregivers only.

If you intend [REDACTED] (b) (4)
[REDACTED] in the future, additional HF data needs may apply. As such, we recommend you consider discussing your plans with the Agency prior to future submissions.

APPENDIX A. LABEL AND LABELING

- Prescribing Information (image not available) received on August 28, 2023. Available in EDR via: <\\CDSESUB1\EVSPROD\nda215842\0038\m1\us\114-labeling\114a-draft-label\proposed-uspi-pdf-tracked-20230828.pdf>

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/s/

EBONY A WHALEY
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09/01/2023 11:30:41 AM

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**Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Medical Policy**

PATIENT LABELING REVIEW

Date: August 31, 2023

To: Brian Proctor
Regulatory Project Manager
Division of Cardiology and Nephrology (DCN)

Through: LaShawn Griffiths, MSHS-PH, BSN, RN
Associate Director for Patient Labeling
Division of Medical Policy Programs (DMPP)

Barbara Fuller, RN, MSN
Team Leader, Patient Labeling
Division of Medical Policy Programs (DMPP)

From: Jessica Chung, PharmD, MS
Patient Labeling Reviewer
Division of Medical Policy Programs (DMPP)

Charuni Shah, PharmD
Regulatory Review Officer
Office of Prescription Drug Promotion (OPDP)

Subject: Review of Patient Labeling: Patient Package Insert (PPI)
and Instructions for Use (IFUs)

Drug Name (established name): RIVFLOZA (nedosiran)

Dosage Form and Route: injection, for subcutaneous use

Application Type/Number: NDA 215842

Applicant: Novo Nordisk, Inc.

1 INTRODUCTION

On September 30, 2022, Novo Nordisk, Inc. submitted for the Agency's review the final part of their Rolling Submission of an original New Drug Application (NDA) 215842 for RIVFLOZA (nedosiran) injection. The proposed indication is for the treatment of primary hyperoxaluria type 1 (PH1) in pediatric ^(b)₍₄₎ years of age and older) and adult patients.

This collaborative review is written by the Division of Medical Policy Programs (DMPP) and the Office of Prescription Drug Promotion (OPDP) in response to requests by the Division of Cardiology and Nephrology (DCN) on November 30, 2022, for DMPP and OPDP to review the Applicant's proposed Patient Package Insert (PPI) and Instructions for Use (IFUs) for RIVFLOZA (nedosiran) injection.

DMPP conferred with the Division of Medication Error, Prevention, and Analysis (DMEPA) and a separate DMEPA review of the IFUs was completed on July 13, 2023.

2 MATERIAL REVIEWED

- Draft RIVFLOZA (nedosiran) injection PPI received on September 30, 2022, revised by the Review Division throughout the review cycle, and received by DMPP and OPDP on August 22, 2023.
- Draft RIVFLOZA (nedosiran) injection, single-dose Prefilled-syringe IFU received on September 30, 2022 and May 3, 2023, revised by the Review Division throughout the review cycle, and received by DMPP and OPDP on August 22, 2023.
- Draft RIVFLOZA (nedosiran) injection, single-dose vial IFU received on September 30, 2022, revised by the Review Division throughout the review cycle, and received by DMPP and OPDP on August 22, 2023.
- Draft RIVFLOZA (nedosiran) injection Prescribing Information (PI) received on September 30, 2022, revised by the Review Division throughout the review cycle, and received by DMPP and OPDP on August 22, 2023.

3 REVIEW METHODS

To enhance patient comprehension, materials should be written at a 6th to 8th grade reading level, and have a reading ease score of at least 60%. A reading ease score of 60% corresponds to an 8th grade reading level. In our review of the IFUs, the target reading level is at or below an 8th grade level.

Additionally, in 2008 the American Society of Consultant Pharmacists Foundation (ASCP) in collaboration with the American Foundation for the Blind (AFB) published *Guidelines for Prescription Labeling and Consumer Medication Information for People with Vision Loss*. The ASCP and AFB recommended using fonts such as Verdana, Arial or APHont to make medical information more accessible for patients with vision loss. We reformatted the IFU documents using the Arial font, size 10.

In our collaborative review of the PPI and IFUs we:

- simplified wording and clarified concepts where possible
- ensured that the PPI and IFUs are consistent with the Prescribing Information (PI)
- removed unnecessary or redundant information
- ensured that the PPI and IFUs are free of promotional language or suggested revisions to ensure that it is free of promotional language
- ensured that the PPI and IFUs meet the criteria as specified in FDA's Guidance for Useful Written Consumer Medication Information (published July 2006)

4 CONCLUSIONS

The PPI and IFUs are acceptable with our recommended changes.

5 RECOMMENDATIONS

- Please send these comments to the Applicant and copy DMPP and OPDP on the correspondence.
- Our collaborative review of the PPI and IFUs are appended to this memorandum. Consult DMPP and OPDP regarding any additional revisions made to the PI to determine if corresponding revisions need to be made to the PPI and IFUs.

Please let us know if you have any questions.

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/s/

JESSICA M CHUNG
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08/31/2023 11:02:01 AM

**FOOD AND DRUG ADMINISTRATION
Center for Drug Evaluation and Research
Office of Prescription Drug Promotion**

******Pre-decisional Agency Information******

Memorandum

Date: August 30, 2023

To: Kirtida Mistry, MD., Clinical Reviewer
Division of Cardiology and Nephrology (DCN)

Brian Proctor, Regulatory Project Manager (DCN)

From: Charuni Shah, Regulatory Review Officer
Office of Prescription Drug Promotion (OPDP)

CC: Sapna Shah, Team Leader, OPDP

Subject: OPDP Labeling Comments for
RIVFLOZA® (nedosiran) injection, for subcutaneous use

NDA: 215842

Background:

In response to DCN's consult request dated November 30, 2022, OPDP has reviewed the proposed Prescribing Information (PI), Patient Package Insert (PPI), and Instructions for Use (IFU) for the original NDA for RIVFLOZA® (nedosiran) injection, for subcutaneous use.

PI/MG:

OPDP's review of the proposed PI is based on the draft labeling provided via email by DCN on August 21, 2023, and our comments are provided below.

OPDP comments on the proposed PPI and IFU will be sent under separate cover, as a combined OPDP and Division of Medical Policy Programs (DMPP) review.

Thank you for your consult. If you have any questions, please contact Charuni Shah at (240)-402-4997 or Charuni.Shah@fda.hhs.gov.

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/s/

CHARUNI P SHAH
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MEMORANDUM
REVIEW OF REVISED LABEL AND LABELING
Division of Medication Error Prevention and Analysis 2 (DMEPA 2)
Office of Medication Error Prevention and Risk Management (OMEPRM)
Office of Surveillance and Epidemiology (OSE)
Center for Drug Evaluation and Research (CDER)

Date of This Memorandum:	August 1, 2023
Requesting Office or Division:	Division of Cardiology and Nephrology (DCN)
Application Type and Number:	NDA 215842
Product Name, Dosage Form, and Strength:	Rivfloza (nedosiran) injection, Vial: 80 mg/0.5 mL Prefilled syringe: 128 mg/0.8 mL and 160 mg/mL
Applicant Name:	Novo Nordisk Inc.
TTT ID #:	2022-2123-1
DMEPA 2 Safety Evaluator:	Ebony Whaley, PharmD, BCPPPS
DMEPA 2 Associate Director for Human Factors:	Lolita Sterrett, PharmD

1 PURPOSE OF MEMORANDUM

The Applicant submitted revised container labels and carton labeling received on July 31, 2023 for Rivfloza. The Division of Cardiology and Nephrology (DCN) requested that we review the revised container labels and carton labeling for Rivfloza (Appendix A) to determine if they are acceptable from a medication error perspective. The revisions are in response to recommendations that we made during a previous label and labeling review.^a

2 CONCLUSION

The Applicant implemented all of our recommendations and we have no additional recommendations at this time. As such, we find the following carton labeling and container labels acceptable:

- the carton labeling and carton labeling inserts submitted on July 31, 2023 for the 80 mg/0.5 mL vial, 128 mg/0.8 mL prefilled syringe (PFS) and 160 mg/mL PFS,

^a Whaley, E. Human Factors Study Results and Label and Labeling Review for Rivfloza (NDA 215842). Silver Spring (MD): FDA, CDER, OSE, DMEPA 2 (US); 2023 JUL 12. TTT ID No.: 2022-2123 and 2022-2125.

- the container labels submitted on July 31, 2023 for the 80 mg/0.5 mL vial and 160 mg/mL PFS, and
- the container label submitted on September 30, 2022 for the 128 mg/0.8 mL PFS.

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/s/

EBONY A WHALEY
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LOLITA G STERRETT
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505(b)(1) NDA Assessment: Immunogenicity Assay Method Validation

Submission Tracking Number (STN):	NDA 215842
Subject:	Assessment of the method validation report for an ELISA method for the detection of anti-nedosiran antibodies in human serum
Date Received:	September 30, 2022
Assessment/Revision Date:	July 17, 2023
Primary Assessor:	Mayumi Takahashi, Ph.D. OPQ/OBP/DBRR4
Secondary Assessor:	Frederick Mills, Ph.D. OPQ/OBP/DBRR4
Applicant:	Novo Nordisk, Inc.
Product:	Nedosiran sodium
Indication:	Treatment of primary hyperoxaluria Type 1
Clinical Division	CDER/OND/OCHEN/DCN

RECOMMENDATION:

An enzyme-linked immunosorbent assay (ELISA) was developed for the detection, confirmation, and titration of anti-nedosiran antibodies in clinical human serum samples. All the validation parameters are appropriately evaluated, and the assay method is specific, precise, selective, and robust for the detection of anti-nedosiran-reactive antibodies in clinical human serum samples. In addition, the method exhibits sufficient drug tolerance and no prozone effect. The ADA assay is appropriately validated, and thus, suitable for its intended purpose of assessing/measuring ADA levels in clinical samples.

Justification:

Nedosiran is a chemically modified double-stranded 36-22 mer small interfering RNA (siRNA), conjugated on the sense strand with triantennary N-acetylgalactosamine (GalNAc) to facilitate uptake by hepatocytes. Appropriate assessments were conducted throughout the development program to determine its immunogenic potential.

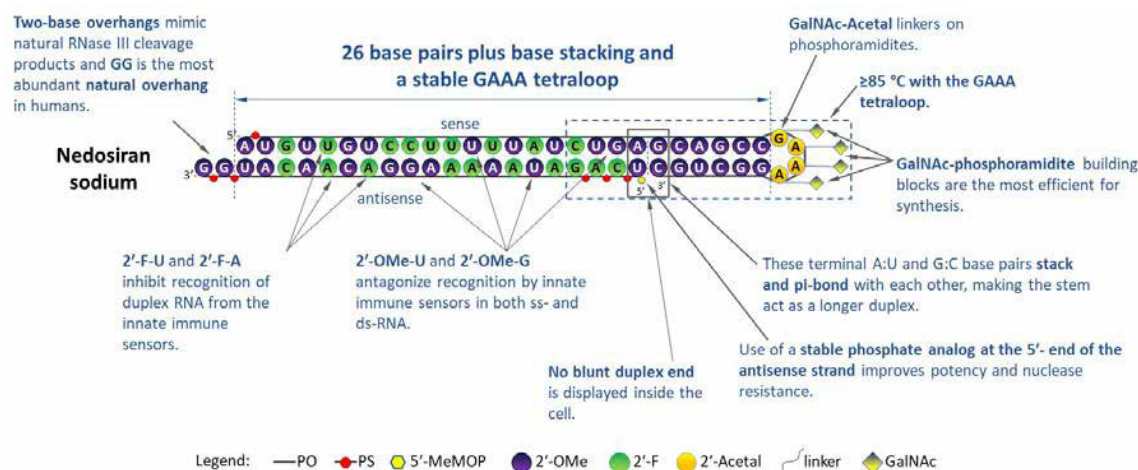
The ELISA method for the detection of anti-nedosiran antibodies (ADA) was developed and validated under Project Code "RQSW2". The assay was used for the detection of ADA in samples collected in patients enrolled in clinical studies DCR-PHXC-101 Part B (PHYOX1), DCR-PHXC-201 (PHYOX2), DCR-PHXC-301 (PHYOX3), and DCR-PHXC-104 (PHYOX4). The Method Validation Report (Project Code RQSW2) along with Method Validation Report Addendum 1 (Project Code RVZQ2) were submitted to the Agency as part of NDA submission for review. The sensitivity, specificity, selectivity, precision, drug tolerance, prozone effect, and ADA stability were evaluated. False positive rates of 5%, 1%, and 0.1% were used for the screening, confirmatory, and titration cut-points, respectively. The ADA assay was appropriately validated in accordance with the 2019 FDA Guidance for Industry "Immunogenicity Testing of Therapeutic Protein Products—Developing and Validating Assays for Anti-Drug Antibody Detection".

ASSESSMENT:

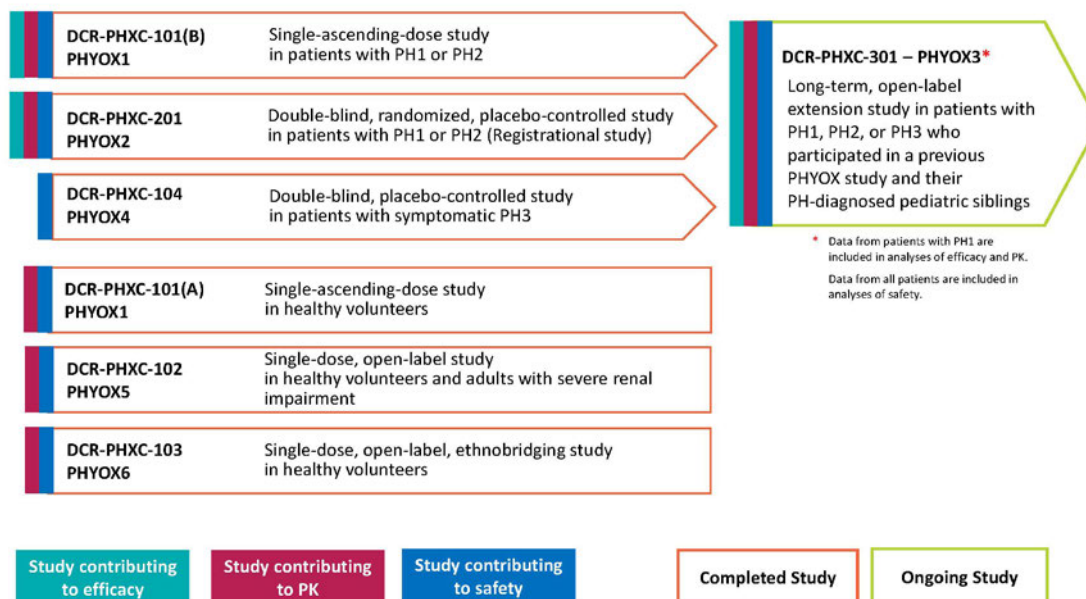
Introduction:

Nedosiran (DCR-PHXC) is a chemically modified double-stranded 36-22 mer small interfering RNA (siRNA), conjugated to GalNAc aminosugar residues for selective delivery to hepatocytes. The sequence of (b) (4) is designed to match the human messenger ribonucleic acid (mRNA) transcripts for lactate dehydrogenase A (LDHA). After SC administration, the GalNAc conjugated to the siRNA bind to asialoglycoprotein receptors (ASGPR) to deliver nedosiran to hepatocytes. After uptake, nedosiran is bound to Dicer protein, and the antisense strand is introduced into the RNAi pathway, selectively reducing the level of LDHA mRNA and, consequently, reducing production of the LDHA. Liver-specific inhibition of LDHA protein production leads to decreased LDH enzyme activity as the endogenous pool of LDHA is naturally turned over. This reduced LDH activity decreases the enzymatic conversion of glyoxylate to oxalate in the liver and, therefore, decreases oxalate levels in the urine and plasma.

The molecular formula of nedosiran sodium is $C_{662}H_{808}F_{19}N_{231}O_{413}P_{57}S_6Na_{57}$ and its molecular weight is 22238.1 Da. It has the following structural features:



Nedosiran is a sterile 170 mg/mL (160 mg/mL free acid) solution of nedosiran sodium in water for injection for subcutaneous injection (SC). It is supplied in a vial or prefilled syringe (PFS). Nedosiran is intended for chronic monthly SC administration in patients with primary hyperoxaluria type 1 (PH1) aged (b) (4) years and older with an eGFR ≥ 30 mL/min/1.73 m². The program of clinical development for nedosiran including ongoing studies is summarized below.



The immunogenicity of nedosiran was evaluated in the clinical program using an assay to detect anti-drug antibodies (ADA) which is described in this assessment memo. The ADA samples were collected in patients with PH in Studies DCR-PHXC-101 Part B (PHYOX1), DCR-PHXC-201 (PHYOX2), and DCR-PHXC-301 (PHYOX3). The ADA samples were also collected in Study DCR-PHXC-104 (PHYOX4) and included in the immunogenicity analysis. However, the PK and ADA data from PHYOX4 were not included in the PK-ADA analysis as all ADA samples from this study were tested with negative ADA, and none of PH patients in this study was a PH1 patient. Across all clinical studies of nedosiran in patients with PH dosed with nedosiran, nedosiran did not induce or boost ADA. Among 59 patients tested with the ADA assay, none developed treatment-emergent ADA. One patient tested positive both pre- and post-administration. The ADA titer of this patient continually reduced by 94% over 12-month treatment period from 2560 at screening, to 1280 on Day 45, 640 in Month 6, and 160 in Month 12. Overall, there was no impact of presence of nedosiran ADA on the PK or PD response of nedosiran. No clinically significant differences in the safety, pharmacokinetic, or pharmacodynamic profiles of nedosiran were observed in patients who were test-positive with the ADA assay during the treatment period.

The enzyme-linked immunosorbent assay (ELISA) for the detection, confirmation, and titration of anti-nedosiran antibodies in human serum (identified as ICDIM 522) was developed and validated by (b) (4) under the Project Code RQSW2. Due to failure to maintain assay performance for unknown reasons and loss of assay sensitivities, additional evaluations were conducted to validate the use of a new lot of affinity purified anti-nedosiran antibody (lot 45410C) as an acceptable positive control for use in the detection of anti-nedosiran antibodies in human serum. The additional evaluations were reported as the RQSW2 validation addendum (Project Code RVZQ2). The previous positive control lot (45009C) was used in clinical studies DCR-PHXC-101, DCR-PHXC-104, DCR-PHXC-201, and DCR-PHXC-301, and the new positive control lot (45410C) is being used in study DCR-PHXC-301.

ADA Assay Methods and Validation:

1. Assay Format and Method Description

The ADA assay is based on an enzyme-linked immunosorbent assay (ELISA). The assay format consists of: (i) a screening assay to detect samples with anti-nedosiran antibodies; (ii) a confirmatory assay to confirm the presence of anti-nedosiran antibodies in samples that tested positive in the screening assay, and; (iii) a titration assay to measure the titers of antibody positive samples.

In the screening, confirmation, and titration assays, human serum samples, positive controls (PCs), and negative control (NC) are diluted with dilution buffer at MRD of 10. For confirmatory analysis, inhibition solution containing nedosiran is added. The diluted samples are added to the 3'NH₂-nedosiran coated and blocked plate. Any anti-drug antibodies (ADA) present in the human serum bound to the coated nedosiran. After washing, bound antibodies are detected by adding HRP-labeled Protein A/G. Bound HRP-labeled Protein A/G was detected by adding TMB substrate. After stopping the TMB reaction with 1N hydrochloric acid, the OD at 450 nM is measured. The OD₄₅₀ values are directly proportional to the amount of ADA present in the human serum.

Assessor's Comment:

- *The multi-tiered testing approach is consistent with the FDA recommendations and is acceptable.*
- *In clinical studies of nedosiran in patients with PH dosed with nedosiran, no treatment emergent ADA including induced ADA and boosted ADA were observed in patients tested with the ADA assay. In patients with positive ADA, no effect on nedosiran concentration (PK) and urinary oxalate excretion profiles (PD) associated with ADA were observed. Therefore, further characterization (e.g. neutralizing antibodies) of ADA positive samples were not conducted. This is acceptable.*
- *The assay can detect ADAs against the whole nedosiran molecule including GalNAc (N-Acetylgalactosamine) moieties. In clinical studies, most of positive ADA cases were observed in pre-dose samples (pre-existing ADAs). Although the domain specificity of ADAs is not examined, these pre-existing ADAs against nedosiran may include antibodies that bind to GalNAc moiety of nedosiran, given that studies suggest that human serum contains large amounts of anti-carbohydrate antibodies including Anti-α-GalNAc (Tn) antibodies (Breiman et al., Front Microbiol. 2021, 12, 641460; Dobrochaeva et al., Mol. Immunol., 2020, 120, 74).*

2. Method Validation

2.1. Summary of method validation performance for ADA assay

Table 1

(b) (4)	Project Code	RQSW2			
	Method ID	ICDIM 522			
Analyte		Anti-nedosiran			
MRD		1:10 in dilution buffer (or inhibitory buffer for confirmation)			
Matrix		Human serum			
Method Description		ELISA			
Sample Volume (µL)		30 µL aliquot			
Sample Storage Temperature		-80 °C			
Screening Assay Cut Point		Floating cut point; CPF = 1.37			
Titer Assay Cut Point		Floating cut point; CPF = 1.49			
Confirmatory Assay Cut Point		% Signal inhibition = 35.0%			
Sensitivity		47.8 ng/mL for screening and 59.9 ng/mL for confirmatory			
Titer Precision		Acceptable, since 12 out of 12 titer curves (100%) were within ± 1 dilution of median last positive dilution			
PC Characteristics		The PC is a rabbit polyclonal antibody raised against nedosiran, a GalNAc-conjugated siRNA, which knocks down LDH.			
PC Concentrations		98.0, 124, 400, and 1600 ng/mL			
Control Intra-Assay Statistics (%)		Level	Conc. (ng/mL)	Precision	
				Uninhibited (OD)	Inhibited (% Inhibition)
		NC	0.00	2.28%	73.3%*
		Screening Low PC	98.0^	3.26%	7.29%
		Confirmatory Low PC	124^	2.26%	2.56%
		Mid PC	400	3.30%	1.61%
Control Inter-Assay Statistics (%)		Level	Conc. (ng/mL)	Precision	
				Uninhibited (OD)	Inhibited (% Inhibition)
		NC	0.00	25.1%	338%
		Screening Low PC	98.0^	12.8%	21.5%**
		Confirmatory Low PC	124^	14.7%	13.8%
		Mid PC	400	17.8%	7.29%
High PC	1600^	19.7%	2.83%		
Thawed Matrix Stability (Hours)		24 hours at room temperature			
Freeze/Thaw Stability (Cycles)		6 cycles thawed at room temperature			
Drug Tolerance		400 ng/mL antibody detected in the presence of 1.00 µg/mL drug; 1600 ng/mL antibody detected in the presence of 25.0 µg/mL drug			

Matrix Interference (Selectivity)	Data for the 20 additional unfortified samples were acceptable, with 16 out of 20 samples (80%) meeting the screening and confirmatory acceptance criteria. Data for the screening low PC were acceptable, with 8 out of 10 samples (80%) meeting the screening and confirmatory acceptance criteria. Data for the 20 additional samples spiked at the confirmatory low PC were acceptable, with 20 out of 20 samples (100%) meeting the screening and confirmatory acceptance criteria. Data for the high PC were acceptable, with 10 out of 10 samples (100%) meeting the screening acceptance criteria and 9 out of 10 samples (90%) meeting the confirmatory acceptance criteria.
Prozone Effect	No apparent hook effect observed at concentrations up to 50,000 ng/mL
Hemolysis	No apparent effect from hemolysis on the detection of anti-nedosiran antibodies.
Lipemia	No apparent effect from lipemia on the detection of anti-nedosiran antibodies.
Post-Validation Run Acceptance	Mean NC should be ≤ 0.0998 OD; sLPC SNR ratio $\geq$ CPF (1.37); cLPC SNR ratio $\geq$ CPF (1.37); HPC SNR ratio ≥ 7.28

Assessor's Comments: As a response to the information request (SN 0016), a validation report amendment was provided (SN 0020) to report the correct sensitivity values of 53.7 ng/mL for screening and 58.0 ng/mL for confirmatory.

2.2. Instruments and Reagents

A list of critical reagents used for the validation study is provided below.

Table 2

Purpose	Material/Reagent	Lot	Concentration	Source	Storage conditions
Confirmatory analysis/drug tolerance	Nedosiran DP	S119007	169 mg/mL	Dicerna	2 to 8°C
Positive control	Rabbit polyclonal anti-nedosiran antibody	45009C	9.22 mg/mL		-80°C
Capture antibody	NH ₂ -Nedosiran	8025R-1P: 8025R-1G	20 µg lyophilized powder		-80°C
Detection reagent	HRP-Protein A/G	VG301424	500 µg/mL	(b) (4)	-20°C

Assessor's Comment: Certificates of analysis for nedosiran DP, rabbit polyclonal anti-nedosiran antibody (lot 45009C), NH₂-nedosiran, and HRP-Protein A/G (lot VG301424) are provided in the method validation report.

Negative Control

Matrix-based negative control pools prepared by pooling 9 individual drug-naïve sample lots with no detectable ADA. The negative control pool was stored at -80°C.

Positive Control

The positive control (PC), rabbit polyclonal anti-nedosiran, was generated by immunizing rabbits with nedosiran. This antigen-specific IgG fraction of polyclonal antibodies was purified from antiserum by Protein A chromatography followed by extensive dialysis against potassium phosphate buffer (0.02 M potassium phosphate, 0.15 M sodium chloride, pH7.2). Matrix-based positive control pools were prepared by spiking anti-nedosiran into the negative control pool (human serum) at 98.0 ng/mL (screening low PC (sLPC)), 124 ng/mL (confirmatory low PC (cLPC)), 400 ng/mL (mid PC (MPC)), and 1600 ng/mL (high PC (HPC)), and are stored at -80°C.

Assessor's Comments:

- *The rabbit polyclonal anti-nedosiran PC lot 45009C was used for the validation of the ELISA method for the detection of anti-nedosiran antibodies in human serum conducted by (b) (4) under Project Code "RQSW2". As described in the introduction section, due to the assay performance failure and loss in assay sensitivities, a new lot of anti-nedosiran PC (lot 45410C) was prepared using the same method described above and qualified for use in the ADA assay (ICDM 522) through the RQSW2 validation addendum to assess drug tolerance, selectivity, specificity (matrix interference), inter- and intra-assay precision, and assay sensitivity. The assessment of the addendum can be found in section 3. Validation Addendum in this memorandum.*
- *The preparation of the rabbit polyclonal anti-nedosiran PC is consistent with the FDA recommendations and is acceptable.*

Equipment and computerized systems used for the validation study are listed in the report.

2.3. Acceptance Criteria

Table 3

Parameter	Acceptance Criteria
Minimum Required Dilution (MRD)	1:10
Assay Acceptance	• Controls must produce mean results relative to their prepared concentrations: HPC > MPC > LPC > NC. • %CV between duplicate well responses for controls and samples: ≤ 20.0%. • At least three NC results must be averaged to calculate a plate specific cut point. • Validation run data may be used to determine the HPC lower bound criteria for post-validation run acceptance. • Validation run data may be used to determine the LPC lower bound criteria for post-validation run acceptance. • Validation run data may be used to determine the NC upper bound criteria for post-validation run acceptance. • High and mid- level controls should be above the screening and confirmatory cut points.

	- Post-cut point runs, multiple LPC concentration levels may be run. At least one of those levels must be above each cut point. - NC inhibition should be below the confirmatory cut point.
Screening Cut Point (SCP)	Report Results
Titration Cut Point (TCP)	Report Results
Confirmatory Cut Point (CCP)	Report Results
Intra-assay Precision	%CV ≤ 20.0%
Inter-assay Precision	%CV ≤ 20.0%
Sensitivity	Report Results (≤ 100 ng/mL)
Titer Precision	Results from ≥ 80% of titer curves be within ± 1 dilution of median last positive dilution
Prozone Effect	High concentration samples should maintain a response ≥ the assay cut point (i.e., remain positive).
Stability	%CV between results at each control level must fall within the established precision of the method. - Samples must produce mean results relative to their prepared concentrations: HPC > LPC ≥ cut point > blank
Effect of Hemolysis	
Effect of Lipemia	
Matrix Interference (Selectivity)	≥ 80% blank sample results < their respective cut point(s). ≥ 80% of spiked matrix samples are expected be ≥ their respective cut point(s).
Drug Tolerance	Report Results
Post-Validation Run Acceptance	Report Results

2.4. Minimum Required Dilution (MRD)

The MRD was determined to be 10.

Assessor's Comments: No data is provided to support the MRD of 1:10. The MRD value is within the acceptable range of 1:5 to 1:100 recommended in the FDA guidance document.

2.5. Cut Point Determination

Serum samples from 50 individual drug-naïve human serum sample lots with no detectable ADA were tested in the screening and confirmatory assays (inhibition). Screening, titration, and confirmatory cut point analysis were performed for 6 independent assays by 4 analysts (for a total of 300 individual drug-naïve human serum sample values).

2.5.1. Screening Cut Point (SCP) and Titration Cut Point (TCP) Factors

Each subject's sample mean signal (S) was divided by the mean signal of the negative control (N) in the screening cut point run to obtain the screening ratio (SNR). Samples with duplicate results with a CV value that exceeded 20.0% were excluded from the analyses (9 samples and 2 NC). Two biological outliers were identified based on their raw response being several standard deviations from the relative mean sample responses and their % inhibition being greater than the expected confirmatory cut point (CCP), and these samples were excluded from all analysis. Several data transformations were attempted, but no transformation produced a normal distribution. Therefore, a non-parametric approach was applied to the SCP determination. The non-transformed

pooled data was evaluated, and 11 statistical outliers were identified using Tukey's Outlier Filter approach ($Q1 - 1.5IQR$ and $Q3 + 1.5IQR$) and were excluded from subsequent analysis. Friedman's test for equivalence of medians indicated that there was a statistically significant difference between the medians across the 6 data panels (p -value = 0.005). Levene's test for homogeneity of variances indicated that there was no statistical evidence of a difference in the variances across the panels (p -value = 0.135). The screening and titer cut point factors were calculated using the 95th and 99.9th upper percentiles, respectively. SCP and TCP factors were determined to be 1.37 and 1.49, respectively.

Assessor's Comment: The Applicant's approach for the determination of SCP and TCP factors is consistent with the 2019 immunogenicity assay guidance and is acceptable.

2.5.2. Confirmatory Cut Point (CCP)

The CCP was evaluated for individual human serum samples in the presence or absence of 50 µg/mL nedosiran. The mean percentage signal inhibition for each sample was calculated ($\% \text{signal inhibition} = 100 \times [1 - (\text{inhibited sample response} / \text{uninhibited sample response})]$). Samples with duplicate results with a CV value that exceeded 20.0% were excluded from the analyses (20 samples). Two biological outliers were identified based on their raw response being several standard deviations from the relative mean sample responses and their % inhibition being greater than the expected CCP, and these samples were excluded from all analysis. Several data transformations were attempted, but no transformation produced a normal distribution. Therefore, a non-parametric approach was applied to the CCP determination. The non-transformed pooled data was evaluated, and 12 statistical outliers were identified using Tukey's Outlier Filter approach and were excluded from subsequent analysis. The CCP was determined by estimating the lower 99th percentile of the ratios (ratio = inhibited sample response/uninhibited sample response). The ratio CCP was then transformed to %inhibition cut point $[(1 - \text{CCP}) \times 100]$, resulting in upper 99th percentile on the %inhibition. The CCP was determined to be 35.0%.

Assessor's Comment: The CCP only requires a modest inhibition of 35%, as opposed to potential non-specificity that might result from requiring higher inhibition. The statistical approach is consistent with 2019 immunogenicity guidance. Therefore, the Applicant's approach for the determination of the CCP is acceptable.

2.6. Assay Precision

Intra-assay Precision of the Screening and Confirmatory Assays

PC samples (HPC, MPC, cLPC, and sLPC) and NC were assessed by testing multiple wells ($n \geq 6$) in a given run. Each sample was analyzed uninhibited and inhibited with nedosiran. Acceptance criterion for intra-assay precision is $\%CV \leq 20\%$.

In the screening assay, intra-assay precision (%CV) were determined to be 3.15% (HPC), 3.30% (MPC), 2.26% (cLPC), and 3.26% (sLPC). The screening intra-assay precision (%CV) for the NC was determined to be 2.28%.

In the confirmatory assay, intra-assay precision (%CV) was determined to be 0.34% (HPC), 1.61% (MPC), 2.56% (cLPC), and 7.29% (sLPC). The confirmatory intra-assay precision (%CV) for the NC was determined to be 73.3% which did not meet the acceptance criterion (%CV $\leq$ 20%).

Assessor's Comments: The %inhibition values of NC are ranging from 0.0 to 8.02, and the average and standard deviation values are 4.62% and 3.39, respectively. In general, smaller values give greater statistical variabilities. In addition, all the %inhibition values of NC are substantially lower than the CCP. Therefore, the intra-assay precision for the NC is acceptable. The %CV values of all PC levels in the screening assay are $\leq$ 20% and met the acceptance criteria. Likewise, the %CV values of all PC levels in the confirmatory assay are $\leq$ 20% and met the acceptance criteria. This is acceptable.

Inter-assay Precision of the Screening and Confirmatory Assays

PC samples (HPC, MPC, cLPC, and sLPC) and NC were assessed by at least 2 analysts through $\geq$ 6 validation runs (49 runs for PCs and 48 runs for NC). Acceptance criterion for inter-assay precision is %CV $\leq$ 20%.

In the screening assay, inter-assay precision (%CV) were determined to be 19.7% (HPC), 17.8% (MPC), 14.7% (cLPC), and 12.8% (sLPC). The screening inter-assay precision (%CV) for the NC was determined to be 25.1%.

In the confirmatory assay, inter-assay precision data (with HM15136 as inhibitor only) was determined to be 2.83% (HPC), 7.29% (MPC), 13.8% (cLPC). The confirmatory inter-assay precision (%CV) for the NC was determined to be 338%.

Assessor's Comments:

- *The %CV value of NC in the screening assay was 25.1%, which is greater than the acceptance criterion of $\leq$ 20%CV. The response values of NC are ranging from 0.0600 to 0.173, and the average and standard deviation values are 0.0862 and 0.0217, respectively (n = 189). Similarly, the %CV value of NC in the confirmatory assay was 338%, which is greater than the acceptance criterion of $\leq$ 20%CV. The response values of NC (% inhibition) are ranging from -78.3 to 26.1, and the average and standard deviation values are 4.40 and 14.9, respectively (n = 42). In general, %CV values are inclined to be high for unnormalized NC values due to lower response. Given that all assay runs that were used for the evaluation met the assay acceptance criteria of HPC > MPC > LPC > NC and at average NC values do not rise above a plate specific screening cut point or CCP, the inter assay precision results for NCs are acceptable.*
- *The %CV values of sLPC %Inhibition in the confirmatory assay were 21.5%, which is greater than the acceptance criterion. However, the sLPC level is only intended for screening assays and, therefore, inter-assay precision of the sLPC is not applicable for the confirmatory assay. This is acceptable.*

2.7. Titer Precision

Titer precision was assessed by titration of pooled normal human serum spiked with the HPC (1600 mg/mL) using two-fold serial dilutions (2 to 64-fold dilutions). The titration precision assays were conducted in 6 independent runs (12 assays) performed over 4

days by ≥ 3 analysts. The titer precision was reported as the median of the titer values across the runs. The titer precision met acceptance criteria: results from $\geq 80\%$ of titer curves be within ± 1 dilution of median last positive dilution (12/12, 100%). The median titer was determined to be 16.0.

Assessor's Comments: Ten assays out of 12 assays gave titer values of 16.0 and the rest of two assays gave titer values of 32.0 and 8.0 (within ± 1 dilution). The determination of titer precision is appropriate.

2.8. Assay Sensitivity

Assay sensitivity was determined by titration of the PC from 16000 to 25.0 ng/mL in pooled human serum using two-fold serial dilutions (2- to 64-fold dilutions). Assay sensitivity PC titrations were tested in the screening and confirmatory assay on total of 6 runs (12 assays) performed over 4 days by ≥ 3 analysts.

Sensitivity for each tier was calculated for each curve using a linear regression to interpolate the concentration at which each curve transects the assay cut points.

The screening sensitivity was determined to be 53.7 ng/mL and the confirmatory sensitivity was determined to be 58.0 ng/mL.

Assessor's Comment:

- The assay sensitivity values were originally determined to be 47.8 ng/mL for screening and 59.9 ng/mL for confirmatory, which were calculated using incorrect cut point. In a response to an information request, the Applicant confirmed that the sensitivity values originally reported were incorrect and provided correct sensitivity values (SN 0020). In addition, The Applicant also confirmed that the relative assay sensitivity (RAS) which was also reported in the original validation report was not used to determine the sensitivity.*
- The Applicant's approach for the evaluation of the sensitivity for the screening and confirmatory assays is appropriate, and sensitivity values for screening and confirmatory assays are well within guidance.*

2.9. Determination of LPC Concentration

The assay sensitivity data was used to estimate the concentrations of LPC. The approximate concentration of the LPC is estimated as the 1% prediction interval of the mean PC concentration equal to the SCP as follows:

$$LPC = \text{mean sensitivity} + (t_{0.01, df} * SD)$$

Where $t_{0.01, df}$ is the critical value of the students t distribution for the 1% prediction interval for the degrees of freedom (df) of the sensitivity and SD is the standard deviation from the mean sensitivities determined.

The validated sLPC value (98.0 ng/mL) was calculated incorrectly and was later determined, to be 100 ng/mL when the correct SCP values was used. The Applicant stated that this discrepancy had no impact on the validation because the validated sLPC value is lower, yet still met acceptance criteria, and was fractionally lower (2.04%)

compared to the correct sLPC concentration of 100 ng/mL. Thus, the validated LPC concentration of 98.0 ng/mL was used during sample analysis.

Similarly, the validated cLPC value (124 ng/mL) was calculated incorrectly using an incorrect CCP of 35.7% rather than 35.0%. It was later determined to be 121 ng/mL if the CCP of 35.0% was used. The Applicant stated that this discrepancy had no impact on the validation because the validated cLPC value is lower, yet still met acceptance criteria, and was fractionally higher (2.5%) compared to the correct cLPC concentration of 121 ng/mL. Thus, the validated cLPC concentration of 124 ng/mL was used during sample analysis.

Assessor's Comment: The differences between the correct value and validated values (100 ng/ml vs 98 ng/ml for sLPC and 121 ng/ml vs 124 ng/mL for cLPC) are very small and not expected to impact interpretation of results.

2.10. Prozone Effect

Prozone effect was assessed with PC levels from 50,000 ng/mL to 24.4 ng/mL (2-fold serial dilution) in pooled human serum. The samples were analyzed in the screening and confirmatory assay. All samples met the acceptance criteria (response at high concentration $\geq$ SCP or $\geq$ CCP). No apparent "hook effect" was observed at concentrations up to 50,000 ng/mL.

Assessor's comments: The response curve reached a plateau at the higher PC concentrations (50,000 ng/mL and 25,000 ng/mL) in both screening and confirmatory assays. All PC levels produce positive results, and the change is small compared to the %inhibition values of these PC levels (94.9% and 96.7%, respectively). Thus, although there is a small decrease in responses at the higher PC concentrations in the confirmatory assay, the change is small compared to the %inhibition values of these PC levels (94.9% and 96.7%, respectively) resulting in a negligible decrease. This is acceptable.

2.11. Drug Tolerance

To determine the drug tolerance levels, nedosiran was titrated at concentrations of 0.0, 1.0, 10.0, 25.0, and 50.0 μ g/mL in pooled matrix spiked with PC at the HPC, MPC, cLPC, and sLPC levels. The sLPC and cLPC levels exhibited drug tolerance below 1.0 μ g/mL nedosiran. The drug tolerance for screening assay was determined to be 1.0 μ g/mL nedosiran at MPC (400 ng/mL) level, and 25.00 μ g/mL nedosiran at the HPC (1600 ng/mL) level.

Assessor's Comments: Although the sLPC and cLPC levels exhibited drug tolerance below 1.0 μ g/mL nedosiran, ADA sampling was performed a minimum of 15 days post-dosing (single- or monthly dosing), the serum concentration of nedosiran in samples is anticipated to be low ($\leq$ 10 ng/mL) and it is unlikely that nedosiran in serum samples would significantly interfere with assay performance (refer to [2.7.2 Summary of Clinical Pharmacology Studies](#)).

2.12. Selectivity (Matrix Interference)

Matrix interference was assessed by testing individual human serum samples unspiked and spiked with PC at the HPC, cLPC, and sLPC levels. Data from initial 10 individual serum samples spiked at the sLPC met screening and confirmatory acceptance criteria (8/10, 80%). The same samples unspiked and spiked at the HPC met the acceptance criteria for screening (10/10, 100%) and confirmatory (9/10, 90%). Data from 20 additional individual serum samples unspiked and spiked at the cLPC met screening and confirmatory acceptance criteria (unspiked: 16/20, 80%, spiked at cLPC: 20/20, 200%).

Assessor's Comments: the matrix interference study data indicate that serum interference in the screening and confirmatory assay performance appear to be minimal. Therefore, the method appears suitable for use to evaluate ADAs in individual human serum samples collected from subjects in clinical studies.

The selectivity of the method was also tested in hemolyzed (containing 5% lysed whole blood) and lipemic (containing ≥ 300 mg/dL triglycerides) samples. All hemolyzed samples spiked at the HPC, cLPC, and sLPC levels were positive in the assay (response $\geq$ SCP and $\geq$ CCP), and the results met acceptance criteria. All lipemic samples spiked at the HPC and LPC levels were positive in the assay, and the results met acceptance criteria.

Assessor's Comments: The ADA assay performance does not seem to be affected by lipemic and hemolyzed serums at the HPC, cLPC and sLPC levels. This assay method appears to be suitable for use for detecting higher and lower levels of ADAs in lipemic and hemolyzed serums.

2.13. Post-Validation Run Acceptance

Post-validation run acceptance (system suitability) was determined using validation data from acceptable, non-cut point runs. System suitability limits targeted an approximate 1% failure rate by calculating a control limit based on the 1% prediction interval of the control values. The upper limit for the NC value was calculated using the degrees of freedom (df) from the t-distribution for a 1% prediction interval ($t_{0.01df}$) based on the number of validation runs using the following formula: $Mean\ NC + (t_{0.01df} * SD)$

The upper and lower limits for the PC ratio values were calculated using the df from the t-distribution for a 1% prediction interval ($t_{0.01, df}$) based on the number of validation runs using the following formula: $Mean\ SNR \pm (t_{0.01df} * SD)$

Upper limit of NC was 0.0998 (n = 72). Lower limit of sLPC SNR, cLPC SNR, and HPC SNR were 1.33, 1.25, and 7.28, respectively.

The Table 4 provides a summary of run acceptance criteria.

Table 4

Parameter	Acceptance Criteria
Run acceptance	Mean NC $\leq$ 0.0998 OD sLPC SNR $\geq$ SCP factor (1.37) cLPC SNR $\geq$ SCP factor (1.37) HPC SNR $\geq$ 7.28 HPC>MPC>LPC>NC

	%CV between duplicate well resposensed for controls and samples should be $\leq 20\%$ NC %Inhibition < CCP
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Assessor Comment:

- *It should be noted that the lower limits of sLPC and cLPC were lower than the SCP factor (1.37). Given the LPC was statistically determined to be $\geq$ SCP (with 99% confidence), the SCP factor is applied to the lower limit of sLPC and cLPC.*
- *The Post-validation run acceptance are reasonable and acceptable.*

2.14. Stability

Stability experiments are conducted on PC samples.

Freeze/thaw stability was assessed by evaluating HPC, cLPC, and sLPC samples that were subjected to 6 freeze/thaw cycles (-80°C /room temperature). Although this study did not include freshly prepared samples, all results nonetheless met the acceptance criteria ($\leq 20\%$ CV, HPC > LPC $\geq$ cut point > blank).

Short-term stability in thawed matrix was assessed by evaluating HPC, cLPC, and sLPC samples that were thawed and stored at room temperature for 24 hours prior to analysis. As with the freeze-thaw study, the room temperature study did not include freshly prepared samples, but all results met the acceptance criteria ($\leq 20\%$ CV, HPC > LPC $\geq$ cut point > blank).

Assessor's Comments: Because multiple freeze-thaw cycles of antibody samples (e.g., PCs and subject samples) and storage at temperature that is higher than recommended storage temperature (-80°C) may affect assay results, information from studies evaluating short-term stability under various conditions may be useful. Although the stability studies did not include control data from freshly prepared samples, the data met the pre-defined acceptance criteria, confirming freeze-thaw and room temperature stability.

2.15. Robustness

Robustness of the assay was evaluated during the assay's development to establish limitations for incubation time and temperatures, pH, and salt concentrations for specific assay buffers. Thus, these robustness parameters are not evaluated during method validation. Parameters demonstrating robustness of the assay during method validation under everyday laboratory conditions include multiple analysts, plate readers, plate washers, buffer preparations (blocking buffer and wash buffer), and plate lots.

Assessor's Comments: The Applicant provided a table summarizing the robustness parameters and acceptability of each run as part of the validation addendum (SN 0020). It appears that the assay is robust to be day to day, lot to lot, instrument to instrument, and analyst to analyst variabilities.

3. Method Validation Addendum (new positive control)

3.1. Background

It was noted on February 2022, that the sLPC failed to produce signal above the plate specific SPC, and the cLPC failed to exhibit signal suppression above the CCP. A series of troubleshooting runs were conducted but failed to identify potential causes of the decline in assay performance. These troubleshooting runs evaluated potential interference from; (i) non-critical reagents, materials, and equipment; (ii) positive control preparation scale, storage time, and antibody stock solution; (iii) HRP-conjugated protein A/G and/or the NH₂-nedosiran coating material. During the assay performance evaluation, the assay sensitivity was recalculated using multiple sensitivity (titer) curves, which showed sensitivity reduction in both the screening and confirmatory tiers of the assay. The sensitivity calculated based on troubleshooting runs was 92.3 ng/mL for the screening assay (64.5% difference from the validated assay screening sensitivity) and 99.4 ng/mL for the confirmatory assay (49.6% difference from the validated assay confirmatory sensitivity). Therefore, a new lot of anti-nedosiran antibodies (lot 45410C) was evaluated and qualified for use in the ADA assay (ICDIM 522) through the RQSW2 validation addendum to assess drug tolerance, selectivity, specificity, inter- and intra-assay precision, and assay sensitivity.

3.2. Summary of method validation addendum

Project Code	RVZQ2		
Method ID	ICDIM 522		
Analyte	Anti-nedosiran antibodies		
Matrix	Human serum		
Method Platform	ELISA		
Drug concentration use in confirmatory assay	50.0 µg/mL		
Sample volume	30 µL aliquot		
Sample storage temperature	-80°C		
Capture reagent	NH ₂ -Nedosiran		
Detection reagent	HRP-labeled Protein A/G		
Screening assay cut point (SCP)	Floating cut point: 1.37		
Titer assay cut point (TCP)	Floating cut point: 1.49		
Confirmatory assay cut point (CCP)	%Signal inhibition: 35.0%		
Negative Control (NC)	Pooled nine individual drug-naïve sample lots with no detectable ADA		
Positive Control (PC)	- Stock solutions of protein A purified anti-nedosiran antibodies (lot:45410C) were supplied by Dicerna and were used prior to the expiration date. - PCs prepared by spiking protein A purified anti-nedosiran antibodies (lot: 45410C) into the NC pool at 98.0, 124, and 1600 ng/mL.		
MRD	1:10		
Titer Precision	Median titer = 16 9 out of 9 titer curves (100%) were within ±1 dilution of the median last positive dilution		
Sensitivity	Screening sensitivity: 53.8 ng/mL Confirmatory sensitivity: 72.0 ng/mL Calculated Proposed Screening LPC: 99.1 ng/mL Calculated Proposed Confirmatory LPC: 186 ng/mL		
Drug Tolerance	Titer	PC Conc. (ng/mL)	Tolerated Drug Conc. (µg/mL)

	Screening		98.0		0.00		
			124		0.00		
			400		1.00		
			1600		10.00		
	Confirmatory		98.0		0.00		
			124		0.00		
			400		1.00		
			1600		5.00		
Control Intra-Assay Precision			Level	Conc. (ng/mL)	Intra-Assay Precision (%CV)		
					Uninhibited OD	Inhibited OD	% Inhibition
			NC	0.00	1.71%	2.22%	25.9%
			sLPC	98.0	3.52%	4.27%	6.51%
			cLPC	124	15.8%	3.16%	10.0%
			HCP	1600	5.63%	12.8%	0.593%
Control Inter-Assay Precision			Level	Conc. (ng/mL)	Inter-Assay Precision (%CV)		
					Uninhibited OD	Inhibited OD	% Inhibition
			NC	0.00	8.98%	7.42%	93.0%
			sLPC	98.0	16.1%	N/A	N/A
			cLPC	124	17.9%	11.0%	17.9%
			HCP	1600	27.0%	11.3%	2.04%
Selectivity and Specificity – Healthy Normal			PC conc. (ng/mL)	Number of Samples Meeting Criteria/ Total Number of Samples in Each Assay			
			0.00	24/30 (80.0%) screened negative and 25/27 (92.6%) confirmed negative			
			98.0	10/10 (100%) screened potential positive and 10/10 (100%) confirmed positive			
			124	10/10 (100%) screened potential positive and 9/9 (100%) confirmed positive			
			186	10/10 (100%) screened potential positive and 10/10 (100%) confirmed positive			
			1600	10/10 (100%) screened potential positive and 10/10 (100%) confirmed positive			

All parameters and reagents used in the validation addendum are the same as those used in the original validation.

3.3. Sensitivity

Assay sensitivity was evaluated with the new PC lot (45410C) in both the screening and confirmatory assays using samples spiked with 1600 ng/mL (undiluted) and serially diluted (2-, 4-, 8-, 16-, 32-, and 64-fold) PC. The screening assay sensitivity calculated was 53.8 ng/mL with a proposed sLPC concentration of 99.1 ng/mL. The confirmatory assay sensitivity calculated was 72.0 ng/mL with a proposed cLPC concentration of 186 ng/mL.

Assessor's Comments: Because there is a potential decrease in the confirmatory sensitivity, the Applicant evaluated both validated and newly calculated cLPC concentrations in the specificity assessment (matrix interference) using 10 individual normal human serum. Given the validated cLPC concentration of 124 ng/mL consistently produced positive results above the confirmatory cut point during the specificity assessment, the Applicant decided to maintain the validated cLPC level of 124 ng/mL for the remaining addendum experiments and for the future evaluations. This is acceptable.

More details of the specificity assessment are described in section 3. In this memorandum.

3.4. Drug Tolerance

Drug tolerance was evaluated with PC levels at 98.0 (sLPC), 124 (cLPC), 400 (MPC), and 1600 ng/mL (HPC) using the new PC lot (45410C). The PC samples were spiked with nedosiran (0.00, 0.500, 1.00, 2.00, 5.00, 10.0, 25.0, and 50.0 µg/mL) and incubated at ambient temperature for one hour prior to freezing overnight. Each sample was analyzed in both screening and confirmatory assays. The sLPC and cLPC levels exhibited drug tolerance below 0.500 µg/mL nedosiran in both the screening and confirmatory assays, which was consistent with results from the original validation. The drug tolerance levels were determined to be 1.0 µg/mL nedosiran at MPC (400 ng/mL) level for screening assay and confirmatory assays, 10.00 µg/mL and 5.00 µg/mL nedosiran at the HPC (1600 ng/mL) level for screening and confirmatory assays, respectively.

Assessor's Comments: There is a decrease in drug tolerance at the HPC level compared to that of original validation (10 µg/mL vs 25 µg/mL for screening). As noted in section 2.11, ADA sampling was performed a minimum of 15 days post-dosing, and the serum concentration of nedosiran in samples is anticipated to be low (≤ 10 ng/mL). Therefore, nedosiran in serum samples may not significantly interfere the assay performance (refer to [2.7.2 Summary of Clinical Pharmacology Studies](#)).

3.5. Assay Precision

Intra-assay Precision of the Screening and Confirmatory Assays

Intra-assay precision was evaluated for screening and confirmatory assays using the negative control (0.00 ng/mL) and positive controls spiked with the PC at 98.0 ng/mL (sLPC level), 124 ng/mL (cLPC level), and 1600 ng/mL (HPC level). Acceptance criterion for intra-assay precision is %CV $\leq$ 20%. All results met the acceptance criterion except for NC % inhibition result for confirmatory assay (25.9%CV).

Assessor's Comments: The %inhibition values of NC are ranging from 6.92 to 13.9, and the average and standard deviation values are 9.93% and 2.58, respectively. All the %inhibition values of NC are well below the CCP. Therefore, the intra-assay precision for the NC is acceptable.

Inter-assay Precision of the Screening and Confirmatory Assays

Inter-assay precision was evaluated by for screening and confirmatory assays using NC, sLPC, cLPC, and HPC samples in multiple replicates (≥ 6 runs) by ≥ 2 analysts. Acceptance criteria for intra-assay precision are: %CV $\leq$ 20%; and negative control %CV may exceed 20.0%. All results met the acceptance criteria except for the %CV value of HPC level for the screening assay (27.0%). The %CV value of cLPC was originally calculated to 25.7%, however, it was noted that the inter-assay data for the uninhibited cLPC level contains a Dixon outlier (cLPC Replicate 1 in run 16RVZQ2). The % CV value of cLPC value was determined to be 17.9% when this Dixon outlier was excluded from the %CV calculation.

Assessor's Comments:

- *Although the %CV of cLPC (25.7%) for the screening assay did not meet the acceptance criteria of $\leq 20.0\%$ when the Dixon outlier is included in the %CV calculation, cLPC is not used for the screening assay, therefore, the data provided is information purpose only. This is acceptable.*
- *It is confirmed from the data provided in the submission that all PC sample replicates consistently produced responses greater than the screening and confirmatory assay cut points and responses in line with their relative preparation concentration: HPC > cLPC > sLPC > SCP > NC. Moreover, few in study patient signals were in the high range (Refer to [Clinical Overview](#) and [tabulated clinical data](#)). Therefore, modestly out of range %CV value of HPC for the screening assay (27.0%) is acceptable.*

3.6. Selectivity (Matrix Interference)

Matrix interference was evaluated by testing individual human serum samples unspiked and spiked with PC at the HPC (1600 ng/mL), the proposed new cLPC (186 ng/mL), the previously validated cLPC (124 ng/mL), and sLPC (98 ng/mL) levels.

Data from initial 10 unspiked individual serum samples did not meet the acceptance criteria ($\geq 80\%$) for screening assay. Data from 20 additional unspiked samples again did not meet the acceptance criteria ($\geq 80\%$) for screening assay. Samples from the 20 additional unspiked individual serum were reanalyzed at twice the number of replicates ($n = 2$) in the screening assay, and 19 out of 20 samples (95%) tested below the screening assay cut point. Overall, data from unspiked samples met the acceptance criteria since 24 out of 30 individual serum samples (80%) tested below the screening assay cut point and 25 out of 27 individual serum samples (92.6%) tested below the confirmatory assay cut point.

All data from the 10 individual samples spiked with PC at the HPC, the proposed new cLPC (186 ng/mL), the previously validated cLPC (124 ng/mL), and sLPC levels met the acceptance criteria for screening (100%) and confirmatory (100%). Since both the proposed new cLPC level (186 ng/mL) and the previously validated cLPC level (124 ng/mL) performed adequately and showed no matrix interference, the validated cLPC concentration was maintained for the remaining validation addendum experiments and will be used as the cLPC in future evaluations.

Assessor's Comments: the matrix interference study data indicate that serum interference to the performance of the new PC in the screening and confirmatory assay appear to be minimal. Therefore, the method and new PC appear suitable for use to evaluate ADAs in individual human serum samples collected from subjects in clinical studies.

Assessment Conclusions:

In the method validation report for the ELISA for the detection, confirmation, and titration of anti-nedosiran antibodies in human serum, the Applicant demonstrated that the assay method is specific, precise, selective, and robust for the detection of anti-nedosiran-

reactive antibodies in clinical human serum samples. Sufficient drug tolerance is reported and there was no prozone effect was observed in the method. False positive rates used for the cut-point determination are in line with the 2019 FDA Guidance. Overall, all the validation parameters are appropriately evaluated, and the ADA assay is appropriately validated. The assay is suitable for its intended purpose of measuring ADA levels in clinical samples.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

MAYUMI N TAKAHASHI
07/17/2023 11:21:50 AM

FREDERICK C MILLS
07/17/2023 12:22:36 PM

HUMAN FACTORS STUDY REPORT AND LABELS AND LABELING REVIEW

Division of Medication Error Prevention and Analysis 2 (DMEPA 2)
Office of Medication Error Prevention and Risk Management (OMEPRM)
Office of Surveillance and Epidemiology (OSE)
Center for Drug Evaluation and Research (CDER)

*** This document contains proprietary information that cannot be released to the public***

Date of This Review:	July 12, 2023
Requesting Office or Division:	Division of Cardiology and Nephrology (DCN)
Application Type and Number:	NDA 215842
Product Type:	Combination product
Drug Constituent Name and Strength	Rivfloza (nedosiran) injection, Vial: 80 mg/0.5 mL Prefilled syringe: 128 mg/0.8 mL and 160 mg/mL
Device constituent:	Prefilled syringe
Rx or OTC:	Rx
Applicant/Sponsor Name:	Novo Nordisk Inc
Submission Date:	9/30/22; 11/8/22; 1/27/23; 5/3/23; 5/24/23
TTT ID #:	2022-2123 and 2022-2125
DMEPA 2 Safety Evaluator:	Ebony Whaley, PharmD, BCPPS
DMEPA 2 Associate Director for Human Factors:	Lolita White, PharmD
DMEPA 2 Deputy Director:	Chi-Ming (Alice) Tu, PharmD, BCPS

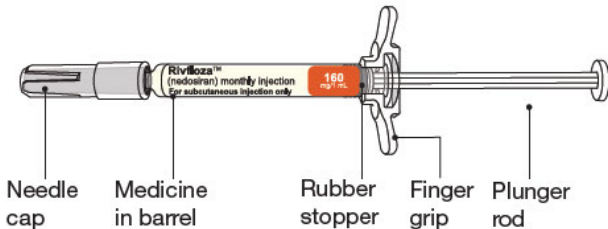
1 REASON FOR REVIEW

This review evaluates the human factors (HF) validation study report and labels and labeling submitted under NDA 215842 for Rivfloza (nedosiran) injection.

1.1 PRODUCT DESCRIPTION

Table 1 presents relevant product information for Rivfloza that Novo Nordisk Inc on submitted on 9/30/22.

Table 1. Product description										
Initial Approval Date	N/A									
Active Ingredient	nedosiran									
Indication	the treatment of primary hyperoxaluria type 1 (PH1) to lower urinary oxalate levels in pediatric (9 years of age and older) and adult patients with relatively preserved kidney function, e.g., eGFR $\geq$ 30 mL/min/1.73 m ² .									
Route of Administration	subcutaneous									
Dosage Form	Injection solution									
Strength	Vial: 80 mg/0.5 mL Prefilled syringe (PFS): 128 mg/0.8 mL, and 160 mg/mL									
Dose and Frequency	The recommended dose consists of once monthly doses administered subcutaneously as shown below. Dose Regimen in Adults and Pediatric Patients ^(b)₍₄₎ years of age and older) <table><tr><th>Age</th><th>Body Weight</th><th>Dosing Regimen</th></tr><tr><td rowspan="2">Adults and adolescents 12 years and older</td><td>Greater than or equal to 50 kg</td><td>160 mg once monthly (PFS, 1 mL)</td></tr><tr><td>Less than 50 kg</td><td>128 mg once monthly (PFS, 0.8 mL)</td></tr></table> ^(b) ₍₄₎		Age	Body Weight	Dosing Regimen	Adults and adolescents 12 years and older	Greater than or equal to 50 kg	160 mg once monthly (PFS, 1 mL)	Less than 50 kg	128 mg once monthly (PFS, 0.8 mL)
Age	Body Weight	Dosing Regimen								
Adults and adolescents 12 years and older	Greater than or equal to 50 kg	160 mg once monthly (PFS, 1 mL)								
	Less than 50 kg	128 mg once monthly (PFS, 0.8 mL)								
How Supplied	Clear, sterile, preservative-free, colorless-to-yellow solution available in single-dose PFS and single-dose vials in cartons containing one unit each.									

	<table><tr><th colspan="5">Presentations</th></tr><tr><th>Presentation</th><th>Total Volume</th><th>Total amount available in presentation</th><th>Concentration</th><th>NDC number</th></tr><tr><td>Single-dose PFS</td><td>1 mL</td><td>160 mg</td><td>160 mg/mL</td><td>NDC 0169-5306-10</td></tr><tr><td>Single-dose PFS</td><td>0.8 mL</td><td>128 mg</td><td>160 mg/mL</td><td>NDC 0169-5307-08</td></tr><tr><td>Single-dose vial</td><td>0.5 mL</td><td>80 mg</td><td>160 mg/mL</td><td>NDC 0169-5308-01</td></tr></table> - The PFS presentations are intended for administration to adults and pediatric patients aged 12 years and older. - The vial presentation is intended for administration to pediatric patients aged (b) (4) to 11 years old	Presentations					Presentation	Total Volume	Total amount available in presentation	Concentration	NDC number	Single-dose PFS	1 mL	160 mg	160 mg/mL	NDC 0169-5306-10	Single-dose PFS	0.8 mL	128 mg	160 mg/mL	NDC 0169-5307-08	Single-dose vial	0.5 mL	80 mg	160 mg/mL	NDC 0169-5308-01
Presentations																										
Presentation	Total Volume	Total amount available in presentation	Concentration	NDC number																						
Single-dose PFS	1 mL	160 mg	160 mg/mL	NDC 0169-5306-10																						
Single-dose PFS	0.8 mL	128 mg	160 mg/mL	NDC 0169-5307-08																						
Single-dose vial	0.5 mL	80 mg	160 mg/mL	NDC 0169-5308-01																						
Storage	Store refrigerated at 36°F to 46°F (2°C to 8°C). Can be stored, if needed, at (b) (4) for a maximum of 28 days (4 weeks). Do not freeze. Store in original carton, away from direct heat and light. Storage Conditions <table><tr><td>Refrigerated 36°F to 46°F (2°C to 8°C)</td><td>(b) (4)</td></tr><tr><td>Until expiration date</td><td>Maximum 28 days (4 weeks)</td></tr></table>	Refrigerated 36°F to 46°F (2°C to 8°C)	(b) (4)	Until expiration date	Maximum 28 days (4 weeks)																					
Refrigerated 36°F to 46°F (2°C to 8°C)	(b) (4)																									
Until expiration date	Maximum 28 days (4 weeks)																									
Container Closure/Device Constituent (including figure)	PFS: (b) (4) 1 mL, glass PFS with a staked ½” needle  Needle cap Medicine in barrel Rubber stopper Finger grip Plunger rod Vial 																									
Intended Users	Adult and pediatric patients and adult lay caregivers																									

Intended Use Environment	Home environment
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1.2 RELEVANT REGULATORY HISTORY RELATED TO THE PROPOSED PRODUCT'S HUMAN FACTORS DEVELOPMENT PROGRAM

- On February 2, 2021, the Applicant submitted an HF validation study (HFVS) protocol for the proposed PFS under IND 132214. We completed our review of the HFVS protocol on May 18, 2021^a and provided recommendations for the Applicant.
- On September 30, 2022, the Applicant submitted the results of the HFVS for the proposed PFS under NDA 215842, which is the subject of this review.

2 MATERIALS REVIEWED

We considered the materials listed in Table 2 for this review.

Table 2. Materials Considered for this Review	
Material Reviewed	Appendix/Section
Product Information/Prescribing Information	Section 1.1
Background Information Previous DMEPA Reviews	Section 1.2
Background Information on Human Factors Engineering (HFE) Process	Appendix A
Human Factors Validation Study Report	Appendix B
Information Requests Issued During the Review	Appendix C
Labels and Labeling	Appendix D

3 OVERALL ASSESSMENT OF MATERIALS REVIEWED

The sections below provide a summary of the HFVS design and our analysis of the study methodology.

3.1 SUMMARY OF STUDY DESIGN

Table 3 presents a summary of the HFVS design. See Appendix C for more details on the study design.

Table 3. Study Methodology for Human Factors (HF) Validation Study for the PFS

^a Yokum, A. Human Factors Protocol Review for nedosiran (IND 132214). Silver Spring (MD): FDA, CDER, OSE, DMEPA (US); 2021 MAY 18. RCM No.: 2021-219.

Study Design Elements	Details
Participants	• Adult patients (primarily from the general population, and 2 were diagnosed with primary hyperoxaluria), n = 16 ○ Injection experienced, n = 8 ○ Injection naïve, n = 8 • Pediatric patients from the general population (aged 12 to 17 years old), n = 16 ○ Injection experienced, n = 8 ○ Injection naïve, n = 8 • Lay adult caregivers, n = 16 ○ Injection experienced, n = 8 ○ Injection naïve, n = 8 For pediatric patient participants, their parent/guardian was welcomed to observe if desired; however, the parent/guardian did not provide help or input during testing.
Training	Participants were not trained.
Test Environment and Materials	The test environment simulated a home use environment and kitchen table setup. The test room included a refrigerator, a sink, injection supplies, injection pad, and cartons of both doses of nedosiran PFS. The Applicant also included two other medication injection products in the refrigerator to facilitate product differentiation and nedosiran dose differentiation. A manikin was in the room for the caregiver sessions.
Sequence of Study	• Differentiation scenario • Simulated-use scenario – administration of either a 128 mg or 160 mg dose ○ All adult patient participants and half of the caregiver participants were assigned the 160 mg dose. The other half of the caregiver participants and all pediatric patient participants were assigned the 128 mg dose. • Root cause analysis (RCA) interview • Knowledge task questions • RCA interview

3.2 DISCUSSION OF METHODOLOGY

- Inclusion of surrogate patient participants: We note 14 of the 16 adult patient participants in the HFVS and all of the pediatric patient participants in the HFVS were surrogate patient participants who did not have PH1. The Applicant indicated they attempted to recruit adult and pediatric patients with PH1 and provided a summary of

their recruitment efforts in the HFVS result report. The Applicant was unable to recruit a sufficient number of adult patient and pediatric patients due to the rarity of PH1. Additionally, the Applicant indicated that patients with PH1 do not have physical or cognitive differences that would impact use of the proposed PFS as compared to the general population. We find the Applicant's justification for use of surrogate adult patient and pediatric patient participants acceptable and find the patient participant methodology does not preclude our review of the HFVS results in this instance.

4 RESULTS AND ANALYSES

We have carefully reviewed each observed event in the HFVS, the Applicant's URR, the participants' subjective feedback, the Applicant's RCA, and the Applicant's comments and proposed mitigations below to determine if the results indicate that the user interface has been appropriately designed to support the safe and effective use of the proposed product.

See Table 4 which describes the study results, the Applicant's analyses of the results, and DMEPA's analyses and recommendations. Additionally, we provide our review of the label and labeling for areas of vulnerability that may lead to medication errors.

Table 4. Analysis of HFVS Results for the PFS									
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URR = use-related risk analysis									
Information Supplied by Applicant*	DMEPA's Findings and Recommendations								
Task: Do not prime out any air bubbles Scenario: Simulated use <table border="1"> <tr> <td>Errors:</td><td>Participant Type:</td></tr> <tr> <td>UE (n=1)</td><td>JE</td></tr> <tr> <td>CC (n=0)</td><td></td></tr> <tr> <td>UD (n=0)</td><td></td></tr> </table> Observed error: Held the uncapped PFS upright and pressed the plunger rod as if priming out air. A drop of liquid appeared at the tip of the needle; no other volume was expelled.	Errors:	Participant Type:	UE (n=1)	JE	CC (n=0)		UD (n=0)		
Errors:	Participant Type:								
UE (n=1)	JE								
CC (n=0)									
UD (n=0)									
Risk associated with Task Errors (Per Applicant URR): Reduced therapeutic effect due to underdose	We note that the presence of small air bubbles is not likely clinically meaningful given the proposed product is a prefilled syringe (e.g., with controls to ensure the dose is accurate) and given the proposed product is administered subcutaneously.								
Relevant RCA/Subjective Feedback/Observation: Negative transfer: was taught to prime out any air bubbles with their current injection device									
Applicant Comment and Proposed Mitigations: The Applicant did not implement revisions in response to participant performance on this task.	We acknowledge the HF validation study participants did not note confusion regarding the IFU Step 5 statement (b) (4) however,								

Table 4. Analysis of HFVS Results for the PFS									
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URRRA = use-related risk analysis									
Information Supplied by Applicant*	DMEPA's Findings and Recommendations								
	we are concerned that this statement does not adequately convey the expected appearance of the drug product (e.g., the air bubbles). As such, we provide a recommendation in Table 5 below. We find this recommendation can be implemented without the submission of additional HF data.								
<u>Task</u>: Select the injection site <u>Scenario</u>: Simulated use <table border="1"> <tr> <td>Errors:</td><td>Participant Type:</td></tr> <tr> <td>UE (n=2)</td><td>AE (n=1), JE (n=1)</td></tr> <tr> <td>CC (n=1)</td><td>CE</td></tr> <tr> <td>UD (n=0)</td><td></td></tr> </table> Observed error(s): • Injected near the inner elbow (similar to intravenous administration) • Injected their upper arm • Initially chose the upper arm, then glanced at the instructions for use (IFU) and changed to the thigh	Errors:	Participant Type:	UE (n=2)	AE (n=1), JE (n=1)	CC (n=1)	CE	UD (n=0)		
Errors:	Participant Type:								
UE (n=2)	AE (n=1), JE (n=1)								
CC (n=1)	CE								
UD (n=0)									
Risk associated with Task Errors (Per Applicant URRRA): • Administration to back of upper arm – slow absorption of drug equivalent to an underdose • Intravenous administration – drug is cleared rapidly equivalent to an underdose	The URRRA does not describe the clinical sequelae that may occur as a result of an underdose caused by intravenous administration.								

Table 4. Analysis of HFVS Results for the PFS	
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URRRA = use-related risk analysis	
Information Supplied by Applicant*	DMEPA's Findings and Recommendations
	In discussion with the review team (i.e., clinical, clinical pharmacology), we note that an underdose that is not of a large magnitude may result in a small loss in efficacy.
Relevant RCA/Subjective Feedback/Observation: - Chose the arm because that is where they normally inject their prescription medications. - Did not think using the upper arm would matter, as long as the injection goes into the fatty tissue. - Noted the IFU does not specifically say “do not inject to the arm” but does show the thigh or abdomen. - The participant who experienced a close call did not explain why they chose the arm.	
Applicant Comment and Proposed Mitigations: - The Applicant did not implement revisions in response to participant performance on this task. - The Applicant stated that if the injection was incorrectly administered in the upper arm, the patient would still receive the full dose.	We note that the injection sites for the proposed product are consistent with other marketed products approved for subcutaneous administration. We note the graphic of the injection sites in IFU Step 6 (b) (4) As such, we provide a recommendation in Table 5 below. We find the recommendation can be implemented without the submission of additional HF data.

Table 4. Analysis of HFVS Results for the PFS									
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URRRA = use-related risk analysis									
Information Supplied by Applicant*	DMEPA's Findings and Recommendations								
Task: Pinch the skin Scenario: Simulated use <table border="1"> <tr> <th>Errors:</th><th>Participant Type:</th></tr> <tr> <td>UE (n = 4)</td><td>CN (n=1), AE (n=1), JE (n=1), JN (n=1)</td></tr> <tr> <td>CC (n=0)</td><td></td></tr> <tr> <td>UD (n=0)</td><td></td></tr> </table> Observed error(s): - Did not pinch the skin at the injection site. Of note, 2 of the 4 participants self-administered into the arm. Risk associated with Task Errors (Per Applicant URRRA): - Slow absorption of drug equivalent to an underdose Relevant RCA/Subjective Feedback/Observation: - Initially saw the instruction to pinch the skin but did not pinch during the injection due to being worried about and focused on the bare needle - The amount of content in the IFU - Did not have a free hand to pinch the skin and realized he would if he had selected the thigh or abdomen - Study artifact: noticed the instruction to pinch but did not think to pinch the injection pad - The following revisions were suggested by study participants: - Put a reminder to pinch the skin prior to removing the needle cap - Make the instructions bold font in the IFU	Errors:	Participant Type:	UE (n = 4)	CN (n=1), AE (n=1), JE (n=1), JN (n=1)	CC (n=0)		UD (n=0)		
Errors:	Participant Type:								
UE (n = 4)	CN (n=1), AE (n=1), JE (n=1), JN (n=1)								
CC (n=0)									
UD (n=0)									

Table 4. Analysis of HFVS Results for the PFS									
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URRRA = use-related risk analysis									
Information Supplied by Applicant*	DMEPA's Findings and Recommendations								
Applicant Comment and Proposed Mitigations: - The Applicant did not implement revisions in response to participant performance on this task. - The Applicant determined the risks associated with use errors with task are acceptably low based on the needle length of ½”. - The Applicant also noted that a skin pinching graphic appears in the IFU under the “giving your injection” section in two locations.	We note a participant recommended to make the pinch the skin instruction bold; however, we note the title of IFU Step 9 is already in bold font. We did not identify areas of improvement and have no recommendations at this time.								
Task: Insert the needle fully into the skin using ~45-degree insertion angle Scenario: Simulated use <table border="1"> <tr> <th>Errors:</th><th>Participant Type:</th></tr> <tr> <td>UE (n=7)</td><td>CN (n=1), AE (n=2), JE (n=2), JN (n=2)</td></tr> <tr> <td>CC (n=0)</td><td></td></tr> <tr> <td>UD (n=0)</td><td></td></tr> </table> Observed error(s): - Inserted the needle into the injection pad using a larger insertion angle (e.g., 80° - 90°) than instructed. - Used the correct insertion angle but did not fully insert the needle into the injection pad.	Errors:	Participant Type:	UE (n=7)	CN (n=1), AE (n=2), JE (n=2), JN (n=2)	CC (n=0)		UD (n=0)		
Errors:	Participant Type:								
UE (n=7)	CN (n=1), AE (n=2), JE (n=2), JN (n=2)								
CC (n=0)									
UD (n=0)									
Risk associated with Task Errors (Per Applicant URRRA): Injection into incorrect injection site (intracutaneous or intramuscular) resulting in slow absorption of drug equivalent to an underdose									

Table 4. Analysis of HFVS Results for the PFS	
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URRRA = use-related risk analysis	
Information Supplied by Applicant*	DMEPA's Findings and Recommendations
Relevant RCA/Subjective Feedback/Observation: - Noticed the IFU instruction but chose a 90° angle due to their own use habits with other injections (e.g., a prefilled pen with a 90° insertion angle) - Chose a larger angle “automatically” and because they had not fully reviewed the IFU - Has seen healthcare providers (HCPs give injections with needles not fully inserted - The following revisions were suggested by study participants: - Show the injection angle and all injection steps on the product carton. - Add the word “fully” next to the words “insert the needle” in the IFU.	
Applicant Comment and Proposed Mitigations: - The Applicant did not implement revisions in response to participant performance on this task. - The Applicant noted the risks are acceptably low because the needle is ½” in length, which increases the likelihood to reach the subcutaneous layer. - The Applicant also noted the IFU includes a 45° angle graphic.	IFU Step 9 includes text and graphic to convey that users should insert the needle at the injection site. However, IFU Step 9’s title and text can be improved to clarify that users should fully insert the needle. As such, we provide recommendations in Table 5 below. We find these recommendations can be implemented without the submission of additional HF data.

Table 4. Analysis of HFVS Results for the PFS									
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URR = use-related risk analysis									
Information Supplied by Applicant*	DMEPA's Findings and Recommendations								
Task: Depress plunger rod fully Scenario: Simulated use <table border="1"> <tr> <td>Errors:</td><td>Participant Type:</td></tr> <tr> <td>UE (n=1)</td><td>JN</td></tr> <tr> <td>CC (n=0)</td><td></td></tr> <tr> <td>UD (n=0)</td><td></td></tr> </table> Observed error(s): - Depressed the plunger rod and delivered approximately 80% of the syringe volume (based on moderator inspection), removed the needle from the injection pad, maintained pressure on the plunger rod, and the remainder of the dose expelled onto the pad. Risk associated with Task Errors (Per Applicant URR): - Reduced therapeutic effect due to underdose Relevant RCA/Subjective Feedback/Observation: - Felt that she had squeezed out all of the medication but realized afterwards that maybe she didn't press all the way down. - Device gave the participant the impression that the dose had been fully injected when it had not. - Participant possibly felt some resistance toward the end of the injection and therefore removed the needle. Applicant Comment and Proposed Mitigations: - The Applicant did not implement revisions in response to participant performance on this task.	Errors:	Participant Type:	UE (n=1)	JN	CC (n=0)		UD (n=0)		
Errors:	Participant Type:								
UE (n=1)	JN								
CC (n=0)									
UD (n=0)									
	Our independent review of the PFS did not identify concerns with depressing the plunger rod.								

Table 4. Analysis of HFVS Results for the PFS									
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URRRA = use-related risk analysis									
Information Supplied by Applicant*	DMEPA's Findings and Recommendations								
The Applicant stated the IFU instructs the user to slowly depress the plunger rod to inject all of the medicine and illustrations are provided. The Applicant also stated the design of the device suggests the plunger rod needs to be pressed so that the medicine can be injected and noted the syringe barrel is clear.	We did not identify areas of improvement and have no recommendations at this time.								
<u>Task</u>: Do not recap needle <u>Scenario</u>: Simulated use <table border="1"> <tr> <th>Errors:</th><th>Participant Type:</th></tr> <tr> <td>UE (n=6)</td><td>AE (n=3), AN (n=1), JE (n=1), JN (n=1)</td></tr> <tr> <td>CC (n=0)</td><td></td></tr> <tr> <td>UD (n=0)</td><td></td></tr> </table> Observed error(s): - Recapped or handled the needle post injection and prior to disposal - No needlesticks occurred Risk associated with Task Errors (Per Applicant URRRA): - Pain Relevant RCA/Subjective Feedback/Observation: - Transfer of use habits with their current injection devices - Unsure why he recapped the needle - Recapped the needle out of concern that others could receive a needle stick. Applicant Comment and Proposed Mitigations:	Errors:	Participant Type:	UE (n=6)	AE (n=3), AN (n=1), JE (n=1), JN (n=1)	CC (n=0)		UD (n=0)		
Errors:	Participant Type:								
UE (n=6)	AE (n=3), AN (n=1), JE (n=1), JN (n=1)								
CC (n=0)									
UD (n=0)									
	We note the proposed PFS does not have a needle safety guard. We also acknowledge the Applicant								

Table 4. Analysis of HFVS Results for the PFS									
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URRRA = use-related risk analysis									
Information Supplied by Applicant*	DMEPA's Findings and Recommendations								
- The Applicant did not implement revisions in response to participant performance on this task. - The Applicant stated that if a needlestick were to occur during recapping the needle, there is no risk of disease transmission and that the IFU states "do not recap the needle" (b) (4) in the "Giving your injection" Section.	stated they previously completed formative testing with a PFS with a needle guard and did not include a needle guard in the PFS final design due to use-related issues related to the needle guard (e.g., users prematurely locked out the PFS which prevented dose delivery). We did not identify areas of improvement and have no recommendations at this time.								
Task: Dispose of PFS in appropriate container Scenario: Simulated use <table border="1"> <tr> <td>Errors:</td><td>Participant Type:</td></tr> <tr> <td>UE (n=1)</td><td>JN</td></tr> <tr> <td>CC (n=0)</td><td></td></tr> <tr> <td>UD (n=0)</td><td></td></tr> </table> Observed error: - Disposed of the used PFS in the household trash Risk associated with Task Errors (Per Applicant URRRA): - Risk of transmission of communicable disease Relevant RCA/Subjective Feedback/Observation: - Unsure where to dispose of the PFS and cap but knew it had to be disposed of right away - Stated that medications at home normally go in the regular trash	Errors:	Participant Type:	UE (n=1)	JN	CC (n=0)		UD (n=0)		
Errors:	Participant Type:								
UE (n=1)	JN								
CC (n=0)									
UD (n=0)									

Table 4. Analysis of HFVS Results for the PFS									
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URRRA = use-related risk analysis									
Information Supplied by Applicant*	DMEPA's Findings and Recommendations								
- Asked about the sharps bin in the study room and was unsure why he did not use it for disposing this PFS - Suggested adding a biohazard symbol to the image of the sharps bin in the IFU.									
Applicant Comment and Proposed Mitigations: - The Applicant did not implement revisions in response to participant performance on this task. - The Applicant noted the IFU has an illustration of a sharps container and states alternatives that can be used if one is not available. The Applicant also noted they removed a previously included biohazard symbol based on a recommendation from the Agency.	IFU Step 12 includes text and graphic to convey the disposal technique. However, IFU Step 12's title and the graphic can be improved to further clarify that the PFS should be disposed of in a sharps container. As such, we provide a recommendation in Table 5 below. We find this recommendation can be implemented without the submission of additional HF data.								
Task: Disposal (How should the syringe and the needle cap be disposed of? [Answer: in sharps-like container and not recapped]) Scenario: Knowledge question <table border="1"> <thead> <tr> <th>Errors:</th><th>Participant Type:</th></tr> </thead> <tbody> <tr> <td>UE (n=1)</td><td>CN</td></tr> <tr> <td>CC (n=0)</td><td></td></tr> <tr> <td>UD (n=0)</td><td></td></tr> </tbody> </table> Observed error: Correctly stated that the used syringe and needle cap should be disposed of in a sharps container but incorrectly stated the needle "should be recapped"	Errors:	Participant Type:	UE (n=1)	CN	CC (n=0)		UD (n=0)		
Errors:	Participant Type:								
UE (n=1)	CN								
CC (n=0)									
UD (n=0)									

Table 4. Analysis of HFVS Results for the PFS	
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URRRA = use-related risk analysis	
Information Supplied by Applicant*	DMEPA's Findings and Recommendations
to protect the needle and make sure that nobody gets a needle stick". Of note, the participant did not attempt to recap the needle during simulated use and properly disposed of the used needle and cap.	
Risk associated with Task Errors (Per Applicant URRRA): • Needlestick injury • Contamination	
Relevant RCA/Subjective Feedback/Observation: • Did not initially see anything describing a capped or uncapped syringe in Step 12 (b) (4) • Noted that Step 12 says, "throw away the used syringe (b) (4) ..." but it does not specify "uncapped and implied the wording in Step 12 could be improved.	
Applicant Comment and Proposed Mitigations: • The Applicant did not implement revisions in response to participant performance on this knowledge task. • The Applicant stated although the word "uncapped" was not in IFU Step 12, the participant did not recap the syringe because it was stated in prior steps.	We acknowledge the RCA and subjective feedback indicated the participant noted IFU Step 12 does not specify that the PFS should be uncapped at the time of disposal. However, we note IFU Step 8 instructions users to throw away the needle cap and IFU Step 11 informs users not to recap the needle. However, we find the instructions regarding the disposal of the needle cap could be improved. As such, we provide a recommendation in Table 5 below. We find this recommendation can be

Table 4. Analysis of HFVS Results for the PFS	
Legend: AE = injection-experienced adult; AN = injection-naïve adult; CC = close call; CE = injection-experienced caregiver, CN = injection-naïve caregiver; JE = injection-experienced adolescent; JN = injection-naïve adolescent; RCA = root cause analysis; UD = use difficulty; UE = use error; URRRA = use-related risk analysis	
Information Supplied by Applicant*	DMEPA's Findings and Recommendations
	implemented without the submission of additional HF data.

4.1 ANALYSIS OF OTHER TASKS WITH USE-RELATED EVENTS NOT INCLUDED IN TABLE 4

The HFVS showed use errors, a use difficulty, and study artifacts with the tasks below:

- Select the correct dose carton (n=3) use errors
- Open the carton (n=1) use difficulty
- Wash hands or correct response to knowledge question (n=1) study artifact

For the 3 use errors, one caregiver participant and 2 adolescent patient participants experienced use errors with the select the correct dose carton task. We note 1 of the 3 use errors occurred due to study artifact because the participant selected the carton that matched their actual weight instead of the carton that matched the mock prescription. Additionally, we generally expect that the pharmacist will be responsible for selecting the correct dose carton.

For the 1 use difficulty, one participant experienced initial difficulty with opening the carton due to the tamper evident seal; this participant was able to open the carton on his own. For the study artifact, one participant did not wash their hands in the simulated use scenario and stated they would normally wash their hands at home in actual use. In addition, this participant did answer the knowledge task question correctly to wash hands prior to injecting.

We reviewed the available participants' subjective feedback, the Applicant's root cause analysis, and Applicant's proposed risk mitigation strategy we determined the residual risk is acceptable. Subsequently, we did not identify further need for risk mitigation strategies at this time to address the use errors related to the aforementioned tasks.

4.2 LABELS AND LABELING

Tables 5 and 6 below include the identified medication error issues with the submitted label and labeling, our rationale for concern, and the proposed recommendation to minimize the risk for medication error.

4.3 THE VIAL PRESENTATION

We note the Applicant submitted HFVS results for the PFS only and did not submit HFVS results for the vial presentation (b) (4)

In response to the Agency's April 19, 2023 Information Request (IR), the Applicant submitted a risk assessment and justification to support their determination that no HFVS data is necessary for the vial presentation; the submission also included a description of the Applicant's intention to provide training on the vial presentation to caregivers. Additionally, in a June 2, 2023 email correspondence, the

Applicant provided a high-level summary of their proposed training for the vial presentation, which includes a training program with in-person visits with an HCP, assessment of preparation and administration proficiency by an HCP, and routine follow-up during the first 6 months of use.

In the June 5, 2023 Late Cycle Meeting, the Applicant confirmed the training program will be provided by the Applicant, the proposed product will be dispensed through a limited network specialty pharmacy, and HCP trainers will utilize a checklist to ensure that caregivers are proficient in the preparation and administration of nedosiran vials. Additionally, in the Late Cycle Meeting, the Applicant noted they anticipate the nedosiran vial will be used by a small patient population of approximately 30 patients over the 3-year timeframe from 2024 to 2026.

Based on internal communication, we learned from the OND clinical review team that clinical impact of wrong dose errors (underdose or overdose) with the vial presentation are only potential injection site reactions and there are no clinically significant concerns. OND also indicated that the ability to administer the nedosiran vial at home once monthly would reduce the burden of having to travel to a monthly medical appointment for administration of nedosiran.

Because no HFVS data was submitted, it is unknown if caregivers can safely and effectively prepare nedosiran vials for administration to pediatric patients in the home setting. If the Review Team finds the risk of the unknown outweighs the public health need, then we recommend limiting the vial presentation to administration by healthcare professionals only. If the Review Team finds the public health need outweighs the risk of the unknown, and choose to approve the vial for caregiver use at home, then we recommend including the following statement to Section 2.2 of the PI:

“Vials: [REDACTED] (b) (4)
[REDACTED] Rivfloza vials are intended for use under the guidance and supervision of a [REDACTED] (b) (4). A caregiver may administer Rivfloza to pediatric patients after proper training in preparing Rivfloza vials for administration if a [REDACTED] (b) (4) determines that it is appropriate, and with medical follow-up as necessary.”

Additionally, to ensure the Applicant’s training program is robust, we recommend the Applicant ensure the training includes didactic, demonstration, and assessment components in Section 5.1 below.

5 CONCLUSION AND RECOMMENDATIONS

Our review of the results of the HF validation study for Rivfloza (nedosiran) injection PFS identified use errors with critical tasks; however, based on our review, we find the residual risks are acceptable or can be further mitigated via additional labels and labeling revisions for these use errors. Thus, in this specific instance, we find the simulated use HFVS results are acceptable provided our recommendations are implemented.

Additionally, our evaluation of the proposed packaging, label, and labeling for the PFS and vial identified areas of vulnerability that may lead to medication errors. Below, we have provided recommendations in Table 5 for the Division and Table 6 for the Applicant. We ask that the Division convey Table 6 in its entirety to the Applicant so that recommendations are implemented within the current review cycle of this NDA 215842.

Table 5: Identified Issues and Recommendations for Cardiology and Nephrology (DCN)			
	Identified Issue	Rationale for Concern	Recommendation
Prescribing Information – General			
1.	The Prescribing Information (PI) contains (i.e., 1.0 mL).	To avoid ten-fold misinterpretation, trailing zeroes should be eliminated from dose expressions. ^b	We recommend removing all instances of trailing zeroes in the PI.
Full Prescribing Information – Section 2 Dosage and Administration			
1.	The Applicant did not submit human factors validation study (HFVS) data to support the safe and effective use by caregivers in preparing nedosiran vials for administration to pediatric patients in the home setting.	Due to lack of HFVS data, it is unknown if caregivers can safely and effectively prepare nedosiran vials for administration to pediatric patients in the home setting.	If the Review Team finds the risk of the unknown outweighs the public health need, then we recommend limiting the vial presentation to administration by healthcare professionals (HCPs) only. If the Review Team find the public health need outweighs the risk of the unknown, and choose to approve the vial for caregiver use at home, then we recommend including the following statement to Section 2.2 of the PI: - “Vials: (b) (4) Rivfloza vials are intended for use under the guidance and supervision of a (b) (4) A caregiver may

^b ISMP’s List of Error-Prone Abbreviations, Symbols, and Dose Designations [Internet]. Horsham (PA): Institute for Safe Medication Practices. 2021 [cited 2023 JAN 31]. Available from: <http://www.ismp.org/tools/errorproneabbreviations.pdf>.

			administer Rivfloza to pediatric patients after proper training in preparing Rivfloza vials for administration if a (b) (4) determines that it is appropriate, and with medical follow-up as necessary.”
Instructions for Use (IFU) – prefilled syringe (PFS)			
1.	The Step 6 graphic (b) (4)	In the human factors (HF) validation study, there were use errors and a close call in which participants did not select the correct injection site. Based on the use-related risk analysis (URRA), administration to the incorrect injection site could result in slow absorption of drug equivalent to an underdose or the rapid drug clearance equivalent to an underdose.	We recommend labeling the injection sites on the Step 6 graphic.
2.	The title and text for Step 9 can be improved to show that users should fully insert the needle.	In the HF validation study, there was a use error in which the participant did not fully insert the needle into the injection pad. Based on the URRA, performing the injection task incorrectly might result in injection into an incorrect injection site (intracutaneous or intramuscular) resulting in slow absorption of drug equivalent to an underdose.	We recommend revising the title of Step 9 from (b) (4) to “Pinch the skin and fully insert the needle” and also revising the Step 9 statement (b) (4) to “Grasp the finger grip (b) (4) and fully insert the needle into the injection site...”.

3.	The first graphic in Step 11 is confusing because the depiction of the exact injection site (b) (4)	Confusion regarding the injection sites might result in wrong technique medication errors.	We recommend revising the first graphic in Step 11 (b) (4)
4.	The disposal information in Step 12 can be improved to clarify the correct disposal technique.	In the HF validation study, a participant noted confusion regarding how to dispose of the PFS. Based on the URRA, performing the disposal task incorrectly might result in transmission of communicable disease.	We recommend revising the title of Step 12 from (b) (4) to now read “Throw away (dispose of) (b) (4) Additionally, we recommend labeling the sharps container in the Step 12 graphic.
5.	Step 12 instructs users (b) (4)	Confusion regarding (b) (4) might result in wrong technique medication errors. In the HF validation study, a participant incorrectly stated the needle should be recapped. Based on the URRA, performing the disposal task incorrectly might result in transmission of communicable disease.	We recommend revising IFU Step 12 from (b) (4) to now read “Put your used Pre-filled Syringe in an FDA cleared sharps...”.
6.	The IFU can be improved to clarify the expected appearance of the drug product (e.g., small air bubbles).	Confusion regarding the expected appearance of the drug product might result in wrong technique or wrong dose medication errors (i.e., in instances where users attempt to remove small air bubbles).	We recommend revising (b) (4) to now read “You may see small air bubbles in the liquid. This is normal”.
Instructions for Use (IFU) – vial			

1.	In the absence of HFVS data for the vial presentation, it is unclear whether lay users (b) (4)	Misinterpretation of or confusion (b) (4) might result in wrong dose errors. (b) (4)	We recommend removing (b) (4) Similarly, under “Frequently Asked Questions”, “Do I inject the full volume of the vial? (b) (4) delete (b) (4) so it reads: (b) (4) child’s prescribed dose. You may need more than one vial (b) (4) for the prescribed dose.”
2.	The Step 6 graphic (b) (4)	Based on the risk assessment, administration to an incorrect injection site could result in intracutaneous or intramuscular injection resulting in reduced therapeutic effect or intravenous injection resulting rapid clearance equivalent to an underdose.	We recommend labeling the injection sites on the Step 6 graphic.
3.	The title and text for Step 12 can be improved to ensure that users fully insert the needle.	Failure to fully insert the needle might result in underdose and wrong technique medication errors.	We recommend revising the title of Step 12 from (b) (4) to “Pinch the skin and fully insert the needle”.
4.	The first graphic in Step 14 is confusing (b) (4)	Based on the risk assessment, administration to an incorrect injection site could result in intracutaneous or intramuscular injection resulting in reduced therapeutic effect or intravenous	We recommend revising the first graphic in Step 14 (b) (4)

		injection resulting rapid clearance equivalent to an underdose.	
5.	The disposal information in Step 15 can be improved to clarify the correct disposal technique.	Based on the risk assessment, performing the disposal task incorrectly might result risk of transmission of communicable disease.	We recommend labeling the sharps container in the Step 15 graphic.

5.1 RECOMMENDATIONS FOR NOVO NORDISK INC

Our review of the results of your human factors (HF) validation study for Rivfloza (nedosiran) injection prefilled syringe (PFS) identified areas of vulnerability in your labels and labeling that may lead to medication errors. We provide recommendations in Table 6, and we recommend that you implement these recommendations and submit the revised labels and labeling. We have determined that in this instance, you may implement these revisions without submitting additional HF validation data for Agency review.

Our review of the labels and labeling for the proposed Rivfloza PFS and vial identified areas of vulnerability that may lead to medication errors. We also provide these best practice labeling recommendations in Table 6.

We also note you intend to implement a training program for the vial presentation of your proposed product. To ensure your training program for the vial presentation is robust, we recommend your training program include didactic instruction, hands-on demonstration, and skill assessment.

Table 6: Identified Issues and Recommendations for Novo Nordisk Inc (entire table to be conveyed to Applicant)			
	Identified Issue	Rationale for Concern	Recommendation
Container label – Prefilled syringe (PFS)			
1.	The product strength (b) (4)	(b) (4) Furthermore, your strength expression is not presented in accordance with USP General Chapter <7>, Labeling.	Express the product strength as total quantity per fraction of mL (i.e., 160 mg/mL) in accordance with USP General Chapter <7>.
Container label – vial			
1.	The container label (b) (4)	The label should inform users to discard unused contents to minimize the risk of the entire contents of the vial being given as a single dose.	Revise (b) (4) to “Single-Dose Vial – Discard Unused Portion”.
2.	The linear barcode is presented in horizontal position.	If the linear barcode is presented in horizontal position, then the barcode may wrap around the curvature of a small vial and will not be scannable.	Consider reorienting the linear barcode to a vertical position to improve the scannability of the barcode. Barcodes placed in a horizontal position may not scan due to vial curvature. ^c
Carton labeling - PFS and vial			
1.	The route of administration statement is not prominent.	If users overlook or are unable to find the route of administration information, there is risk of wrong route of administration errors.	Increase the prominence of the route of administration statement and relocate it to immediately below the strength statement

^c Neuenschwander M. et al. Practical guide to bar coding for patient medication safety. Am J Health Syst Pharm. 2003 Apr 15;60(8):768-79.

			and above the “1 x # mL Sterile (b) (4) statement.
2.	The (b) (4) statement is not in the correct format.	The (b) (4) statement should be aligned with the Prescribing Information.	To ensure consistency with the Prescribing Information, revise the statement, (b) (4) to read “Recommended Dosage: See prescribing information.”
3.	The area on the carton for documenting the room temperature storage start date has a dark background color, which could impact the visibility of text handwritten in blue or black ink.	Reduced readability or visibility of the handwritten room temperature storage start date might contribute to deteriorated drug medication errors.	Consider revising or lightening the background color of the area on the carton for documenting the room temperature storage start date.
4.	Non-critical information appears on the principal display panel (PDP) and contributes to overcrowding.	When the carton labeling is crowded, text size and prominence are generally decreased, and important information may be difficult to read or easily overlooked.	Delete the statement (b) (4) from the PDP. We note this information is already on the back panel.
5.	The proprietary name may not be interpretable. (b) (4)	The artwork (b) (4) and may interfere with the interpretation of the proprietary name.	Revise the artwork of the proprietary name (b) (4)
Carton labeling - PFS			

1.	The product strength (b) (4)	(b) (4) Furthermore, your strength expression is not presented in accordance with USP General Chapter <7>, Labeling.	Express the product strength as total quantity per fraction of mL (i.e., 160 mg/mL) in accordance with USP General Chapter <7>.
2.	The total volume included in the net quantity statement for the 128 mg/0.8 mL PFS is not correct.	The carton labeling should accurately describe the product contents.	Revise the net quantity statement for the 128 mg/0.8 mL PFS from (b) (4) to “1 x 0.8 mL”.
Carton labeling - vial			
1.	The carton labeling (b) (4)	The labeling should inform users to discard unused contents to minimize the risk of the entire contents of the vial being given as a single dose.	Revise (b) (4) to “Single-Dose Vial – Discard Unused Portion”.
Carton labeling insert - vial			
1.	The carton labeling insert states (b) (4)	Confusion regarding dosing might result in wrong dose errors.	Remove (b) (4)

APPENDICES

APPENDIX A. BACKGROUND INFORMATION ON HUMAN FACTORS ENGINEERING PROCESS

The background information can be accessed in EDR via:

<\\CDSESUB1\EVSPROD\nda215842\0003\m5\53-clin-stud-rep\535-rep-effic-safety-stud\ph\5354-other-stud-rep\drn-nedosiran-vt-503\drn-nedosiran-vt-503.pdf>

APPENDIX B. HUMAN FACTORS VALIDATION STUDY RESULTS REPORT

The HF study results report can be accessed in EDR via:

<\\CDSESUB1\EVSPROD\nda215842\0003\m5\53-clin-stud-rep\535-rep-effic-safety-stud\ph\5354-other-stud-rep\drn-nedosiran-vt-503\drn-nedosiran-vt-503.pdf>

APPENDIX C. INFORMATION REQUESTS ISSUED DURING THE REVIEW

On November 3, 2022, we issued an Information Request (IR) to request the Applicant provide the patient and lay caregiver HFVS results for the proposed vial presentation and product samples. The Applicant provided a response on November 8, 2022 that can be accessed in EDR via:

<\\CDSESUB1\EVSPROD\nda215842\0004\m1\us\111-info-amend\ir-response-submitted-20221108.pdf>

On January 25, 2023, we issued an IR to request the Applicant provide rationale for the vial IFU instruction [REDACTED] (b) (4)

[REDACTED] The Applicant provided a response on January 27, 2023 that can be accessed in EDR via:

<\\CDSESUB1\EVSPROD\nda215842\0012\m1\us\111-info-amend\ir-response-submitted-20230127.pdf>

On April 19, 2023, the Agency issued an IR to request the Applicant provide a proactive risk assessment of the vial presentation and their determination regarding whether an HFVS should be conducted under simulated use conditions with representative users performing necessary tasks. The Applicant provided a response on May 3, 2023 that can be accessed in EDR via:

<\\CDSESUB1\EVSPROD\nda215842\0025\m1\us\111-info-amend\ir-response-submitted-20230503a.pdf>

APPENDIX D. LABELS AND LABELING

D.1 List of Labels and Labeling Reviewed

Using the principles of human factors and Failure Mode and Effects Analysis,^d along with postmarket medication error experiences with similar products, we reviewed the following Rivfloza labels and labeling submitted by Novo Nordisk Inc.

- Container labels received on 9/30/22
- Carton labeling received on 5/24/23
- Carton labeling insert received on 9/30/22
- PFS Instructions for use (image not shown) received on 9/30/22
 - Available from: <\\CDSESUB1\EVSPROD\nda215842\0003\m1\us\114-labeling\114a-draft-label\proposed-pfs-ifu-pdf.pdf>
- Vial Instructions for use (image not shown) received on 5/3/23
 - Available from: <\\CDSESUB1\EVSPROD\nda215842\0025\m1\us\114-labeling\114a-draft-label\proposed-vial-ifu-word.docx>
- Prescribing Information (image not shown) received on 9/30/22
 - Available from: <\\CDSESUB1\EVSPROD\nda215842\0003\m1\us\114-labeling\114a-draft-label\proposed-uspi-pdf.pdf>

^d Institute for Healthcare Improvement (IHI). Failure Modes and Effects Analysis. Boston. IHI:2004.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

EBONY A WHALEY
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DEPARTMENT OF HEALTH & HUMAN SERVICES **Public Health Service**

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Division of Pediatrics and Maternal Health Review

Date: 5/15/2023 **Date consulted:** 3/2/2023

From: Katherine Kratz, M.D., Medical Officer, Maternal Health
Division of Pediatrics and Maternal Health (DPMH)

Through: Miriam Dinatale, D.O., Team Leader, Maternal Health, DPMH

Lynne P. Yao, MD, OND, Division Director, DPMH

To: Division of Cardiology and Nephrology (DCN)

Drug: Rivfloza (nedosiran)

NDA: NDA 215842

Applicant: Dicerna Pharmaceuticals, Inc.

Subject: Pregnancy and Lactation Labeling

Indication: Indicated for the treatment of primary hyperoxaluria type 1 (PH1)

Materials

Reviewed:

- DPMH consult request dated 3/2/2023. DARRTS Reference ID: 5135347.
- Applicant's submitted background package and proposed labeling for NDA 215842. Sequence Number (SN) 0003, submitted on 9/30/2022.
- Applicant's 120-Day Safety Update for NDA 215842. SN 0011, submitted on 1/27/2023.
- DPMH Maternal Health review of pregnancy and lactation subsections of labeling for NDA 214103 for Oxlumo (lumasiran) by Miriam Dinatale, DO, dated 8/26/2020. DARRTS Reference ID: 4661962.

- DPMH Maternal Health review of pregnancy and lactation subsections of labeling for NDA 214012 for Leqvio (inclisiran) by Christos Mastroyannis, MD, dated 10/19/2020. DARRTS Reference ID: 4687201.
- United States Prescribing Information (USPI) for Onpattro (patisiran).
- USPI for Givlaari (givosiran).
- USPI for Oxlumo (lumasiran)
- USPI for Leqvio (inclisiran).

Consult Question: “Please review PLLR [Pregnancy and Lactation Labeling Rule] aspects of labeling.”

I. INTRODUCTION AND BACKGROUND

On September 30, 2022, the applicant, Dicerna Pharmaceuticals, Inc., submitted a 505(b)(1) New Drug Application (NDA) for Rivfloza (nedosiran) for the treatment of PH1. DCN consulted DPMH on March 2, 2023 to assist with the Pregnancy and Lactation subsections of labeling.

Relevant Regulatory History

- Four small interfering RNA (siRNA) drug products have been approved by FDA: patisiran, givosiran, lumasiran, and inclisiran. The indication for each of these drugs is listed below under each specific drug name.
 - Class labeling does not include a boxed warning for embryo-fetal toxicity or a contraindication for pregnancy or lactation. See Appendix A for additional details regarding pregnancy and lactation labeling for other drugs in this class.
 - Lumasiran is the only approved siRNA for the treatment of PH1.
 - Among the siRNAs, patisiran, givosiran, and lumasiran are conjugated to N-Acetylgalactosamine (GalNAc) like nedosiran. As explained under the Pregnancy section of this review (below), nedosiran caused developmental and maternal toxicities in rabbits, but not in rodents. Patisiran and givosiran also demonstrated developmental and maternal toxicities in rabbits and not in rodents.¹ Lumasiran did not cause developmental or maternal toxicities in rabbits or rodents.
 - The patisiran labeling states, “patisiran-LC [lipid complex] (0, 0.3, 1, or 2 mg/kg), administered to pregnant rabbits every week during the period of organogenesis, resulted in embryofetal mortality and reduced fetal body weight at the mid and high doses, which were associated with maternal toxicity.” The relation of these mid and high doses to the maximum recommended human dose (MRHD) is not specified in the labeling.
 - The givosiran labeling states, “Administration of givosiran was maternally toxic based on decreased body weight gain at all dose levels tested and resulted in increased postimplantation loss starting at 1.5 mg/kg/day. An increased incidence of skeletal variations of the sternebrae was observed at 20 mg/kg. The 1.5 mg/kg/day dose in rabbits is 5 times the maximum recommended human dose (MRHD) of 2.5 mg/kg/month normalized to 0.089 mg/kg/day, based on body surface area. In a

¹ USPIs for Onpattro (patisiran) and Givlaari (givosiran).

combined fertility and embryo-fetal development study in female rats, givosiran was administered subcutaneously at doses of 0.5 to 5 mg/kg/day during organogenesis (gestational days 6-17). The 5 mg/kg/day dose (9 times the normalized MRHD based on body surface area) was associated with a skeletal variation (incomplete ossification of pubes) and produced maternal toxicity.”

- Patisiran:
 - 2018: FDA approved Onpattro (patisiran) under NDA 210922 for the treatment of the polyneuropathy of hereditary transthyretin-mediated amyloidosis in adults.
 - 2019: DPMH reviewed the Post-Marketing Requirement (PMR) for a Pregnancy Surveillance Study for Onpattro (patisiran, NDA 210922). DPMH did not conduct a consult review for patisiran labeling.
- Givosiran:
 - 2019: FDA approved Givlaari (givosiran) under NDA 212194 for the treatment of adults with acute hepatic porphyria. DPMH did not conduct a consult review for givosiran labeling.
- Lumasiran:
 - 2020: FDA approved Oxlumo (lumasiran) under NDA 214103 for the treatment of PH1. Although both lumasiran and nedosiran are indicated for the treatment of PH1, lumasiran has a different mechanism of action than nedosiran. Lumasiran reduces levels of glucolate oxidase (GO) by targeting hydroxyacid oxidase 1 (HAO1) messenger RNA (mRNA) in hepatocytes whereas nedosiran reduces levels of lactate dehydrogenase (LDH) by targeting LDHA mRNA in hepatocytes. The labeling for lumasiran does not contain a boxed warning or warnings or precautions related to embryofetal toxicity. DPMH conducted a consult review of the lumasiran labeling.
- Inclisiran:
 - 2021: FDA approved Leqvio (inclisiran) under NDA 214012 for the treatment of adults with heterozygous familial hypercholesterolemia (HeFH) or clinical atherosclerotic cardiovascular disease (ASCVD), who require additional lowering of low-density lipoprotein cholesterol (LDL-C) as an adjunct to diet and maximally tolerated statin therapy. DPMH conducted a consult review of inclisiran labeling.

Drug Characteristics²

Drug class	Double-stranded siRNA
Mechanism of action	Nedosiran is an siRNA conjugated to GalNAC to ensure preferential uptake by hepatocytes via asialoglycoprotein receptors. Once inside the hepatocyte, nedosiran targets the <i>LDHA</i> mRNA isoenzyme and reduces lactate dehydrogenase (LDH) thereby reducing production of oxalate by the liver.
Dosage forms	1) a vial with 0.5 mL extractable volume; 2) a pre-filled syringe with a deliverable volume of 0.8 mL, containing 128 mg of drug;

² NDA 215842, Proposed United States Prescribing Information (USPI) under review by DCN.

	3) a pre-filled syringe with a deliverable volume of 1.0 mL, containing 160 mg of drug
Proposed Dosing	≥ 12 years old and ≥ 50 kg: 160 mg (1 mL) subcutaneously (SC) once monthly ≥ 12 years old and < 50 kg: 128 mg SC (0.8 mL) once monthly (b) (4)
Molecular weight	22,238 Daltons
Half-life	15 hours
% protein bound	85.6%
Bioavailability	Not specified

II. REVIEW

PREGNANCY

Primary hyperoxaluria and Pregnancy³

- PH1 and pregnancy has been previously reviewed by DPMH.⁴
- Briefly, it is a rare inborn error of glyoxylate metabolism, affecting 1-3 people per one million in North America and Europe, in which oxalate is overproduced and deposited as calcium oxalate in various organs.
- Increased urinary excretion of oxalate leads to kidney injury and end-stage kidney disease if left untreated.
- The median age at diagnosis is approximately 5 to 5.5 years of age but ranges from less than one year of age to over 50 years of age.
- Current medical management involves increased fluid intake; inhibition of calcium oxalate precipitation with potassium citrate, sodium citrate, neutral phosphate, or magnesium oxide; dietary oxalate restriction; pyridoxine supplementation; and lumasiran.
- A recent publication⁵ identified 46 females with PH in the Rare Kidney Stone Consortium over 17 years (from January 1, 2003 to November 30, 2020) who had at least one pregnancy. The data from this cohort study suggested that pregnancy was not associated with kidney function decline in females with PH, and there did not appear to be an increased risk of maternal and infant complications.

Nonclinical Experience⁶

In an embryofetal development study in mice (study 18-DEV-013), pregnant females were administered 0 (0.9% saline), 500, 1000, or 2000 mg/kg nedosiran subcutaneous (SC) injection on gestational day (GD) 6, 8, 10, 12, and 14, and DCR-m355a, the mouse analog of nedosiran, 10 mg/kg on GD 3 and 10. No maternal or developmental toxicity was noted in this mouse study at 500 mg/kg (14.5x the maximum recommended human dose (MHRD)), 1000 mg/kg (29x the

³ Niaudet, P et al. Primary hyperoxaluria. UpToDate. Feb 2023. Topic 6141. Version 54.0

⁴ DPMH Maternal Health review of pregnancy and lactation subsections of labeling for NDA 214103 for Oxumo (lumasiran) by Miriam Dinatale, DO, dated 8/26/20. DARRTS Reference ID: 4661962.

⁵ Miao J, Mehta RA, Norby S, Seide B, Milliner DS, Lieske J, Kattah A. Comparison of clinical features of pregnant and non-pregnant females with primary hyperoxaluria. J Nephrol. 2022 Jul;35(6):1771-1774. doi: 10.1007/s40620-022-01253-9. Epub 2022 Jan 18. PMID: 35041195; PMCID: PMC9694097.

⁶ NDA 215842, Toxicology Written Summary, Module 2.6.6, pages 19-37.

MRHD), or 2000 mg/kg (58x the MRHD). Based on these results, the maternal and developmental NOAEL for nedosiran was 2000 mg/kg.

In an embryofetal development study in New Zealand White (NWZ) rabbits (study 19-L1360-L-018), subcutaneous administration of nedosiran on GD 7, 9, 11, 13, 15, 17, and 19 at doses of 2, 6, or 20 mg/kg/dose resulted in maternal toxicity in the form of body weight loss and lower food consumption in the 6 and 20 mg/kg/dose groups following administration of the first dose. Higher post-implantation loss and lower numbers of live fetuses occurred at ≥ 6 mg/kg/dose (0.7x the MRHD), and fetal cardiovascular and skeletal malformations occurred at 20 mg/kg/dose (2x the MRHD). Based on these results, a dose level of 2 mg/kg/dose (0.2x the MRHD) was considered to be the NOAEL for maternal toxicity and for embryo/fetal development in rabbits. According to the applicant, the human relevance of the rabbit findings is unclear, but based on the lack of similar toxicity observed in mice, the rabbits appear to be particularly sensitive to the bulk chemical and physical properties of nedosiran.

Reviewer comment:

Administration of a mouse-specific, pharmacologically active form of nedosiran to mice did not result in any maternal toxicity or fetal loss. In rabbits, maternal toxicity, post-implantation loss, and fetal malformations were seen; however, nedosiran is not pharmacologically active in rabbits, so the human relevance of these findings is unclear. DPMH discussed findings from the rabbit study 19-L1360-L-018 with the DCN Pharmacology/Toxicology (P/T) and clinical teams. The P/T and clinical teams stated that it is likely that nedosiran has off-target effects in rabbits that will not be seen in humans. Although the maternal toxicity, fetal losses, and fetal malformations occurred in the rabbits treated with 0.2-2x the MRHD of nedosiran, the DCN clinical team noted that it is difficult to compare animal drug exposures to human drug exposures because the animals were dosed more frequently than humans.

Two other siRNAs, patisiran and givosiran, also demonstrated developmental toxicities in the presence of maternal toxicity in reproduction studies in rabbits. Similar to the approach taken for other siRNAs, the DCN P/T and clinical teams recommended including these rabbit data in subsection 8.1 of labeling along. Additionally, the DCN P/T and clinical teams recommended adding a statement that the cause for the embryo-fetal toxicity in rabbits is unclear. Elevating these findings to a higher-level warning/precaution or contraindication in labeling was not recommended. DPMH agrees with the DCN P/T and clinical teams' recommendations.

In a postnatal development study in mice (study 19-L1360-M-027), there were no adverse effects at any dose level.

The reader is referred to the Pharmacology/Toxicology review by Dr. Belay Tesfamariam for further details.

Review of Pharmacovigilance Database

There was one pregnancy reported during the nedosiran clinical development program (study DCR-PHXC-301).⁷ The participant received nedosiran from (b) (6) to (b) (6). When the participant received nedosiran on (b) (6), her screening urine pregnancy test was negative. The participant reported forgetting to take her oral contraceptive for 2-3 days in (b) (6) and using condoms inconsistently. On (b) (6), the participant took a home urine pregnancy test because her menses were late, and the pregnancy test was negative. On (b) (6), the participant had a positive serum beta-hcg pregnancy test and discontinued nedosiran. The participant experienced a spontaneous abortion (SAB) at approximately 5 weeks of pregnancy. The SAB was considered unrelated to nedosiran.

Review of Literature

Applicant's review:

The applicant did not submit a literature review related to nedosiran and pregnancy.

DPMH review:

DPMH conducted a search of published human studies in PubMed and Embase, using the search terms “small interfering RNA” OR “lumasiran” OR “nedosiran” AND “pregnancy,” “pregnancy outcomes,” “birth defects,” “stillbirth,” and “spontaneous abortion.” No publications were found.

DPMH also searched Micromedex,⁸ Reprotox,⁹ TERIS,¹⁰ and Shepard's¹¹ for siRNA and lumasiran. No information was found.

Reviewer comment:

In terms of clinical data, there was only one pregnancy reported by the applicant during the development program for nedosiran, which resulted in an SAB. DPMH determined that the SAB was not likely related to nedosiran's effect on the fetus for the following reasons:

- Based on the timing of the positive pregnancy test, conception likely occurred approximately 2 weeks after the last dose of nedosiran when the drug would have been eliminated from plasma based on the pharmacokinetic (PK) half-life, assuming elimination is complete after 6 half-lives = 15 hours x 6 half-lives = 90 hours.*
- The asialoglycoprotein receptor, required for uptake of GalNAc-conjugated siRNAs such as nedosiran, is not expressed appreciably in the human fetal liver until near*

⁷ Applicant's 120-Day Safety Update for NDA 215842. SN 0011, submitted on 1/27/2023.

⁸ Truven Health Analytics information, <http://www.micromedexsolutions.com>. Accessed 3/27/2023.

⁹ Reprotox Website: www.Reprotox.org. Accessed 3/27/2023.

¹⁰ TERIS database, Truven Health Analytics, Micromedex Solutions. Accessed 3/27/2023.

¹¹ Shepard's database, Truven Health Analytics, Micromedex Solutions. Accessed 3/27/2023.

- delivery.^{12,13} If the drug target is not expressed in the developing human fetal liver, then it is unlikely that the drug will result in fetal toxicity.
- The pre- and post-natal development studies in mice demonstrated that the active mouse analog of nedosiran, DCR-m355a, reduced maternal expression of LDHA but did not change LDHA expression in pup livers and did not cause embryonic toxicity.¹⁴ This suggests that nedosiran does not reach its target in fetal mice.

See the Discussion and Conclusions section below for recommendations regarding labeling of subsection 8.1.

LACTATION

Nonclinical Experience

In study 19-L1360-M-027,¹⁵ nedosiran was administered via SC injection to female mice on Lactation Days (LD) 1, 8, 15, and 20, and DCR-m355a (the mouse analog of nedosiran) was administered via SC injection on LD 5, 12, and 19. According to the applicant, “There was no significant change in Ldha mRNA expression in PND 4 pup livers suggesting no meaningful fetal or lactation exposure to DCR-m355a.”¹⁶

Reviewer comment:

DPMH discussed the reported findings from study 19-L1360-M-027 with the P/T Team. The P/T Team agrees with the applicant’s conclusion that the study suggests that lactation exposure in animals appears to be clinically insignificant. Whether this is true in humans is unknown.

The reader is referred to the Pharmacology/Toxicology review by Dr. Belay Tesfamariam for further details.

Review of Pharmacovigilance Database

There are no reports of lactation in the clinical studies.

Review of Literature

Applicant’s review:

The applicant did not submit a literature review of nedosiran and lactation.

DPMH review:

¹² Yoshida S, Furuhashi M, Suganuma N. Expression of asialoglycoprotein receptor in human fetal liver. *Endocr J*. 1999 Feb;46(1):67-73. doi: 10.1507/endocrj.46.67. PMID: 10426569.

¹³ Haase N, Foster DJ, Cunningham MW, Bercher J, Nguyen T, Shulga-Morskaya S, Milstein S, Shaikh S, Rollins J, Golic M, Herse F, Kräker K, Bendix I, Serdar M, Napieczynska H, Heuser A, Gellhaus A, Thiele K, Wallukat G, Müller DN, LaMarca B, Dechend R. RNA interference therapeutics targeting angiotensinogen ameliorate preeclamptic phenotype in rodent models. *J Clin Invest*. 2020 Jun 1;130(6):2928-2942. doi: 10.1172/JCI99417. PMID: 32338644; PMCID: PMC7260005.

¹⁴ NDA 215842, Toxicology Written Summary, Module 2.6.6, page 39.

¹⁵ NDA 215842, Toxicology Written Summary, Module 2.6.6, pages 37-40.

¹⁶ NDA 215842, Toxicology Written Summary, Module 2.6.6, page 39.

DPMH conducted a search for published human studies in PubMed and Embase, using the search terms: “small interfering RNA” OR “lumasiran” OR “nedosiran” AND “lactation” and “breastfeeding.” No publications were found.

In addition, DPMH conducted a search for siRNA and lumasiran in Micromedex, Hale’s *Medications and Mothers’ Milk*,¹⁷ Reprotox, the Drugs and Lactation Database (LactMed),¹⁸ and Briggs *Drugs in Pregnancy and Lactation: A Reference Guide to Fetal and Neonatal Risk*.¹⁹ No information was found.

Reviewer comment:

In studies in mice, exposure to nedosiran via milk was insignificant. There are no human data on the presence of nedosiran in milk or the effects of nedosiran on milk production or on breastfed infants. See the Discussion and Conclusions section below for labeling recommendations for subsection 8.2.

FEMALES AND MALES OF REPRODUCTIVE POTENTIAL

Nonclinical Experience²⁰

In a fertility study in mice (study 19-DEV-001), there were no nedosiran- or DCR-m355a (the mouse analogue of nedosiran)-related effects on any male mating and fertility parameter, sperm motility, sperm density, or sperm morphology, reproductive organ weights (testes, epididymides, prostate, and seminal vesicles), and no macroscopic observations detected at necropsy examination. In female mice, there were no nedosiran- or DCR-m355a-related effects on estrous cycling, mating or fertility or ovarian and or uterine parameters, and there were no macroscopic observations.

In a 39-week repeat dose study with an 8-week recovery period in monkeys (study 17-DEV-005), dose-related minimal to moderate basophilic hypertrophy of the testes was noted. During the 8-week recovery period, there was minimal but ongoing reversibility of the hypertrophy in the testes.

Reviewer comment:

DPMH discussed the reported findings from study 17-DEV-005 with the DCN P/T team. Per the P/T team, “the findings of basophilic hypertrophy of macrophages in the testes of sexually mature monkeys may not suggest impairment of fertility. In the dedicated study on fertility and embryonic development in male mice (19-DEV-001), there were no nedosiran- or DCR-m355a- (mouse active analog) related effects on mating and fertility parameters, sperm motility, sperm density, sperm morphology, or reproductive organ weights. The findings in the monkey testes were still evident during an 8-week recovery period, which may be due to complete knockdown of LDHA mRNA for 4-6 weeks and may

¹⁷ Hale, Thomas W. *Hale’s Medications & Mothers’ Milk* 2021: A Manual of Lactational Pharmacology. 19th ed. New York: Springer Publishing Company, 2020. www.halesmeds.com

¹⁸ Drugs and Lactation Database (LactMed). Accessed 7/26/22.

¹⁹ Briggs, Gerald G., Craig V. Towers, and Alicia B. Forinash. *Briggs Drugs in Pregnancy and Lactation: a Reference Guide to Fetal and Neonatal Risk*. 12th edition. Philadelphia, PA: Lippincott Williams & Wilkins, 2021. Print.

²⁰ NDA 215842, Toxicology Written Summary, Module 2.6.6, pages 23-25.

need several weeks to months for recovery.” The P/T team did not recommend inclusion of the monkey findings in labeling. DPMH agrees.

In an *in vitro* study with human hepatocytes (study 18-DEV-007),²¹ nedosiran did not inhibit or induce CYP enzymes.

The reader is referred to the Pharmacology/Toxicology review by Dr. Belay Tesfamariam for further details.

Review of Pharmacovigilance Database

The applicant did not report any cases of infertility in clinical trials conducted for nedosiran.

Review of Literature

Applicant’s review:

The applicant did not submit a literature review of nedosiran and fertility.

DPMH review:

DPMH conducted a literature search for studies in humans using PubMed and Embase, using the search terms “small interfering RNA” OR “lumasiran” OR “nedosiran” AND “fertility,” “contraception,” “oral contraceptives,” and “infertility.” DPMH also conducted a search in Micromedex, Reprotox, and TERIS.²² No publications were identified.

Reviewer comment:

See the Discussion and Conclusions section regarding the labeling of subsection 8.3.

III. DISCUSSION AND CONCLUSIONS

Pregnancy

There was one report of a pregnancy during the clinical development of nedosiran, but the information from that pregnancy was insufficient to determine any clinical risks with use during pregnancy. In studies in mice, there were no adverse effects on pregnancy or embryo-fetal development related to nedosiran in mice at 14.5x to 58x the MRHD; however, in rabbits, there was evidence of maternal toxicity (weight loss at 0.2x the MRHD) and developmental toxicity (fetal loss at 0.7x the MRHD and cardiovascular and skeletal abnormalities at 2x the MRHD). Given the uncertain clinical relevance of these rabbit findings and the difficulty involved with translating animal drug exposures to human exposures, DPMH agrees with the DCN team that these findings do not warrant an embryo-fetal toxicity warning in section 5 of the labeling. DPMH recommends labeling nedosiran in a similar fashion to the other drugs in the class that demonstrated maternal toxicity in rabbits, namely patisiran (maternal toxicity with associated developmental toxicity- embryofetal mortality and reduced fetal body weight) and givosiran (maternal toxicity with associated decreased body weight gain, postimplantation loss, and skeletal variations), which means including text related to the animal findings in labeling under the Risk Summary and Animal Data in subsection 8.1.

²¹ NDA 215842, Reports of Hepatic Metabolism and Drug Interaction Studies, Module 5.3.2.2, page 48.

²² TERIS database, Truven Health Analytics, Micromedex Solutions. Accessed 3/27/2023.

Over a period of 17 years, 46 pregnancies have been reported in the literature in females with PH. Given the rare nature of this condition, a pregnancy registry would not be feasible for this population. However, since there are no human data with use of nedosiran in pregnant women, DPMH recommends issuing a post-marketing requirement to conduct a single-arm pregnancy safety study to collect information regarding the use of nedosiran in pregnant women.

Lactation

There is no information regarding the use of nedosiran in lactating humans. Based on the drug's large molecular weight, it is unlikely that nedosiran will accumulate in human milk. In mice, the exposure to nedosiran through milk was insignificant. Given that no information has been identified that would preclude breastfeeding, DPMH recommends using the standard risk/benefit language in subsection 8.2 of nedosiran labeling.

Given that PH1 is a rare disease, it would not be feasible to conduct a lactation study in this population. DPMH discussed with DCN the possibility of issuing a PMR to conduct a clinical lactation study in healthy volunteers. During that discussion, it was decided that it would be difficult to enroll breastfeeding individuals in a clinical lactation study due the long pharmacodynamic (PD) half-life of nedosiran. Per the DCN Clinical Pharmacology team, "The estimated PD half-life of nedosiran is very long. While not directly measured, the applicant indicated that when patients rolled over from the single dose Phase 1 study (-101) to the open label study (-301), not all patients had returned to at least 80% of pretreatment urinary oxalate baseline at the time of rollover,²³ which was ranging between 10-23 months after the single dose in (-101)." This long PD half-life would likely preclude women from continuing to breastfeed after receiving a dose of nedosiran. DPMH and DCN think that very few healthy, lactating individuals who had weaned their infants would be willing to enroll in a clinical lactation study that would require them to pump breast milk for the duration of the study given the very long PD half-life of nedosiran.

Females and Males of Reproductive Potential

There are no data on nedosiran and the effects of fertility or contraception in humans. The study in mice did not demonstrate reproductive toxicity. The study in monkeys demonstrated testicular hypertrophy that was minimally reversible over the course of 8-weeks. Because the testicular hypertrophy seen in monkeys is not likely to impact fertility, there will not be an embryo-fetal toxicity warning in labeling, there is no drug-drug interaction between nedosiran and hormonal contraceptives and labeling for other drugs in the class do not contain subsection 8.3, DPMH recommends omitting subsection 8.3.

IV. LABELING RECOMMENDATIONS

DPMH proposed text for labeling of subsections 8.1 and 8.2 (see below). DPMH discussed our labeling recommendations with DCN on 4/26/23. DPMH refers to the final NDA action for final labeling.

²³ IND 132214 for DCRPHXC (nedosiran). Sponsor Response to Type A FDA preliminary comments Ref ID: 4926959, SN 0105, submitted 2/4/2022.

Appendix A: Information for other drugs in the same class

Drug Information	Warnings and Precautions	Subsection 8.1	Subsection 8.2	Subsection 8.3	PMR Study
Onpattro (patisiran) -First in class -Approved 2018. -indicated for the treatment of polyneuropathy of hereditary transthyretin-mediated amyloidosis. - double-stranded siRNA that causes degradation of mutant and wild-type TTR mRNA through RNA interference, which results in a reduction of serum TTR protein and TTR protein deposits in tissues.	None	<u>Risk Summary</u> There are no available data on ONPATTRO use in pregnant women to inform a drug-associated risk of adverse developmental outcomes. ONPATTRO treatment leads to a decrease in serum vitamin A levels, and vitamin A supplementation is advised for patients taking ONPATTRO. Vitamin A is essential for normal embryofetal development; however, excessive levels of vitamin A are associated with adverse developmental effects. The effects on the fetus of a reduction in maternal serum TTR caused by ONPATTRO and of vitamin A supplementation are unknown [see <i>Clinical Pharmacology</i> (12.2), <i>Warnings and Precautions</i> (5.2)]. In animal studies, intravenous administration of patisiran lipid complex (patisiran-LC) to pregnant rabbits resulted in developmental toxicity (embryofetal mortality and reduced fetal body weight) at doses that were also associated with maternal toxicity. No adverse developmental effects were observed when patisiran-L or a rodent-specific (pharmacologically active) surrogate were administered to pregnant rats (<i>see Data</i>). <u>Data</u> <i>Animal Data</i> Intravenous administration of patisiran LC (0, 0.15, 0.50, or 1.5 mg/kg) or a rodent-specific (pharmacologically active) surrogate (1.5 mg/kg) to female rats every week for two weeks prior to mating and continuing throughout organogenesis resulted in no adverse effects on fertility or embryofetal development. Intravenous administration of patisiran-LC (0, 0.1, 0.3, or 0.6 mg/kg) to pregnant rabbits every week during the period of organogenesis produced no adverse effects on embryofetal development. In a separate study, patisiran-LC (0, 0.3, 1, or	8.2 Lactation <u>Risk Summary</u> There is no information regarding the presence of ONPATTRO in human milk, the effects on the breastfed infant, or the effects on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for ONPATTRO and any potential adverse effects on the breastfed infant from ONPATTRO or from the underlying maternal condition. In lactating rats, patisiran was not detected in milk; however, the lipid components (DLin-MC3-DMA and PEG2000-C-DMG) were present in milk.	None	Yes. There is an ongoing pregnancy surveillance study.

		2 mg/kg), administered to pregnant rabbits every week during the period of organogenesis, resulted in embryofetal mortality and reduced fetal body weight at the mid and high doses, which were associated with maternal toxicity. Intravenous administration of patisiran-LC (0, 0.15, 0.50, or 1.5 mg/kg) or a rodent-specific surrogate (1.5 mg/kg) to pregnant rats every week throughout pregnancy and lactation resulted in no adverse developmental effects on the offspring.			
Givlaari (givosiran) -Approved 2019 -indicated for the treatment of adults with acute hepatic porphyria - double-stranded small interfering RNA that causes degradation of aminolevulinate synthase 1 (ALAS1) mRNA in hepatocytes through RNA interference, reducing the elevated levels of liver ALAS1 mRNA.	None	8.1 Pregnancy Risk Summary In animal reproduction studies, subcutaneous administration of givosiran to pregnant rabbits during the period of organogenesis resulted in adverse developmental outcomes at doses that produced maternal toxicity (see Data). There are no available data with GIVLAARI use in pregnant women to evaluate a drug-associated risk of major birth defects, miscarriage, or adverse maternal or fetal outcomes. Consider the benefits and risks of GIVLAARI for the mother and potential adverse effects to the fetus when prescribing GIVLAARI to a pregnant woman. <u>Data</u> <i>Animal Data</i> In an embryo-fetal development study in pregnant rabbits, givosiran was administered subcutaneously at doses of 0.5, 1.5, and 5 mg/kg/day during organogenesis (gestational days 7-19) or 20 mg/kg as a single administration on gestation day 7. Administration of givosiran was maternally toxic based on decreased body weight gain at all dose levels tested and resulted in increased postimplantation loss starting at 1.5 mg/kg/day. An increased incidence of skeletal variations of the sternebrae was observed at 20 mg/kg. The 1.5 mg/kg/day dose in rabbits is 5 times the maximum recommended human dose (MRHD) of 2.5 mg/kg/month normalized to 0.089 mg/kg/day, based on body surface area. In a combined fertility	8.2 Lactation Risk Summary There are no data on the presence of GIVLAARI in human milk, the effects on the breastfed child, or the effects on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for GIVLAARI and any potential adverse effects on the breastfed child from GIVLAARI or from the underlying maternal condition.	None	No

		and embryo-fetal development study in female rats, givosiran was administered subcutaneously at doses of 0.5 to 5 mg/kg/day during organogenesis (gestational days 6-17). The 5 mg/kg/day dose (9 times the normalized MRHD based on body surface area) was associated with a skeletal variation (incomplete ossification of pubes) and produced maternal toxicity. In a pre- and postnatal development study, givosiran administered subcutaneously to pregnant rats On gestation days 7, 13, and 19 and postnatal days 6, 12, and 18 at doses up to 30mg/kg did not produce maternal toxicity or developmental effects in the offspring.			
Leqvio (inclisiran) -Approved 12/2021 -Indicated as an adjunct to diet and maximally tolerated statin therapy for the treatment of adults with heterozygous familial hypercholesterolemia (HeFH) or clinical atherosclerotic cardiovascular disease (ASCVD), who require additional lowering of low-density lipoprotein cholesterol (LDL-C). -siRNA directed to to PCSK9 (proprotein convertase subtilisin kexin type 9) mRNA	None	8.1 Pregnancy <u>Risk Summary</u> Discontinue LEQVIO when pregnancy is recognized. Alternatively, consider the ongoing therapeutic needs of the individual patient. Inclisiran increases LDL-C uptake and lowers LDL-C levels in the circulation, thus decreasing cholesterol and possibly other biologically active substances derived from cholesterol; therefore, LEQVIO may cause fetal harm when administered to pregnant patients based on the mechanism of action [see <i>Clinical Pharmacology</i> (12.1)]. In addition, treatment of hyperlipidemia is not generally necessary during pregnancy. Atherosclerosis is a chronic process and the discontinuation of lipid-lowering drugs during pregnancy should have little impact on the outcome of long-term therapy of primary hyperlipidemia for most patients. There are no available data on the use of LEQVIO in pregnant patients to evaluate for a drug-associated risk of major birth defects, miscarriage or adverse maternal or fetal outcomes. In animal reproduction studies, no adverse developmental effects were observed in rats and rabbits with subcutaneous administration of inclisiran during organogenesis at doses up to 5 to 10 times the maximum	8.2 Lactation <u>Risk Summary</u> There is no information on the presence of inclisiran in human milk, the effects on the breastfed infant, or the effects on milk production. Inclisiran was present in the milk of lactating rats in all dose groups. When a drug is present in animal milk, it is likely that the drug will be present in human milk (<i>see Data</i>). Oligonucleotide-based products typically have poor oral bioavailability; therefore, it is considered unlikely that low levels of inclisiran present in milk will adversely impact an infant's development during lactation. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for LEQVIO and any potential adverse effects on the breastfed infant from LEQVIO or from the underlying maternal condition.	None	No

		recommended human dose (MRHD) based on body surface area (BSA) comparison (<i>see Data</i>). No adverse developmental outcomes were observed in offspring of rats administered inclisiran from organogenesis through lactation at 5 times the MRHD based on BSA comparison (<i>see Data</i>).	<u>Data</u> In lactating rats, inclisiran was detected in milk at mean maternal plasma:milk ratios that ranged between 0.361 and 1.79. However, there is no evidence of systemic absorption in the suckling rat neonates.		
Oxlumo (lumasiran) -approved 11/2020 -indicated for the treatment of primary hyperoxaluria type 1 (PH1) to lower urinary and plasma oxalate levels in pediatric and adult patients -reduces levels of glycolate oxidase (GO) enzyme by targeting the hydroxyacid oxidase 1 (HAO1) messenger ribonucleic acid (mRNA) in hepatocytes through RNA interference.	None	8.1 Pregnancy <u>Risk Summary</u> There are no available data with the use of OXLUMO in pregnant women to evaluate a drug-associated risk of major birth defects, miscarriage, or adverse maternal or fetal outcomes. No adverse effects on pregnancy or embryo-fetal development related to OXLUMO were observed in rats at 45 times and in rabbits at 90 times the maximum recommended human dose in women (<i>see Data</i>). <u>Data</u> <u>Animal Data</u> In an embryo-fetal development study in pregnant rats, lumasiran was administered subcutaneously at doses of 3, 10, and 30 mg/kg/day during organogenesis (gestational days 6-17). Administration of lumasiran resulted in no effects on embryo-fetal survival or fetal body weights and no lumasiran-related fetal malformations were observed. The 30 mg/kg/day dose in rats is 45 times the maximum recommended human dose (MRHD) for women of 3 mg/kg/month normalized to 0.1 mg/kg/day, based on body surface area. In an embryo-fetal development study in female rabbits, lumasiran was administered subcutaneously at doses of 3, 10, and 30 mg/kg/day during organogenesis (gestational days 7-19). There were decreases in maternal food consumption and decreases in maternal body weight gains at doses $\geq$ 3 mg/kg/day. There were no lumasiran-related fetal findings identified at doses up to 30 mg/kg/day (90 times the normalized MRHD based on body surface area). In a postnatal development study, lumasiran administered	8.2 Lactation <u>Risk Summary</u> There are no data on the presence of OXLUMO in human milk, the effects on the breastfed child, or the effects of the drug on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for OXLUMO and any potential adverse effects on the breastfed child from OXLUMO or from the underlying maternal condition.	None	No

		subcutaneously to pregnant female rats on gestational days 7, 13, 19 and on lactation days 6, 12, and 18 through weaning at doses up to 50 mg/kg did not produce maternal toxicity or developmental effects in the offspring.			
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/s/

KATHERINE G KRATZ
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MIRIAM C DINATALE
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LYNNE P YAO
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Clinical Inspection Summary

Date	April 20, 2023
From	Tina Chang, M.D., Medical Officer Good Clinical Practice Assessment Branch (GCPAB) Division of Clinical Compliance Evaluation (DCCE) Office of Scientific Investigations (OSI)
To	Kirtida Mistry, M.D., Clinical Reviewer Aliza Thompson, M.D., M.S., Deputy Director Brian Proctor, Senior Regulatory Project Manager Division of Cardiology and Nephrology (DCN)
NDA #	215842
Applicant	Novo Nordisk Inc.
Drug	Rivfloza (nedosiran)
NME (Yes/No)	Yes
Proposed Indication(s)	Treatment of primary hyperoxaluria Type 1
Consultation Request Date	December 5, 2022
Summary Goal Date	July 28, 2023
Action Goal Date	September 30, 2023
PDUFA Date	September 30, 2023

I. OVERALL ASSESSMENT OF FINDINGS AND RECOMMENDATIONS

Dr. John Lieske was inspected in support of NDA 215842 covering one clinical study, DCR-PHXC-201. Despite some minor protocol deviations at Dr. Lieske's clinical site, the study appears to have been conducted adequately, and the data generated by this clinical investigator (CI) site appears acceptable in support of this NDA.

II. BACKGROUND

NDA 215842 was submitted in support of the use of Rivfloza (nedosiran) for treatment of primary hyperoxaluria Type 1, a rare disease characterized by a specific enzyme deficiency causing an overproduction of liver oxalate leading to an increase of urinary oxalate excretion and kidney stones. The sponsor submitted a single Phase 3 study to support this NDA. The following briefly describes the protocol:

DCR-PHXC-201 "A Phase 2 Placebo-Controlled, Double-Blind, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of DCR-PHXC Solution for Injection (subcutaneous use) in Patients with Primary Hyperoxaluria"

Protocol DCR-PHXC-201 was a Phase 2, randomized, double-blind, placebo-controlled efficacy and safety study of nedosiran for the treatment of primary hyperoxaluria in subjects with genetically confirmed Type 1 or Type 2 primary hyperoxaluria (PH1, PH2), at least 6 years of age and older. The primary objective was to assess the efficacy of nedosiran in

reducing urinary oxalate (Uox) burden in patients with primary hyperoxaluria (PH).

Sites: The study was conducted at 19 sites in France, Spain, Italy, Netherlands, Germany, United Kingdom, Australia, United States, Canada, Lebanon, and Japan.

Subjects: A total of 35 subjects were randomized (i.e., 23 subjects received nedosiran and 12 received placebo) and 33 subjects completed the study (i.e., 22 who received nedosiran and 11 who received placebo completed the study).

Study initiation and completion dates: 28 October 2019 (first patient, first visit); 29 June 2021 (last patient, last visit)

Database lock date of Part 2 of the study: 23 July 2021

Subjects with primary hyperoxaluria were randomly assigned in a 2:1 ratio to receive nedosiran or placebo (0.9% saline) once a month on Days 1, 30, 60, 90, 120, 150, and 180 as a subcutaneous injection into the abdomen or thigh over a 6-month treatment period. Adults and adolescents (aged 12-17 years) weighing ≥ 50 kg were to receive a dose of 170 mg monthly, while adolescents weighing less than 50 kg would receive a lower dose of 136 mg. The measurement of oxalate in the urine was via a 24-hour urine collection. The total study time for each subject was approximately 7 months.

Primary Efficacy Endpoint: 24-hour urinary oxalate (Uox) excretion, measured as the area under the curve (AUC) of percent change from baseline over the last 3 months of treatment from Day 90 to Day 180 and adjusted per 1.73 m^2 body surface area.

The measurement of oxalate in the urine was via 24-hour urine collection. Subjects reported the collection start and stop date times on a 24-hour collection schedule worksheet. During screening, subjects performed two 24-hour collections at home. The average of these two screening collections was determined to be the baseline result. Collection of the two 24-hour urine samples during Screening should have occurred on consecutive days; however, should that not have been feasible, no more than 8 days were to elapse between collections. Subjects were provided urine collection devices to store the urine in a refrigerator until pick-up by courier. The urine was then sent to (b) (4). The (b) (4) reports were sent to the clinical investigator sites electronically after database lock (23 July 2021). Ongoing assessment of Uox continued at select visits throughout the study and should have been within ± 7 days of the scheduled study visit; except for Day 180 samples, which should have been collected within the 7 days prior to the visit. The AUC endpoint was measured over the last 3 months (Day 90, Day 120, Day 150, and Day 180) of treatment.

Rationale for Site Selection

The CI Dr. John Lieske (Site #101) was selected for GCP inspection using a risk-based approach that considered numbers of enrolled subjects (7 out of 35 enrolled subjects), large treatment effect on the primary endpoint, and prior inspectional history.

III. RESULTS (by site):

1. Dr. John Lieske

Site #101

Mayo Clinic

200 First Street SW

Rochester, MN 55905-0001

PDUFA Inspection Dates: February 27- March 3, 2023

At this site, 8 subjects were screened, 7 were randomized, and 6 subjects completed the study. Subject (b) (6) (randomized to placebo) was discontinued due to an elevated creatinine.

Source documents were reviewed for all 8 subjects that were screened. Records reviewed included, but were not limited to, protocol versions, FDA 1572s, financial disclosures, informed consent forms, clinical source records, case report forms, adverse event forms, protocol deviations, eligibility records, investigational product accountability, study staff delegation and training, sponsor/monitor correspondence, primary efficacy endpoint source records and sponsor data listings. Subjects' source records for the primary endpoint consisted of printed laboratory results, paper worksheets reporting start and stop times for 24-hour urine collection, and electronic medical records reporting subject's height and weight.

The source records documenting the raw values for the 24-hour urinary oxalate lab results from the central laboratory for all timepoints (i.e., screening x 2, Day 90, Day 120, Day 150, and Day 180) and the height and weight for each subject were verified against the sponsor's data line listings for all 7 randomized subjects. No discrepancies were noted.

For 2 of the 7 randomized subjects, 3 adverse events were not reported to the sponsor in accordance with the study protocol. Subject (b) (6) randomized to nedosiran reported soreness at the injection site and mild facial flushing on Day 1. Subject (b) (6) randomized to nedosiran reported during his Day 60 visit that he felt a rapid heart rate that started following the Day 30 dose and persisted for a few days. No further details were available from the source records.

Reviewer's comment: Facial flushing and rapid heart rate are not included in the proposed labeling by the sponsor and are presented here for the Review Divisions' consideration.

{See appended electronic signature page}

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CC:

Central Doc. Rm.
Review Division /Division Director/
Review Division /Medical Team Leader/
Review Division /Project Manager/
Review Division/MO/
OSI/Office Director/
OSI/DCCE/ Division Director/
OSI/DCCE/Branch Chief/
OSI/DCCE/Team Leader/
OSI/DCCE/GCP Reviewer/
OSI/ GCP Program Analysts/
OSI/Database PM/

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

SUYOUNG T CHANG
04/20/2023 12:14:29 PM

PHILLIP D KRONSTEIN
04/20/2023 12:43:34 PM

JENN W SELLERS
04/20/2023 01:59:51 PM

Memorandum of Immunogenicity Review

IND:	IND 132214, SDN 117
IND Phase:	3
Subject:	Immunogenicity Consult Review
Regulatory Pathway:	505(b)(1) approval pathway
Submission Date:	09/21/2022
Review/Revision Date:	11/14/2022
Primary Reviewer:	Faruk Sheikh, PhD, OPQ/OBP/DBRR II
Secondary Reviewer:	Harold Dickensheets, PhD, OPQ/OBP/DBRR II
RPM:	Brian Proctor
Responsible Division:	CDER/OND/ODEI/ Division of Cardiology and Nephrology
Consults:	Immunogenicity
Applicant:	Dicerna Pharmaceuticals, Inc.
Product:	DCR-PHXC (Nedosiran)
Route of Admin:	Subcutaneous injection
Dose regimen:	(b) (4) for ≥ 12 years of age weighing ≥ 50 kg (b) (4) for ≥ 12 years of age weighing < 50 kg
Proposed Indication:	Treatment of Oxaluria
Action Due Date:	11/16/2022

RECOMMENDATION: The validation of anti-Nedosorpin antibody binding assay is acceptable. This validated assay could be used in clinical sample analysis.

BACKGROUND:

Dicerna Pharmaceuticals, Inc., a wholly owned subsidiary of Novo Nordisk submitted IND 132214 on 26 February 2018 for DCR-PHXC, small interfering RNA (siRNA) Solution for Injection being developed for the treatment of primary hyperoxaluria (PH). The DCR-PHXC-siRNA has received modifications at various levels of the molecule, and the impact of these modifications in product immunogenicity is unknown. To evaluate the immunogenicity of this product, on January 20, 2021 (SDN 78) the Sponsor submitted their immunogenicity assessment plan including a plan for anti-drug antibody (ADA) assay method validation, which was found to be acceptable. However, OBP provided additional recommendations for the development and validation of the immunogenicity assays, including revisions to the immunogenicity sampling schedule for proposed Phase 2 and future studies (review ref: [4753030](#)). The recommendations were communicated to the Sponsor in an advice letter on 06/06/2022 (DARRTS ref: [4994860](#)).

In the current submission, the Sponsor submitted the validation reports of an ELISA method for the detection of anti-Nedosiran antibodies in human serum ([SDN 117, 09/21/2022](#)). In addition,

the Sponsor provided responses to OBP immunogenicity comments described above in this current submission. The assay validation reports and the responses to immunogenicity comments are reviewed below.

RECOMMENDED COMMENTS FOR SPONSOR: None at this time.

REVIEW:

A. Validation of an anti-Nedosiran antibody assay in human serum

1. Validation Title: Validation of an ELISA Method for the Detection of Anti-Nedosiran Antibodies in Human Serum – New Positive Control.
2. Project Code: RVZQ2
3. Assays validated: Screening, confirmation, and titration
4. Matrix: Human serum
5. Negative Control (NC):
Nine individual normal sample lots were pooled to use as NC sample (Lot numbers, and expiration dates are provided).
6. Positive Control (PC) antibody: Protein A purified anti-Nedosiran antibody (Lot: 45410C). This is a rabbit polyclonal antibody raised against nedosiran, a GalNAc-conjugated siRNA. This antibody was used for preparation of the low PC (LPC), mid PC (MPC) and high PC (HPC) controls.
7. Positive control samples: Positive control samples were prepared by spiking PC antibodies into the NC pool at 98.0 ng/mL (screening LPC), 124 ng/mL (confirmatory LPC), 400 ng/mL (MPC) and 1600 ng/mL (HPC).

Reviewer comment: The LPC concentration was determined based on the sensitivity values obtained during sensitivity assays targeting a 1% rejection rate. The concentration of the confirmatory LPC (cLPC) was adjusted relative to the sensitivity of the confirmation assay, which is usually equal or higher than the screening assay LPC concentration (sLPC) ([RQSW2: Method Validation Report § Sensitivity](#)). The sLPC and cLPC concentrations were used appropriately.

8. MRD: 1:10 in dilution buffer or inhibition solution.

Reviewer comment: The Sponsor did not provide data justifying the use of MRD 10 for the development and validation of this binding antibody assay. According to the FDA guidance¹, the samples are diluted to maintain a reasonable ability of the assay to detect antibodies by an assay and the MRD typically ranges from 1:5 to 1:100. The validation studies reported in this memo suggest that the contribution of non-specific signals is minimum and reasonable to detect anti-drug antibodies by this assay at MRD 10, therefore, MRD 10 is acceptable.

¹ Immunogenicity Testing of Therapeutic Protein Products —Developing and Validating Assays for Anti-Drug Antibody Detection (2019) at <https://www.fda.gov/RegulatoryInformation/Guidances/default.htm>.

9. Method used: ELISA, Capture Reagent - NH₂-Nedosiran (NH₂-DCR-L1360)
Detection Reagent - HRP-Labeled Protein A/G

Method Description: The anti-Nedosiran antibodies in human serum are detected using an ELISA immunoassay. In this assay, NH₂-Nedosiran is coated on plates, washed and after which unoccupied binding sites on the plates are blocked with blocking buffer (1.00 M NaCl in 1X PBS with 3% Dry Milk) at 26 °C ± 2 °C for 1 to 3 hours on a plate shaker at 600 rpm. Human serum samples, positive and negative control samples are diluted in dilution buffer at MRD and then added to the blocked plate and incubated at 26 °C ± 2 °C for 90 minutes. After ADA binds to the immobilized Nedosiran, the plates are washed, and the bound antibodies are detected by adding HRP-labeled protein A/G. The plates are washed, and the ADA-bound HRP-labeled protein A/G is detected by the addition of TMB substrate. The reaction is stopped with 1N HCl and measured at OD₄₅₀ nM, providing a signal that is proportional to the amount of ADA present in the human serum.

10. Validation studies:

10.1 Screening Assay Cut Point:

- 1) Serum samples from 50 drug-naïve normal human donors were used. Data were compiled from 6 independent assays performed by 4 analysts (total of 300 individual sample values).
- 2) The screening and titration assay cut points were set using signal to noise ratio (SNR=sample mean signal/NC mean signal).
- 3) Biological and statistical outliers were selected and removed and tested for normality.
- 4) The non-transformed data were not normally distributed; therefore, the data were evaluated using log transformation and tested for normality (Shapiro-Wilk test). After removal of outliers, it was found that the log-transformed data was not normally distributed (statistically significant p-value of <0.05 for Panel 6).
- 5) Therefore, the nonparametric approach was utilized using the non-transformed pooled data for the determination of screening assay cut point.
- 6) These data were evaluated for statistical outliers using Tukey's Outlier Filter. The median, lower and upper statistical outlier limits, and the resulting statistical outliers are provided in Table 5 and displayed graphically in Figure 3. Eleven results were determined to be statistical outliers and were excluded from subsequent analyses (Table 5 and shown in Fig 3 below).

Table 5
Statistical Outlier Determination Using Non-Transformed Pooled Data
Analysis was performed using data from Panels 1-6 (New Panel 5).

Panel	Run ID	Sample	Outlier					Lower Limit	Upper Limit
			SNR	Q1	Median	Q3	IQR		
1	3RQSW2	40	2.086	0.866	0.983	1.132	0.266	0.468	1.531
2	5RQSW2	6	1.910	0.866	0.983	1.132	0.266	0.468	1.531
2	7RQSW2	40	1.889	0.866	0.983	1.132	0.266	0.468	1.531
3	9RQSW2	6	1.749	0.866	0.983	1.132	0.266	0.468	1.531
3	11RQSW2	40	1.661	0.866	0.983	1.132	0.266	0.468	1.531
4	13RQSW2	6	2.261	0.866	0.983	1.132	0.266	0.468	1.531
4	13RQSW2	8	1.772	0.866	0.983	1.132	0.266	0.468	1.531
4	15RQSW2	40	2.097	0.866	0.983	1.132	0.266	0.468	1.531
5	31RQSW2	40	2.199	0.866	0.983	1.132	0.266	0.468	1.531
6	27RQSW2	6	1.757	0.866	0.983	1.132	0.266	0.468	1.531
6	23RQSW2	40	1.915	0.866	0.983	1.132	0.266	0.468	1.531

- 7) The non-transformed data were evaluated to assess whether the medians were equal across the panels using Friedman's test. The statistical test on equivalence of medians indicated that there was a statistically significant difference between the medians across the panels (p-value = 0.005).
- 8) The non-transformed data were also evaluated to assess whether the variances were equal across the panels using Levene's test. The test results indicated that there was no statistical evidence of a difference in the variances across the panels (p-value = 0.135).
- 9) Therefore, the screening and titration cut point values (CPVs) were determined using percentiles of the non-transformed pooled data.
- 10) The CPVs established using data from Panels 1-6 (New Panel 5) associated with the 95th percentile for screening and 99.9th percentiles for titration assay and these values were applied to each of the runs performed within this study, which is appropriate and acceptable (Figure 3), where data from all 18 runs; 3 runs for each panel was used.
- 11) The screening cut point factor (SCPF) for the 95th upper percentile was determined to be 1.37 (shown in red line in Figure 3).
- 12) The titer CPF for the 99.9th upper percentile was determined to be 1.49 (shown in blue line in Figure 3).

CPVs Established Using Data from Panels 1-6 (New Panel 5)

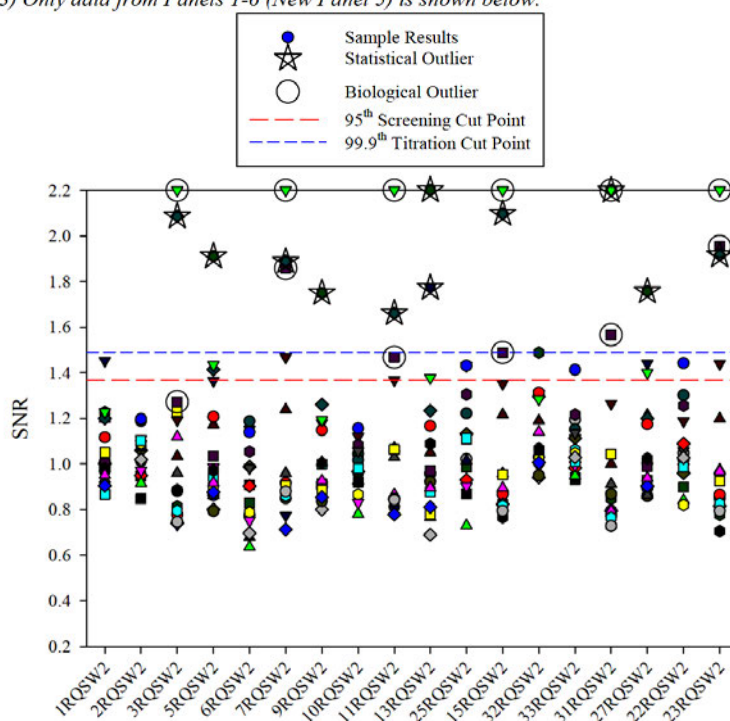
Total Number of Results	Number of Results Excluding Statistical Outliers	Estimates for Different Upper Percentiles		
		95 th Screening CPV	99 th CPV	99.9 th Titration CPV
279	268	1.37	1.45	1.49

Floating screening and titer assay cut points for in-study runs were calculated as:

$$\text{Floating Assay Cut Point} = \text{mean NC for in-study run} \times \text{CPF}$$

Figure 3
Cut Point Performance

Notes: (1) Different symbols/colors represent different samples.
(2) SNR values that exceed the y-axis are displayed at the top of the graph.
(3) Only data from Panels 1-6 (New Panel 5) is shown below.



Assessor comment: The Sponsor used serum samples from 50 normal individuals to determine SCP. The response signal was determined in six panels for each sample (total 300 data points), which are not copied here (Attachment I, Appendix D). These data were used to statistically determine the screening cut point at a 5% false-positive rate (at 95th percentile) and for titration assay cut-point at 0.1% false-positive rate (99.9 percentile) according to the FDA guidance (2019, cited in Footnote 1, above). The false positive rate (FPR) for the screening assay cut-point results, was verified from the resulting data points to be $16/268 = 5.9\%$, when back-calculated using the mean SNR values reported in the submission from six panels of 50 samples, after excluding 11 statistical outliers (Table 5, above). The calculated FPR is within the expected range of 2-11%² for an antibody screening assay justifying the appropriateness of the screening assay cut point determination and ensures proper assay performance; therefore, these results are acceptable.

The FDA guidance for immunogenicity recommends verifying the validated screening assay cut point if normal human serum samples were used in SCP determination, using pre-dose serum samples from target population to minimize the effect of assay variabilities that may arise due to potential inherent differences between normal and diseased human serum samples. Due to constraints with the availability of disease state matrix that reflects the patient population, the

² Goodman, Cowen, Devanarayan et al. (2018): Feedback from the European Bioanalysis Forum: focus workshop on current analysis of immunogenicity: best practices and regulatory hurdles. Conference report, Bioanalysis Vol 10, No 4, 197-204.

Sponsor did not use serum samples from disease state matrix. Nevertheless, the Sponsor's plan indicates that they will determine in-study cut-points to analyze clinical samples. Therefore, verification with disease-state matrix may not be needed and was not performed during assay validation studies.

10.2 Confirmatory Assay Cut Point:

The confirmatory cut point (CCP) was determined by analyzing same 50 individual drug-naïve healthy human serum sample lots as those used in screening assay cut point determination assay and spiked with 50 µg/mL of drug (inhibited).

The CCP data analysis was performed using 300 data sets generated from Panels 1-4, 6 and new Panel 5 using serum samples from 50 individuals. Originally, 8 samples data within Panel 5 showed high variability (CV% > 20%). Therefore, the Sponsor repeated Panel 5 data analysis and reported as Panel 5 (new) in the table.

Normality was determined using non-transformed data from panels 1-6, including new panel 5 data. The analysis found that the non-transformed data was not normally distributed (statistically significant p-value of <0.05 for Panel 6). Therefore, log-transformed data were generated and Shapiro-Wilk test for normality test was applied after removing the outliers (using Tukey's Outlier Filter). The resulting data suggest that the log-transformed data was not normally distributed. Therefore, the CCP was determined using percentiles of the non-transformed data. Samples 39 and 44 were determined to be biological outliers and these samples were excluded from all analyses.

The percent inhibition for each sample is calculated as:

$$\% \text{ Inhibition} = 100 \times (\text{Inhibited sample response}) / (\text{Uninhibited sample response}).$$

The CCP was determined by estimating at the 95th, 99th, and 99.9th percentiles on the percent inhibition (as shown in table below).

CPVs Established Using Data from Panels 1-6 (New Panel 5)

Total Number of Results	Number of Results Excluding Statistical Outliers	Estimates for Different Upper Percentiles					
		95 th		99 th		99.9 th	
		Ratio	% Inhibition	Ratio	% Inhibition	Ratio	% Inhibition
268	256	0.724	27.6%	0.650	35.0%	0.644	35.6%

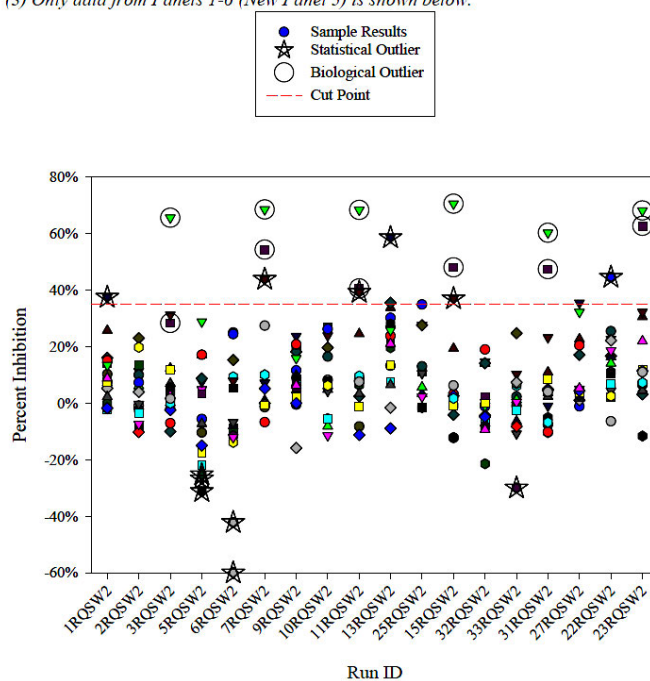
Initially, 12 datapoints (six each for samples 39 and 44) were excluded from the analysis as biological outlier and 20 datapoints were rejected due to higher variability (CV% > 20%) resulting to 268 samples. In addition, 12 more samples were identified as outlier by statistical outlier determination using non-transformed pooled data (Table 5, below). Individual %inhibition data were calculated, and the data are presented in the submission (Appendix E, attachment I), which are not presented here.

Table 5
Statistical Outlier Determination Using Non-Transformed Pooled Data
Measurement Shown as Percent Inhibition
Analysis was performed using data from Panels 1-6 (New Panel 5).

Panel	Run ID	Sample	Outlier Inhibition	Q1	Median	Q3	IQR	Lower Limit	Upper Limit
1	1RQSW2	8	37.554%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%
2	5RQSW2	4	-26.724%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%
2	5RQSW2	6	-25.271%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%
2	5RQSW2	12	-31.206%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%
2	6RQSW2	19	-42.188%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%
2	6RQSW2	34	-65.217%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%
2	7RQSW2	50	43.882%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%
3	11RQSW2	50	39.316%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%
4	13RQSW2	8	58.621%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%
4	15RQSW2	50	37.037%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%
5	33RQSW2	24	-29.968%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%
6	22RQSW2	31	44.604%	-1.180%	5.257%	13.574%	14.754%	-23.311%	35.705%

The confirmatory percent inhibition cut point established using data from Panels 1-6 (New Panel 5) associated with the 99th upper percentile (at 1% false positive rate as per FDA 2019 guidance) was applied to each of the runs tested within this study. The resulting cut point is provided graphically in Figure 3, where data from all 18 runs; 3 runs for each panel is used.

Figure 3
Cut Point Performance
Notes: (1) Different symbols/colors represent different samples.
(2) Percent Inhibition values that exceed the y-axis are displayed at the bottom of the graph.
(3) Only data from Panels 1-6 (New Panel 5) is shown below.



Assessor comment: The Sponsor determined CCP from percent inhibition results at 1% false positive rate as per guidance³. During manual assessment of each percent inhibition data from individual responses after removal of these 12 data point from 256, only 2 samples were found to have the %inhibition results greater than CCP (CCP= 35). Therefore, back calculation suggests that the rate of false positive data generated from the confirmatory assay was 2/256 = 0.78% justifying the appropriateness of the CCP determination and ensuring proper assay performance. Therefore, the CCP determination is acceptable.

10.3 Assay Precision:

10.3.1 Intra-Assay Precision

Three PC (at concentration 98.0, 124, and 1600 ng/mL of the analyte) and NC samples were used in this assay in multiple analyses (n = 6) of each pool in a given run (Ref study: rvzq2-validation report addendum-1). Each sample was analyzed uninhibited (for screening assay) and inhibited with drug (for confirmatory assay). The intra-assay control data were compiled from validation Runs 21RVZQ2, 24RVZQ2, and 20RVZQ2 and are presented in Table 1 below.

Acceptance criteria for intra-assay precision %CV was ≤20.0%.

Table 5. Intra-Assay Precision

Run ID	Uninhibited				Inhibited							
	21RVZQ2	24RVZQ2	20RVZQ2		21RVZQ2	24RVZQ2	20RVZQ2					
Screening Assay Cut Point (OD)	0.114	0.103	0.107		0.114	0.103	0.107					
Confirmatory Cut Point (% Inh.)	35.0	35.0	35.0		35.0	35.0	35.0					
	sLPC (98.0 ng/mL)	cLPC (124 ng/mL)	HPC (1600 ng/mL)	NC (0.00 ng/mL)	sLPC (98.0 ng/mL)	cLPC (124 ng/mL)	HPC (1600 ng/mL)	NC (0.00 ng/mL)				
	IA 1	IA 2	IA 3	IA 0	IA-I 1	IA-I 2	IA-I 3	IA-I 0				
	(OD)	(OD)	(OD)	(OD)	(OD)	(% Inh.)	(OD)	(% Inh.)	(OD)	(% Inh.)	(OD)	(% Inh.)
	0.144	0.229	1.44	0.0800	0.0730	49.3	0.0730	68.1	0.0690	95.2	0.0725	9.38
	0.137	0.166	1.70	0.0825	0.0760	44.5	0.0780	53.0	0.0705	95.9	0.0710	13.9
	0.142	0.161	1.51	0.0810	0.0700	50.7	0.0730	54.7	0.0720	95.2	0.0720	11.1
	0.145	0.163	1.60	0.0795	0.0790	45.5	0.0745	54.3	0.0675	95.8	0.0740	6.92
	0.146	0.156	1.60	0.0785	0.0740	49.3	0.0710	54.5	0.0700	95.6	0.0700	10.8
	0.153	0.166	1.63	0.0800	0.0720	52.9	0.0740	55.4	0.0925	94.3	0.0740	7.50
N	6	6	6	6	6	6	6	6	6	6	6	6
Mean	0.144	0.173	1.580	0.0803	0.0740	48.7	0.0739	56.7	0.0736	95.3	0.0723	9.93
S.D.	0.00508	0.0273	0.0890	0.00137	0.00316	3.17	0.00233	5.67	0.00939	0.565	0.00160	2.58
%C.V.	3.52	15.8	5.63	1.71	4.27	6.51	3.16	10.0	12.8	0.593	2.22	25.9*

Legend:

% Inh. % Inhibition

* Outside acceptance criteria. Data were considered acceptable. See the [Discussion](#) section for details.

Assessor Comment: The OD results for all PC samples were above the plate-specific cut points, whereas NC sample results were below assay cut-points for all sample results as expected. Similarly, %inhibition for all PC control samples were above the CCP (CCP=35%) and all negative control samples had %inhibition results below the CCP.

³ Immunogenicity Testing of Therapeutic Protein Products —Developing and Validating Assays for Anti-Drug Antibody Detection (2019) at <https://www.fda.gov/RegulatoryInformation/Guidances/default.htm>.

The intra-assay precision data met the acceptance criteria ($CV\% \leq 20.0\%$) for all assay runs except for the inhibited NC data, which had a %CV of 25.9% rather than $\leq 20.0\%$. This is not unexpected for NC samples that contain no analyte for inhibition by the PC antibody. Nevertheless, the inhibited NC results were considered acceptable since the NC mean % inhibition (9.93%) was below the CCP of 35.0% for all NC samples. The Intra-Assay Precision demonstrated adequately and is acceptable.

10.3.2 Inter-Assay Precision

Four PC and NC samples were used in this assay in multiple validation runs performed by at least 2 analysts throughout the validation. Each sample was analyzed uninhibited and inhibited with drug. The inter-assay control data for NC were compiled from 189 uninhibited results and 42 inhibited results, whereas, for PC samples, the inter-assay data were compiled from multiple runs for each PC samples. The data are presented in Tables 2A and 2B, a part of tables 2A and 2B is copied from the submission below.

Acceptance criteria for inter-assay precision %CV was $\leq 20.0\%$.

Table 6A. Inter-Assay Precision – NCs

Run ID	Uninhibited	Inhibited	
	NC (0.00 ng/mL)	NC-I	
	(OD)	(OD)	% Inhibition
2RVZQ2	0.0645	0.0600	6.98
	0.0660		
	0.0650		
	0.0625		
3RVZQ2	0.0665	0.0665	2.44
	0.0675		
	rcv		
	0.0705		
29RVZQ2	0.0695	0.0630	6.49
	0.0650		
	0.0675		
	0.0675		
N	64	16	16
Mean	0.0736	0.0693	5.93
S.D.	0.00661	0.00514	5.52
%C.V.	8.98	7.42	93.0*

Legend:

rcv Replicate coefficient of variance unacceptable

N/A Not applicable or no recorded result

* May exceed 20.0% per the validation plan ([Appendix A](#)).

Table 6B. Inter-Assay Precision – PCs

Run ID	Uninhibited	Inhibited		Uninhibited	Inhibited		Uninhibited
	HPC (1600 ng/mL)	HPC-I		cLPC (124 ng/mL)	CLPCI		sLPC (98.0 ng/mL)
	(OD)	(OD)	% Inhibition	(OD)	(OD)	% Inhibition	(OD)
2RVZQ2	0.855	0.0740	91.3	0.111	0.0620	44.4	0.107
	0.851			0.113			0.102
3RVZQ2	0.916	0.0665	92.7	0.114	0.0655	43.8	0.108
	0.914			0.119			0.103
25RVZQ2	1.68	0.0810	95.1	0.184	0.0635	68.5	0.170
	1.65			0.219			0.170
26RVZQ2	1.28	N/A	N/A	N/A	N/A	N/A	0.131
	1.31			N/A			0.127
29RVZQ2	1.10	0.0655	94.7	0.131	0.0630	53.5	0.114
	1.36			0.140			rcv
N	34	16	16	30	16	16	33
Mean	1.22	0.0768	93.1	0.156	0.0702	53.5	0.136
S.D.	0.330	0.00865	1.90	0.0400	0.00768	9.55	0.0219
%C.V.	27.0	11.3	2.04	25.7	11.0	17.9	16.1

Legend:

N/A Not applicable or no recorded result.

rcv Replicate coefficient of variation unacceptable.

Assessor Comment: The inter-assay precision data for all assay runs for positive control samples (HPC, cLPC and sLPC) met the acceptance criteria (CV% ≤ 20.0%) except for the uninhibited assay runs for HPC (N=34) and cLPC (N=30) samples that resulted in CV% of 27% and 25.7% respectively. Visual inspection of each data showed that the OD value for one of two readings for uninhibited cLPC values were relatively higher for samples ID 16RVZQ2 (0.315 versus 0.166) and 25RVZQ2 (0.184 versus 0.219) out of 30 samples, that may have caused the higher CV% of 25.7%. Nevertheless, all samples showed good degree of %inhibition (average of 16 = 53.5%) with a CV% = 17.9%. The OD values of 17 HPC samples (34 readings) were in the range of 0.719 to 0.185 with a mean of 0.122 suggesting that an assay variability exists. However, 94% HPC samples (N=16), showed %inhibition results above 90% with a mean % inhibition of 93.1% and a %CV of 2.04%. Therefore, based on overall data presentation, the inter-assay results for HPC samples could be considered acceptable.

For NC samples, the inter-assay precision met the acceptance criteria for uninhibited and inhibited samples (CV% were 8.98% and 7.42% respectively). Although NC samples met the CV% for OD, the %inhibition data did not meet the acceptance criteria of CV% < 20%, suggesting that high variability exists. However, visual inspection of the %inhibition data for each of 16 assay runs showed that all the assay runs had %inhibition much less than the CCP, as expected. Therefore, the NC samples inter-assay precision data are considered acceptable.

The inter-assay precision was demonstrated and acceptable.

10.4 Assay Sensitivity:

The sensitivity was estimated by analyzing a serially diluted HPC control (1600 ng/mL) diluted to 2, 4, 8, 16, 32 and 64-fold to span the assay cut point. Each sample was analyzed uninhibited and inhibited with drug, and a total of 12 sensitivity assessments were conducted. These data are submitted in Table 3A (not copied here), which are used to determine the sensitivity, as the lowest concentration of the anti-drug antibody at which the control consistently produced a positive response, i.e., greater than or equal to the assay cut point. The sensitivity calculation data are presented in [Table 3A-3C](#) (RQSW2: Method Validation Report, Pages 35-41), which are not copied in this memo.

The overall sensitivity for this validation was determined to be 47.8 ng/mL for screening and 59.9 ng/mL for confirmatory.

The Sponsor calculated LPC concentrations for each Tier from the sensitivity data, which are presented in [Table 3C](#) (RQSW2: Method Validation Report, Page 39-41), which are not copied in this memo.

Assessor Comment: The assay sensitivity (for screening and confirmatory assay) was appropriately determined as the concentration of the control antibody at which the assay result is expected to be above the cut points at least 95% of the time and the assay results met all acceptance criteria and determined to be 47.8 ng/mL for screening assay and 59.9 ng/mL for confirmatory assay which is within an expected range of 100ng/mL as per guidance⁴, therefore, these results are acceptable.

The sLPC and cLPC concentrations were calculated based on assay sensitivity to 98ng/mL and 124ng/mL (table 3B) respectively, however, the Sponsor stated, when re-calculated at the cut-points for screening and confirmatory assays, these values turned up to be 100ng/mL and 121ng/mL respectively for sLPC and cLPC, which are about 2-2.5% deviation from corresponding validation samples, but are not expected to impact on the validation (validation data met acceptance criteria). Therefore, the Sponsor planned to use the validated concentrations (98ng/mL for sLPC and 124ng/mL for cLPC) during sample analysis. This approach is acceptable.

10.5 Titer Precision:

Titer precision was estimated in the screening assay of sensitivity Runs by analyzing a serially diluted surrogate control (1600 ng/mL) diluted 2-, 4-, 8-, 16-, 32- and 64-fold. The titer CPF (1.49), which was calculated based on the screening cut point at the 99.9% upper prediction interval, was used to calculate the titer plate cut point for each sensitivity run. The titer precision was reported as the median of the titer values across the runs. Titer precision results are presented in [Table 4](#) (RQSW2: Method Validation Report, Page 42), which are not copied in this memo.

⁴ Immunogenicity Testing of Therapeutic Protein Products —Developing and Validating Assays for Anti-Drug Antibody Detection (2019) at <https://www.fda.gov/RegulatoryInformation/Guidances/default.htm>.

Acceptance criteria: Results from $\geq 80\%$ of titer curves be within ± 1 dilution of median last positive dilution.

Assessor Comment: The titer precision met acceptance criteria ($CV\% > 20\%$); 12 out of 12 curves (100%) were also within ± 1 dilution of the median last positive dilution. Therefore, these data are acceptable.

10.6 Prozone Effect:

The prozone effect was evaluated in Runs 48RQSW2 by analyzing a prepared 50,000 ng/mL anti-drug antibody sample undiluted and diluted two-fold at 2-, 4-, 8-, 16-, 32-, 64-, 128-, 256-, 512-, 1024- and 2048-fold. Results are presented in Table 5.

Acceptance criteria: High concentration samples containing anti-Nedosiran above the assay sensitivity level should maintain a response $\geq$ the assay cut point (remain positive).

Table 5. Prozone Effect

Plate Cut Point (OD) = 0.107, CCP = 35.0%												
Uninhibited												
Sample ID	PZ 1 Dil 1	PZ 1 Dil 2	PZ 1 Dil 4	PZ 1 Dil 8	PZ 1 Dil 16	PZ 1 Dil 32	PZ 1 Dil 64	PZ 1 Dil 128	PZ 1 Dil 256	PZ 1 Dil 512	PZ 1 Dil 1024	PZ 1 Dil 2048
Anti-Nedosiran Conc.	50,000 ng/mL	25,000 ng/mL	12,500 ng/mL	6,250 ng/mL	3,125 ng/mL	1,563 ng/mL	781 ng/mL	391 ng/mL	195 ng/mL	97.7 ng/mL	48.8 ng/mL	24.4 ng/mL
	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)
Result	4.00	4.00	3.64	2.92	1.44	0.692	0.409	0.237	0.160	0.119	0.101	0.0860
Inhibited												
Sample ID	PZ-I 1 Dil 1	PZ-I 1 Dil 2	PZ-I 1 Dil 4	PZ-I 1 Dil 8	PZ-I 1 Dil 16	PZ-I 1 Dil 32	PZ-I 1 Dil 64	PZ-I 1 Dil 128	PZ-I 1 Dil 256	PZ-I 1 Dil 512	PZ-I 1 Dil 1024	PZ-I 1 Dil 2048
Anti-Nedosiran Conc.	50,000 ng/mL	25,000 ng/mL	12,500 ng/mL	6,250 ng/mL	3,125 ng/mL	1,563 ng/mL	781 ng/mL	391 ng/mL	195 ng/mL	97.7 ng/mL	48.8 ng/mL	24.4 ng/mL
	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)	(OD)
Result	0.205	0.131	0.0975	0.0890	0.0850	0.0820	0.0775	0.0760	0.0740	0.0720	0.0750	0.0780
% Inhibition	94.9	96.7	97.3	97.0	94.1	88.1	81.1	67.9	53.8	39.5	25.7	9.30

Assessor Comment: The data in Table above indicates that the mean signal values follow the dilution effect. For inhibited samples, the %inhibition data also follows the dilution effect up to 12,500ng/mL and then gets lower to 96.7% and 94.9% for 25,000ng/mL and 50,000ng/mL, however, the OD values for the corresponding %Inhibition data follow a linear relationship. Therefore, no apparent "hook effect" was observed at concentrations up to 50,000 ng/mL in assay results, and thus met acceptance criteria. Acceptable.

10.7 Stability:

10.7.1 Freeze/thaw stability

Freeze/thaw stability (F/T) was evaluated in Run 50RQSW2 by analyzing a set of low- and high-level controls that were subjected to 6 freeze/thaw cycles versus freshly prepared controls. Results are presented in [Table 6](#) (RQSW2: Method Validation Report, Page 44), which is not copied in the review memo.

Assessor Comment: The freeze/thaw stability data met the criteria for demonstrating freeze-thaw stability of the analyte.

10.7.2 Room Temperature-

A set of low (sLPC and cLPC) and HPC controls were thawed and stored at room temperature for 24 hours prior to analysis and compared with freshly prepared controls. Results are presented in [Table 7](#) (RQSW2: Method Validation Report, Page 45), which is not copied in the review memo.

Assessor Comment: The room temperature stability data met the criteria for demonstrating room temperature stability for up to 24 hours of the analyte.

10.8 Effect of Hemolysis:

The effect of hemolysis on the detection of anti-nedosiran antibody was evaluated by analyzing a blank, LPC (sLPC and cLPC) and HPC samples prepared in hemolyzed matrix containing 5% fully lysed whole blood. Each sample was analyzed uninhibited (HEMPC) and inhibited with drug (HEM-I). The results are presented in Table 8.

Table 8. Effect of Hemolysis

Run 47RQSW2										
Plate Cut Point (OD) = 0.0985, CCP = 35.0%										
PC Conc.	0.00 ng/mL Anti-Nedosiran		98.0 ng/mL Anti-Nedosiran		124 ng/mL Anti-Nedosiran		1600 ng/mL Anti-Nedosiran			
Tier	Uninhibited	Inhibited	Uninhibited	Inhibited	Uninhibited	Inhibited	Uninhibited	Inhibited	Uninhibited	Inhibited
Sample ID	HEMPC 0	HEM-I 0	HEMPC 1	HEM-I 1	HEMPC 2	HEM-I 2	HEMPC 3	HEM-I 3	HEMPC 3	HEM-I 3
	(OD)	(OD) % Inhibition	(OD)	(OD) % Inhibition	(OD)	(OD) % Inhibition	(OD)	(OD) % Inhibition	(OD)	(OD) % Inhibition
Result	0.0720	0.0625 13.2	0.1160	0.0635 45.0	0.132	0.0655 50.2	0.882	0.0680 92.3	0.882	0.0680 92.3

Assessor Comment: Based on the definitions from the Global Bioanalysis Consortium, the plasma should contain ≤2% lysed blood for this assay, or plasma containing more than 2% lysed blood is considered hemolyzed plasma⁵. The Sponsor used 5% hemolyzed blood in evaluating the effect of hemolysis in this assay, therefore, this assay design is suitable. The assay results showed that no effect from hemolysis (up to 5%) on the detection of anti-nedosiran antibodies was observed using NC, sLPC, cLPC and HPC samples demonstrating the 'Effect of hemolysis' in ADA assay performances. These data are acceptable.

10.9 Effect of Lipemia:

The effect of lipemia on the detection of anti-nedosiran antibody was evaluated by analyzing a blank, LPC (sLPC and cLPC) and HPC samples prepared in human serum containing > 300 mg/dL triglycerides. Each sample was analyzed uninhibited (LIPPC) and inhibited with drug (LIP-I). The results are presented in Table 9.

⁵ Ingelse B. et. al., European Bioanalysis Forum: recommendation on dealing with hemolyzed and hyperlipidemic matrices. Bioanalysis (2014) 6(23), 3113–3120.

Table 9. Effect of Lipemia

Run 47RQSW2												
Plate Cut Point (OD) = 0.0985, CCP = 35.0%												
PC Conc.	0.00 ng/mL Anti-Nedosiran			98.0 ng/mL Anti-Nedosiran			124 ng/mL Anti-Nedosiran			1600 ng/mL Anti-Nedosiran		
Tier	Uninhibited	Inhibited		Uninhibited	Inhibited		Uninhibited	Inhibited		Uninhibited	Inhibited	
Sample ID	LIPPC 0	LIP-I 0		LIPPC 1	LIP-I 1		LIPPC 2	LIP-I 2		LIPPC 3	LIP-I 3	
	(OD)	(OD)	% Inhibition	(OD)	(OD)	% Inhibition	(OD)	(OD)	% Inhibition	(OD)	(OD)	% Inhibition
Result	0.0710	0.0635	10.6	0.1170	0.0640	45.1	0.1300	0.0635	51.2	0.907	0.0685	92.4

Assessor Comment: This assay was performed using hyperlipidemic plasma samples containing 300mg/dL triglycerides. In general, hyperlipidemic plasma refers to plasma with a high fat content, containing over 3.4 mmol/L of triglycerides which equates to 300 mg/dL⁶. Therefore, the Sponsor's approach of evaluating the effect of lipemia using human serum samples containing 300mg/dL triglycerides is acceptable. No effect from lipemia (up to 300 mg/dL triglycerides) on the detection of anti-nedosiran antibodies was observed using NC, sLPC, cLPC and HPC samples demonstrating the effect of lipemia in ADA assay performances. These data are acceptable.

10.10 Matrix Interference/Selectivity:

Matrix interference/Selectivity was evaluated by analyzing human serum samples from individual donors (N=20) as unspiked (SP) and prepared with LPC level (cLPC or sLPC) anti-drug antibody concentrations (SP-I). These results are presented in 'rqsw2 - method validation report – addendum 1', [Table 7C](#) (Page 33) and [Table 7D](#) (Page 34), showing selectivity calculated as %inhibition results.

Assessor comment: The percent inhibition results from 20 samples are presented in [Table 7C](#) (ICDIM 522 Validation Report, (b) (4) Project Code RQSW2), of which, the data for 3 samples were not reported and one sample had %inhibition results above the CCP. The confirmatory assay data using normal serum samples (blank) spiked with cLPC met acceptance criteria since 16 out of 17 samples (94%) tested below the CCP (CCP=35%) demonstrating selectivity for confirmatory assay for blank serum samples from healthy individuals. These data are acceptable.

The results in [Table 7D](#) (ICDIM 522 Validation Report, (b) (4) Project Code RQSW2) showed that one of 20 samples had a high OD (above SCP, outside of the acceptance criteria), while 19 out of 20 samples (95%) tested below the SCP for the assay (plate-specific CP= 0.0971). Therefore, screening assay met acceptance criteria for selectivity in blank individual samples.

Similarly, the Sponsor also evaluated human serum samples from ten healthy normal individual donors spiked with PC antibodies at the HPC level (1600 ng/mL), cLPC1 level (186 ng/mL), validated cLPC level (124 ng/mL), and sLPC level (98.0 ng/mL) and analyzed uninhibited and inhibited with drug in Runs 16RVZQ2 through 19RVZQ2.

⁶ Ingelse B. et. al., European Bioanalysis Forum: recommendation on dealing with hemolyzed and hyperlipidemic matrices. Bioanalysis (2014) 6(23), 3113–3120.

The Sponsor used a new cLPC at 186ng/mL in addition to the validated cLPC at 124ng/mL, the reason is not understood, however, results from all PC levels are presented in Tables 8A to 8D (rvzq2 - method validation report – addendum 1, which are not copied in this memo). I evaluated all data and found that screening and confirmatory assay data from all PC levels met the acceptance criteria and showed no matrix interference, therefore demonstrating the selectivity of the assay for the surrogate PC samples.

10.11 Drug Tolerance:

The potential interference of nedosiran on the detection of anti-nedosiran in study samples were evaluated using sLPC, cLPC, MPC, and HPC samples prepared with 0.00, 1.00, 10.0, 25.0, and 50.0 µg/mL nedosiran and incubated at 37 °C for at least one hour and frozen overnight prior to analysis. Samples were analyzed in the screening assay only. The Sponsor stated that the study samples that will be analyzed are collected when circulating drug concentrations are low, therefore, they reported screening assay results only and this deviation will not impact on the validation. Results are presented in [Table 12](#) (ICDIM 522 Validation Report, (b) (4) Project Code RQSW2), which are not copied here.

Assessor comment: Drug tolerance is defined by the highest drug concentration that retains a positive response for each anti-drug antibody level. The Sponsor used various concentrations of nedosiran in this assay in presence of sLPC, cLPC, MPC, and HPC samples, which is suitable for this assay. The results in Table 12 suggest that the HPC (1600 ng/mL PC) could be detected as positive in presence of 25.0 µg/mL drug and the MPC at 400 ng/mL PC could be detected as positive in presence of up to 1.00 µg/mL drug.

The elimination half-life of nedosiran in plasma after a single dose ranged from 4.6 to 13.8 hours⁷. Since the Sponsor proposes to use a monthly dose of the drug in clinical studies (maximum dose is proposed to be 110mg, which is equivalent to 10.0 µg/mL), levels of circulating drugs are expected to be very low in immunogenicity samples when samples are collected before dose. Therefore, no interference is expected on the detection of anti-nedosiran antibodies in presence of circulating nedosiran at the proposed dose. These data are acceptable.

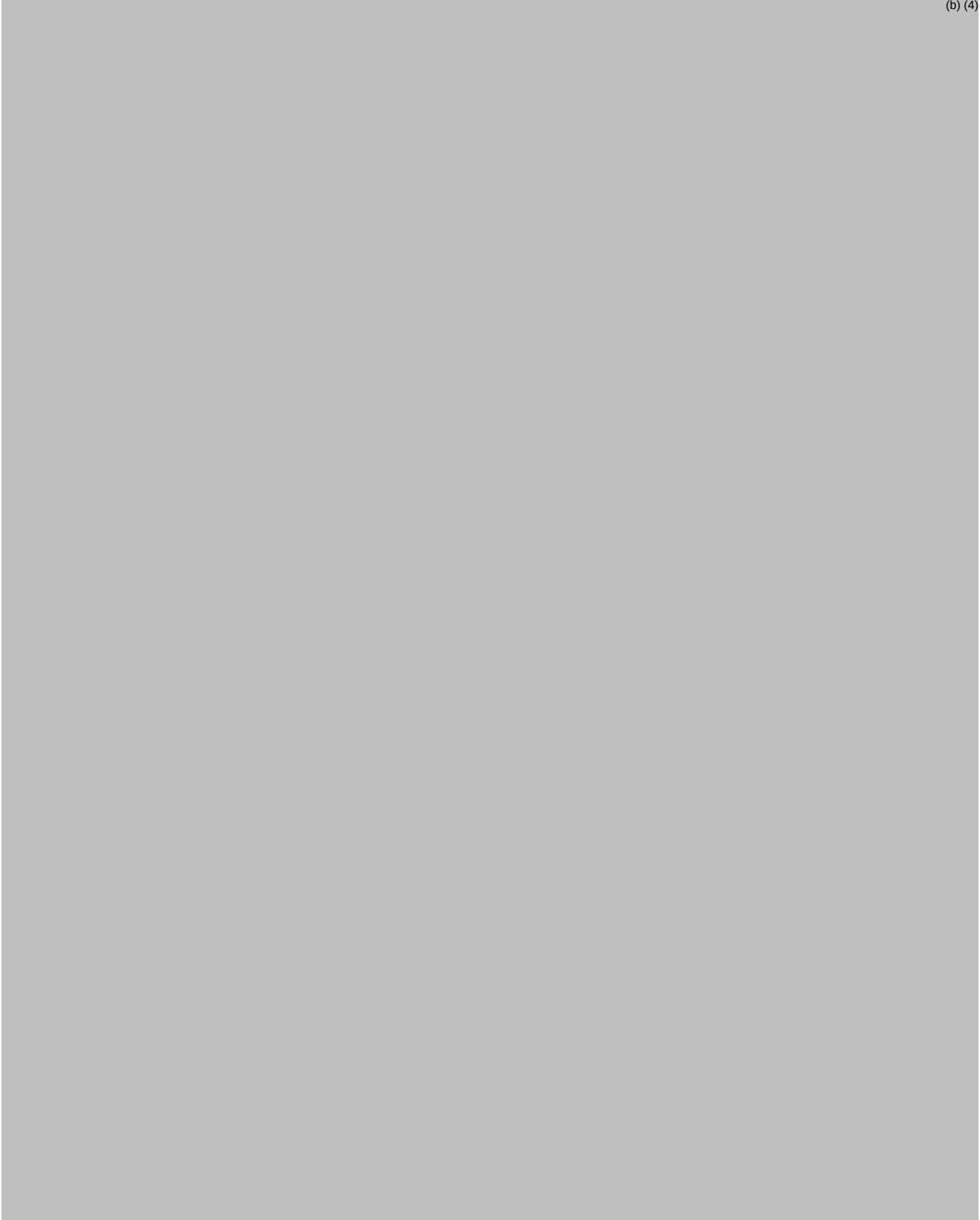
11. Assessor Conclusion:

The anti-Nedosiran antibody assay for the detection of antibodies to Nedosiran drug in human serum samples is validated. The validation process included cut point determination (screening and confirmatory), sensitivity, titer precision, intra- and inter-assay precision, matrix interference (selectivity), effect of hemolysis, effect of lipemia, drug tolerance, prozone or hook effect, and freeze/thaw stability. The validation of the binding antibody assay for antibodies to

⁷ Hoppe B. et. al., Safety, pharmacodynamics, and exposure-response modeling results from a first-in-human phase 1 study of nedosiran (PHYOX1) in primary hyperoxaluria. Kidney International Volume 101, Issue 3, March 2022, Pages 626-634.

nedosiran is acceptable, and the method is applicable for the detection and confirmation of anti-nedosiran antibodies in human serum samples.

(b) (4)



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