CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214012Orig1s000

PRODUCT QUALITY REVIEW(S)

QUALITY ASSESSMENT

Recommendation: Approval

NDA 214012
Review 2

Drug Name/Dosage Form	Leqvio (inclisiran) injection
Strength	284 mg/1.5 mL
Route of Administration	Subcutaneous injection
Rx/OTC Dispensed	Rx
Applicant	Novartis Pharmaceuticals
US agent, if applicable	-

SUBMISSION(S) REVIEWED	DOCUMENT DATE	DISCIPLINE(S) AFFECTED
Resubmission, and amendments	Resubmission: 07/1/2021 (Seq. 008) Amendments: 7/29/2021 (Seq. 0062), 8/18/2021 (Seq. 0063), 9/03/2021 (0064), 9/21/2021 (Seq. 66), 10/12/2021 (0067), 10/26/2021 (0070), 11/5/2021 (Seq. 0072), 11/19/2021 (0073), and 11/23/2021 (0074) and 11/29/2021 (0075).	Quality modules 3, 1.14, and 1.11

Quality Review Team

Discipline	Reviewer/Secondary	Branch/division
Drug Substance	Daniel Jansen/ Suong Tran	Branch III/Division of New Drug Active pharmaceutical
Drug Product	Muthukumar Ramaswamy/Mohan Sapru	Branch V/New Drug Products III
Process/ Facility	Vidya Pai/ Rose Xu	Branch II/ Office of Pharmaceutical Manufacturing Assessment (OPMA)
Microbiology	Avital Shimanovich/ Erika Pfeiler/John Metcalfe	OPMA
Regulatory Business Process Manager	Hamet Toure/Norwin Kakon	Branch I/Regulatory Business Process Management I
Application Technical Lead	Muthukumar Ramaswamy	Branch V/New Drug Products III
Environmental Analysis (EA)	Muthukumar Ramaswamy/Mohan Sapru	Branch V/New Drug Products III of New Drug Products

Quality Review Data Sheet

1. RELATED/SUPPORTING DOCUMENTS

A. DMFs:

DMF #	Type	Holder	Item Referenced	Status	Date Assessment Completed	Comments
(b) (4)	III		(b) (4)	Active	9/24/2019	LOA dated 4/10/2021
	III			Active	*	LOA dated 5/6/2021
	V			Active	5/03/2019	LOA dated 5/6/2021
	V			Active	11/30/2022	LOA dated 4/23/2021
	V			Active	11/30/2021	LOA dated 4/23/2021
	III			Active	*	LOA dated 4/23/2021

*Sufficient information provided in the NDA

B. Other Documents: *IND, RLD, or sister applications*

DOCUMENT	APPLICATION NUMBER	DESCRIPTION
IND	127589	Inclisiran injection

2. CONSULTS: None

DISCIPLINE	STATUS	RECOMMENDATION	DATE	REVIEWER

Executive Summary

I. Recommendations and Conclusion on Approvability

The recommendation from the Office of Pharmaceutical Quality (OPQ) for NDA 214012 is approval, which includes an acceptable recommendation for the facilities listed in the application.

Summary of Complete Response Issues and Resubmission Information
On December 18, 2020, the NDA 214012 received complete recommendation from FDA for facility related issues observed at the drug product manufacturing facility, (b) (4) . There were no outstanding issues related to drug substance, drug product, manufacturing process, and microbiology sections of the original NDA submission. Please refer to OPQ integrated quality assessment dated 10/1/2020 and 12/17/2020 in DARRTS.

The applicant resubmitted the application on July 1, 2021. In this resubmission, the applicant replaced the drug product manufacturing facility, (b) (4) with Sandoz GmbH facility located at Langkampfen, Austria (FEI:3004828473). The applicant also replaced the drug product analytical testing site used for release and stability from (b) (4) with Sandoz GmbH, Langkampfen, Austria (FEI:3004828473) and Sandoz GmbH, Kundl, Austria (FEI: 3002806523).

To support the introduction of the new finished product manufacturing and testing facilities, the applicant provided the following information in Module 3:

a) Manufacturing process and microbiological control information for the drug product manufactured at Sandoz GmbH.
b) Manufactured 3 commercial scale batches at Sandoz GmbH and provided the batch release and 3 months of accelerated and long-term stability data for the 3 commercial batches.
c) Drug product quality comparison between batches used in phase 3 inclisiran studies and the to-be-marketed finished product batches manufactured at Sandoz GmbH site in Austria.

II. Summary of Quality Assessments

A. Product Overview

Inclisiran is a small interfering RNA (siRNA) duplex containing a 21-nucleotide long sense strand linked to a triantennary acetyl galactosamine (GalNAc) on the 3' end and a 23-nucleotide long anti-sense strand. Inclisiran sodium is a new molecular entity.

Inclisiran contains modifications at the 2'-ribose moieties as well as phosphodiester backbone to improve stability of the siRNA in vivo. The inclusion of the GalNAc ligand on the sense strand facilitates the uptake of siRNA by hepatocytes. In hepatocytes, the anti-sense strand of the siRNA duplex inhibits the production of proprotein convertase subtilisin/kexin type 9 (PCSK9) protein and thus contributes the lowering of LDL-C levels in the circulation.

The proposed product, Leqvio (inclisiran) injection, 189 mg/mL is a clear, colorless to pale yellow sterile solution provided as a single-dose 1.5 mL pre-filled syringe. Each syringe contains 284 mg inclisiran (present as 300 mg inclisiran sodium). Leqvio is for subcutaneous administration by healthcare professional.

Leqvio is formulated in water for injection as a preservative free solution with a target pH of 7. Liqvio should be stored at 20°C to 25°C (68°F to 77°F) (b) (4). Excursions permitted between 15°C and 30°C (59°F and 86°F).

Proposed Indication(s) including Intended Patient Population	Treating adults with primary hyperlipidemia (including heterozygous familial hypercholesterolemia (HeFH) or clinical atherosclerotic cardiovascular disease (CVD), who require additional lowering of to reduce low-density lipoprotein cholesterol (LDL-C)
Duration of Treatment	3 doses for the 1st year and then twice a year dosing schedule.
Maximum Daily Dose	284 mg inclisiran (provided as 300mg inclisiran sodium)
Alternative Methods of Administration	Not applicable

B. Quality Assessment Overview

Leqvio is a drug device combination product. Leqvio is packaged in Type (b) (4) glass syringe fitted with a 27G staked needle and (b) (4) on one end and a (b) (4) plunger on the other end. The final product is provided in a carton.

There are no changes to the drug substance, drug product composition, drug product manufacturing process, drug product release and end-of-shelf-life specifications, analytical procedure, and drug product primary container closure system in the resubmission.

However, the resubmission contains a minor update to the container closure system. In addition to the (b) (4) syringe as originally proposed in the original submission, the applicant plans to use a (b) (4) syringe for use in drug product manufacturing. Since the product contact surface of the components associated with the primary container closure system remains the same, the proposed (b) (4) modification has no impact to the performance of the syringe. In addition, the applicant also proposed minor changes to the secondary packaging components as discussed in the Section 3.2.P.7.1,

which are acceptable based on review. Dr. Ramaswamy reviewed the container closure information and concluded that the proposed changes are acceptable.

Although the overall manufacturing process used at Sandoz GmbH and (b) (4) for manufacturing the drug product remains the same, minor changes were implemented for adaptation of the process to the equipment available at the new facility. Briefly, the drug product is (b) (4) (b) (4) (b) (4) The applicant updated batch formula for manufacturing the drug product at (b) (4) scale ((b) (4) (b) (4) syringes).

The manufacturing process and process control information provided in the resubmission was reviewed by Dr. Vidya Pai. Her review concluded that the proposed drug product manufacturing process controls are adequate to support the NDA. Please refer to Dr. Pai's process review in Panorama dated 11/22/2021.

Dr. Shimanovich and Dr. Erika Pfeiler reviewed the microbiology information. This include information related to (b) (4), sterility, container closure integrity, endotoxin, (b) (4) media fill studies, hold times, and post-approval stability commitment in the application. They also reviewed the (b) (4) and validation information provided in Type V DMF (b) (4) (b) (4) (b) (4). The microbiology review concluded that the proposed microbiological controls are adequate to support the NDA. Refer to Dr. Pfeiler's microbiology review dated 9/08/2021 and 11/30/2021 in Panorama.

The resubmission contains a minor CMC update to drug substance starting material specification. Dr. Daniel Jensen reviewed this information and concluded that it is adequate. For additional details, please refer to Dr. Jensen's CMC review dated 9/21/2021 (resubmission) in Panorama.

To support the introduction of new drug product manufacturing and testing facilities, the applicant provided batch release data, 3 months of long-term and accelerated stability data for 3 commercial scale batches manufactured at Sandoz GmbH, Austria at (b) (4) to (b) (4) scale. The applicant also relied on the 12 to 24 months of long-term stability data available for 8 (b) (4) commercial and clinical product development batches to propose the commercial product shelf-life life of 24 months at 25°C.

Dr. Ramaswamy reviewed the drug product stability information provided in the resubmission. Since the product formulation/composition, the container closure system and the manufacturing process are the same, the applicant's approach to seeking shelf-life based on (b) (4) long-term stability data for commercial batches is

reasonable. Based on review of the long-term stability data for [redacted] (b) (4) drug product batches and the acceptable stability profile comparison between Sandoz commercial scale batches and the [redacted] (b) (4) clinical development batches, *a shelf life of 24 months is granted for the drug product when stored at 25°C/60% RH in the proposed commercial packaging*. Please refer to the drug product review dated 11/16/2021 in Panorama for additional information.

Control Strategy: The critical quality attributes of the product are controlled through batch records instructions, process design, excipient specifications including bioburden and endotoxin control, drug substance specification, component specifications for incoming container closure components, [redacted] (b) (4) techniques, in-process controls for the [redacted] (b) (4), bioburden, visual verification of [redacted] (b) (4), product pH verification, [redacted] (b) (4), and adequate finished product specification. The proposed control strategy is adequate to assure the quality of the product

Facility compliance information: Facility compliance information for the drug product and drug substance manufacturing and testing facilities was reviewed by Dr. Vidya Pai. The facility review concluded that the facilities associated with this application are compliant. The Panorama screen shot facility assessment recorded on 11/30/2021 is shown below.

Latest Submission Manufacturing Status for NDA-214012-Original-1

Latest Overall Manufacturing Inspection Recommendation	Inspection Requested	Inspection Completed	pOAI/OAI Alerts	Pending Profile
Approve Completion Date: 11/18/2021 NDA-214012-ORIG-1-RESUB-61	0	2	No	No

Hint: Click Facility ID link to open Facility Program
Click Facility Name to open Facility Status View: Detail
To refresh the report, please click Refresh under Page Options.
Page Options can be found next to the "?" icon located at the top right

Facility ID	FEI	Facility DUNS	Facility Name	Latest Facility Status /As of Date / Recommendation Reason	Profile Code	Facility Drug Supply Chain Role	Compliance Status / Alert Date	Compliance Status Change Reason
110001452	3004828473	301698247	SANDOZ GMBH (SCHAFTENAU)	Approve Facility 11 /10 /2021 Based on File Review	IDD INJECTABLE DELIVERY DEVICE	**Product Supply Chain:** MANUFACTURER: INCLISIRAN PACKAGER: INCLISIRAN SECONDARY PACKAGER: INCLISIRAN LABELER: INCLISIRAN RELEASE TESTER: INCLISIRAN STABILITY TESTER: INCLISIRAN	Compliant	
				Approve Facility 11 /18 /2021 Based on File Review	SVS STERILE-FILLED SMALL VOLUME PARENTERAL DRUGS	**Product Supply Chain:** MANUFACTURER: INCLISIRAN PACKAGER: INCLISIRAN SECONDARY PACKAGER: INCLISIRAN LABELER: INCLISIRAN RELEASE TESTER: INCLISIRAN STABILITY TESTER: INCLISIRAN	Compliant	

(b) (4)				Approve Facility 5 /14 /2020 District Recommendation	CSN NON-STERILE API BY CHEMICAL SYNTHESIS	**Substance Supply Chain:** MANUFACTURER: INCLISIRAN RELEASE TESTER: INCLISIRAN STABILITY TESTER: INCLISIRAN	Compliant
110004423	3002806523	300220969	SANDOZ GMBH	Approve Facility 9 /27 /2021 Based on File Review	LCP LABORATORY, CHEMICAL/PHYSICAL TESTING	**Product Supply Chain:** RELEASE TESTER: INCLISIRAN STABILITY TESTER: INCLISIRAN STERILITY TESTER: INCLISIRAN	Compliant
				Approve Facility 9 /27 /2021 Based on File Review	LMS LABORATORY, MICROBIOLOGICAL - STERILITY TESTING	**Product Supply Chain:** RELEASE TESTER: INCLISIRAN STABILITY TESTER: INCLISIRAN STERILITY TESTER: INCLISIRAN	Compliant
(b) (4)				Approve Facility 9 /3 /2020 District Recommendation	CSN NON-STERILE API BY CHEMICAL SYNTHESIS	**Substance Supply Chain:** MANUFACTURER: INCLISIRAN	Compliant
				No Evaluation Necessary 11 /18 /2021	IDD INJECTABLE DELIVERY DEVICE	**Product Supply Chain:** STABILITY TESTER: INCLISIRAN SECONDARY PACKAGER: INCLISIRAN RELEASE TESTER: INCLISIRAN PACKAGER: INCLISIRAN MANUFACTURER: INCLISIRAN LABELER: INCLISIRAN	Compliant
				No Evaluation Necessary 11 /18 /2021	SVS STERILE-FILLED SMALL VOLUME PARENTERAL DRUGS	**Product Supply Chain:** STABILITY TESTER: INCLISIRAN SECONDARY PACKAGER: INCLISIRAN RELEASE TESTER: INCLISIRAN PACKAGER: INCLISIRAN MANUFACTURER: INCLISIRAN LABELER: INCLISIRAN	Compliant
				No Evaluation Necessary 11 /18 /2021	LCP LABORATORY, CHEMICAL/PHYSICAL TESTING	**Product Supply Chain:** RELEASE TESTER: INCLISIRAN	Compliant

Thus, the overall manufacturing inspection recommendation (OMIR) from the Office of Process Manufacturing Assessment (OPMA) for this NDA is approval. For additional details, please refer to Dr. Pai's review dated 11/22/2021

Environmental assessment: The applicant sought exemption from environmental impact analysis per 21CFR 25.31(b) as the action on this NDA may not significantly affect the quality of the human environment. Dr. Ramaswamy granted categorical exclusion from submitting environmental assessment. Please refer to drug product review dated 11/16/2021 for additional information.

Post-marketing Commitment (PMC):

a) Drug product specification: The applicant introduced Sandoz, GmbH as the new drug product manufacturing facility. The applicant's manufacturing experience for drug product is limited. The current drug product specification for purity and impurity content is based on the upper confidence limit (mean ± 3SD) derived from limited batch release data available for drug substance batches used in the original submission. FDA recommended the applicant to reevaluate and tighten the specification for drug product based on batch release and stability data available from drug product batches manufactured at Sandoz GmbH from at least 10 drug substance batches. Novartis committed to submit the re-evaluation and potential tightening of the purity and impurity content specifications for the finished product by the end of November 2023.

b) Leachable study data for site-specific drug product batches manufactured at Sandoz GmbH was not available in the resubmission. Since there is no change to the container closure system, the original leachable data available considered supportive of the product. Lack of leachable data for Sandoz GmbH drug product batches would be considered as a low risk. The applicant was asked to provide a commitment to complete the end of the shelf-life leachable study information to support product acceptability during shelf-life. Novartis committed to provide the end of 24-month

shelf-life leachable study data from the three commitment stability batches manufactured at Sandoz GmbH by January 2024.

Container and Carton Label Review: The dosage form description, strength, established name, NDC #, Lot #/Expiry, and storage conditions are adequately described in the carton and container label, which meets relevant regulatory requirements for labeling. Refer to the Dr. Carver's labeling review dated 9/4/2020 for additional information. It contains one labeling comment related to change the descriptors of the sodium salt from "284 mg inclisiran (equivalent to 300 mg inclisiran sodium)" to "284 mg inclisiran (provided as 300 mg inclisiran sodium)".

On November 22, 2021, Novartis explained that the revisions requested by FDA would not be implementable until post-approval due to major COVID-related supply chain delays in receiving timely carton/container labeling, primarily tray lidding foiling. Novartis already has pre-printed text as originally proposed to FDA in the Complete Response Resubmission. *Novartis committed to applying the FDA revisions (changes from "equivalent to" to "present as") 4 months post-approval under the change category, changes-being-effected-0 (CBE-0) supplement. I agree with the request, as the delay in the implementation of the proposed label change is a low risk.*

OVERALL ASSESSMENT AND SIGNATURES:

At present, there are no outstanding deficiencies related to the drug substance, drug product, process, microbiology, facility, environmental analysis, and container/ carton label sections of the NDA, the OPQ overall recommendation for NDA *214012 is approval*.

Muthukumar Ramaswamy, Ph.D. 11/30/2021

Application Technical Lead Name and Date

Muthukumar Ramaswamy

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NDA 214012 (**NAI**) –Adequate

Drug Substance: Inclisiran

Review of Resubmission Amendment (received on 07/01/2021):

Brief summary of amendment information:

This amendment includes minor updated information on the drug substance:

- Table 1-8 Specifications for (b) (4) in Section 3.2.S.2.3 Control of Materials was updated to reflect changes to acceptance criteria (from NMT (b) (4) to NMT (b) (4)) of one of the non-critical impurities (b) (4) (b) (4)

Review assessment: The original drug substance review found that the information provided in the NDA was adequate. This is a minor change to starting material specification that will not affect the overall quality of the drug substance or specified/unspecified impurities.

The drug substance information remains **Adequate.**

Daniel Jansen

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Su (Suong) Tran

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CHAPTER VII: MICROBIOLOGY

IQA NDA Assessment Guide Reference

Product Information	
NDA Number	214012
Assessment Cycle Number	02
Drug Product Name/ Strength	Inclisiran/300 mg/dose
Route of Administration	Subcutaneous
Applicant Name	Novartis Pharmaceuticals Corporation
Therapeutic Classification/ OND Division	Division of Diabetes, Lipid Disorders, and Obesity
Manufacturing Site	The amendment replaces the prior manufacturing site with the proposed Sandoz site: Sandoz GmbH 3004828473 Biochemiestrasse 10, Langkampfen, Austria, 6336
Method of Sterilization	(b) (4)

Assessment Recommendation: **Adequate**

Assessment Summary:

Document(s) Assessed	Date Received
Original submission (eCTD seq. 0058)	07/01/2021
IR Response	08/17/2021
IR Response	09/03/2021

List Submissions being assessed (table): See table

Highlight Key Issues from Last Cycle and Their Resolution: N/A

Remarks: In the previous cycle, the microbiology review was adequate. However, the inspection resulted in an OAI at the previous DP manufacturing site. Therefore, the subject supplement proposed a new DP manufacturing site.

Note that the original assessor of this application was Avital Shimanovich. Since she departed the Agency, Erika Pfeiler assessed the IR responses.

Concise Description of Outstanding Issues: N/A

Supporting Documents: N/A

Microbiological Assessment of the Proposed Change

The applicant proposed to replace the previously proposed manufacturing site, (b) (4), with the Sandoz site in Austria. Although the first review cycle was deemed adequate by microbiology for manufacturing at the (b) (4) site, the PAI of the (b) (4) site resulted in a withhold of the subject application. In the Type A meeting following the CRL, the applicant requested to submit an amendment to replace the previously proposed site at (b) (4) which was a CMO with a manufacturing site owned and operated by the applicant. The subject amendment describes the manufacturing process and validation at the Sandoz site.

S DRUG SUBSTANCE

N/A, no changes. The drug substance is a non-sterile chemically modified double stranded 21-23mer small interfering RNA conjugated to N-GalNAc.

P.1 DESCRIPTION OF THE COMPOSITION OF THE DRUG PRODUCT

- **Description of the drug product –** The drug product is a clear, sterile solution in a 2.25 mL pre-filled syringe (PFS).

- **Composition of the drug product –** The following table provides the composition of the drug product:

Ingredient	Function	Amount per PFS
Inclisiran Sodium, in-house	300 mg	API
WFI, USP	q.s. to 1.5 mL	Diluent
Sodium Hydroxide, NF	q.s. to pH	pH adjustor
Phosphoric Acid, NF	q.s. to pH	pH adjustor

- **Description of the container closure system –** The following table provides a description of the container closure system (CCS):

	Description	Supplier (b) (4)
Syringe		
Needle		
(b) (4)		
Plunger		

Assessment: *N/A, there are no changes to this section. This information is provided for ease of review.*

P.2 PHARMACEUTICAL DEVELOPMENT

P.2.5 MICROBIOLOGICAL ATTRIBUTES

Container/Closure and Package Integrity

Assessment: *N/A, the container closure system (CCS) has not been changed.*

P.3 MANUFACTURE

P.3.1 MANUFACTURERS

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Primary Microbiology Assessor Name and Date:
Avital Shimanovich, PhD, 07/23/2021
Erika Pfeiler, Ph.D., 09/07/2021
Secondary Assessor Name and Date: John Metcalfe, PhD, 09/07/2021

Erika Pfeiler

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John Metcalfe

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Comments: I concur.

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M E M O R A N D U M

DATE: 30 November 2021

TO: NDA 214012

FROM: Erika Pfeiler, Ph.D.

THROUGH: John Metcalfe, Ph.D.

SUBJECT: Updates to DMFs submitted on 01 July 2021 (SDN 61)

In the amendment submitted on 01 July 2021, the applicant provided updated letters of authorization for (b) (4) DMFs (b) (4) as an update from the previously submitted LOA for DMF (b) (4) which was provided in the first review cycle. (Note that the file name in the SDN 61 submission for LOA (b) (4) is the same as the previously submitted LOA for DMF (b) (4).) This change in letters of authorization was not documented or assessed in microbiology assessment N214012MR02.

The (b) (4) is described in the LOAs as (b) (4). Recent assessments of the respective DMFs found in microbiology assessments D (b) (4) M22R01, D (b) (4) M05R01, and D (b) (4) M06R01 demonstrate the suitability of these (b) (4) .

(b) (4) were described in the updated section 3.2.P.3 submitted on 01 July. However, since (b) (4) , this represents a low risk to product sterility and no further information will be requested from the applicant.

There are no changes to microbiology's previous recommendation for approval for this application.

END

Erika Pfeiler

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John Metcalfe

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/s/

MUTHUKUMAR RAMASWAMY
11/30/2021 04:23:54 PM

QUALITY ASSESSMENT

Recommendation: Complete Response

NDA 214012
Review 1

Drug Name/Dosage Form	Leqvio (inclisiran) injection
Strength	284 mg/1.5 mL
Route of Administration	Subcutaneous injection
Rx/OTC Dispensed	Rx
Applicant	Novartis Pharmaceuticals
US agent, if applicable	-

SUBMISSION(S) REVIEWED	DOCUMENT DATE	DISCIPLINE(S) AFFECTED
Pre-submission, Original submission, and amendments	Original submission: 12/23/2019 Amendments: 2/20/2020, 3/16/2020 and 4/14/2020 and 4/24/2020 (Stability), 6/03/2020 (Microbiology), 6/19/2020, 6/24/20 (Label), 8/7/20 (drug product), 8/14/2020 (Facility) and 9/29/20.	Quality modules 3, 1.14, and 1.11

Quality Review Team

Discipline	Reviewer/Secondary	Branch/division
Drug Substance	Daniel Jansen/ Suong Tran	Branch III/Division of New Drug Active pharmaceutical
Drug Product	Theodore Carver / David J Claffey	Branch V/New Drug Products III
Process/ Facility	Kumar Janoria/ Vidya Pai	Branch II/ Office of Pharmaceutical Manufacturing Assessment (OPMA)
Microbiology	Avital Shimanovich/ John Metcalfe	OPMA
Regulatory Business Process Manager	Leeza Rahimi/ Hamet Toure	Branch I/Regulatory Business Process Management I
Application Technical Lead	Muthukumar Ramaswamy	Branch V/New Drug Products III
Environmental Analysis (EA)	Theodore Carver/ David J Claffey	Branch V/New Drug Products III of New Drug Products

Quality Review Data Sheet

1. RELATED/SUPPORTING DOCUMENTS

A. DMFs:

DMF #	Type	Holder	Item Referenced	Status	Date Assessment Completed	Comments
(b) (4)	III		(b) (4)	Active	9/24/2019	LOA dated 4/10/2019
	III			Active	*	LOA dated 5/6/2019
	V			Active	5/03/2019	LOA dated 5/6/2019
	V			Active	1/02/2020	LOA dated 4/10/2019
	III			Active	*	LOA dated 4/10/2019

*Sufficient information provided in the NA

B. Other Documents: *IND, RLD, or sister applications*

DOCUMENT	APPLICATION NUMBER	DESCRIPTION
IND	127589	Inclisiran injection

2. CONSULTS: None

DISCIPLINE	STATUS	RECOMMENDATION	DATE	REVIEWER

Executive Summary

I. Recommendations and Conclusion on Approvability

The Office of Pharmaceutical Quality (OPQ) recommends a **complete response** action due to OPMA's 'withhold approval' recommendation because of deficiencies related to the (b) (4) drug product manufacturing site.

Summary of Complete Response Issues
The drug product is manufactured at (b) (4) located at (b) (4). This facility was inspected by FDA in (b) (4) and a 6-item 483 was issued. A pre-approval inspection of this facility was recommended by the facility review team to verify the corrective actions and confirm the required (b) (4). Due to travel restrictions, a 704(a)(4) records review was initiated in lieu of a PAI. The 704(a)4 records request and review process identified several deficiencies related to (b) (4) (b) (4) Therefore, facility review concluded that a pre-approval inspection and/or adequate response to post-action letter (PAL) is needed before this application may be approved. The overall manufacturing inspection recommendation (OMIR) from the Office of Process Manufacturing Assessment (OPMA) for this NDA in Panorama is "withhold approval'. Thus, the overall facility assessment recommendation from OPMA for this NDA is inadequate due to facilities deficient for inadequate response to 704(a)(4) request.

Action Letter Approvability Deficiencies:

During a review of records requested under section 704(a)(4) of the Federal Food, Drug, and Cosmetic Act, and provided by (b) (4) (b) (4) manufacturing facility, the FDA noted objectionable conditions. These objectionable conditions will be conveyed to the representative of the facility within 10 business days of this Complete Response Letter. Satisfactory resolution of these objectionable conditions is required (e.g., preapproval inspection and/or adequate facility responses addressing these conditions) before this application may be approved.

If it is determined that an inspection is needed to approve your application, please note that FDA continues to monitor the public health situation as well as travel restrictions. We are actively working to define an approach for scheduling outstanding inspections, once safe travel may resume and based on public health need and other factors.
For more information, please see the FDA guidances related to COVID 19. These guidances can be found at https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-related-guidance-documents-industry-fda-staff-and-other-stakeholders

Additional comments (Not approvability deficiency)
Include an identification (ID) test in the drug product specification that has higher specificity for inclisiran. Although the ID tests in the current specification, duplex retention time by IPRP-HPLC and ID by infrared spectrum, are orthogonal tests, they may not provide an adequate level of discrimination with respect to similar siRNA products that may be manufactured at the same facility. Similar siRNA products may also have similar IR spectra and duplex retention times. The test for identity by single strand molecular weight, which is used as an ID test for the inclisiran drug substance, has more discriminating power and would be appropriate for use as an additional ID test for the drug product.

II. Summary of Quality Assessments

A. Product Overview

Inclisiran is a small interfering RNA (siRNA) duplex containing a 21-nucleotide long sense strand linked to a triantennary acetyl galactosamine (GalNAc) on the 3' end and a 23-nucleotide long anti-sense strand. Inclisiran sodium is a new molecular entity The proposed product, Leqvio (inclisiran) injection, 189 mg/mL is a clear, colorless to pale yellow sterile solution provided as a single-use 1.5 mL pre-filled syringe. Each syringe contains 284 mg inclisiran (equivalent to 300 mg inclisiran sodium).

Leqvio is for adults with primary hyperlipidemia (including heterozygous familial hypercholesterolemia (HeFH) or clinical atherosclerotic cardiovascular disease (CVD), who require additional lowering of to reduce low-density lipoprotein cholesterol (LDL-C). Leqvio is for subcutaneous administration by healthcare professional. After initial dosing, the proposed product is for dosing once every 6 months.

Inclisiran contains modifications at the 2'ribose moiety as well as phosphodiester backbone to stability of the siRNA in vivo. The inclusion of a triantennary acetyl galactosamine (GalNAc) ligand on the sense strand of siRNA facilitates the uptake of siRNA by hepatocytes. In hepatocytes, the anti-sense strand of the siRNA duplex inhibits the production of proprotein convertase subtilisin/kexin type 9 (PCSK9) protein by directing the breakdown of mRNA for PCSK9 protein and thus contributes the lowering of LDL-C levels in the circulation.

Leqvio is formulated in water for injection as a preservative free solution with a target pH of 7. Liqvio should be stored at 20°C to 25°C (68°F to 77°F) (b) (4). Excursions permitted between 15°C and 30°C (59°F and 86°F)

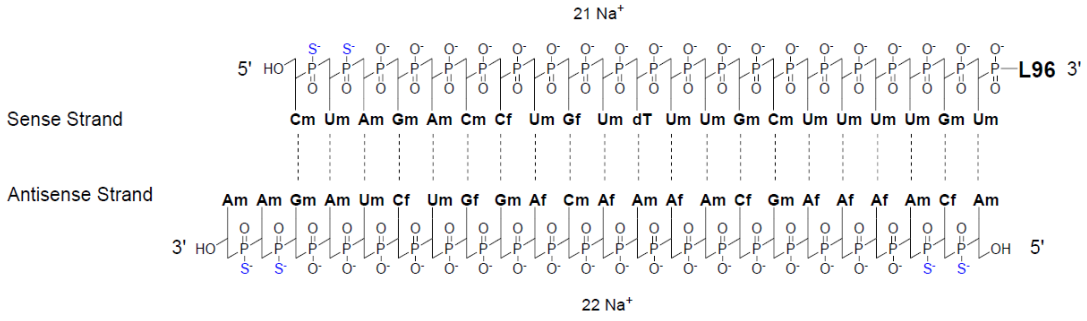
Proposed Indication(s) including Intended Patient Population	Treating adults with primary hyperlipidemia (including heterozygous familial hypercholesterolemia (HeFH) or clinical atherosclerotic cardiovascular disease (CVD), who

	require additional lowering of to reduce low-density lipoprotein cholesterol (LDL-C)
Duration of Treatment	3 doses for the 1st year and then twice a year dosing schedule.
Maximum Daily Dose	284 mg inclisiran (provided as 300mg inclisiran sodium)
Alternative Methods of Administration	Not applicable

B. Quality Assessment Overview

Drug Substance

Inclisiran is a chemically modified small interfering RNA (siRNA) duplex. The siRNA duplex contains a 21-nucleotide sense strand conjugated with triantennary acetyl galactosamine, (GalNAc) on the 3' end. The anti-sense strand contains 23 nucleotides. With one exception, the siRNA contains modifications at the 2' ribose moiety (as a 2'-fluoroadenosine -2'-fluoro-cytidine or 2'-fluoro-guanosine or a 2'-O-methyl adenosine, 2'-O-methyl -cytidine or 2'-O-methyl guanosine or 2'-O-methyl-uridine) to confer stability. In addition, six of the terminal phosphodiester backbones are modified to 3'-5'-phosphorothioate linkage to protect the siRNA from nucleases. The structure of inclisiran as illustrated in the NDA is reproduced below.



Inclisiran Sodium

Af, Cf, Gf = 2'-F ribonucleotides
Am, Cm, Gm, Um = 2'-OMe ribonucleotides
dT = thymidine
s = phosphorothioate
L96 = Triantennary N-acetyl galactosamine moeity

The molecular formula and molecular weight of inclisiran salt form is respectively $C_{529}H_{664}F_{12}N_{176}Na_{43}O_{316}P_{43}S_6$ and 17,284.75 g/mol. Inclisiran sodium is a white to pale white powder and is freely soluble in water.

Inclisiran sodium is (b) (4) (b) (4) (b) (4) the drug substance as a white to pale white powder. The structure and properties of the drug substance has been well characterized using state-of-the-art analytical methods.

Dr. Daniel Jensen reviewed the chemistry, manufacturing, and control information for the drug substance including manufacturing process description, the proposed starting material specifications, control strategy for impurities, characterization data for drug substance and its impurities, reference standard information, test method descriptions and methods validation, drug substance specifications, and stability data. His review concluded that the drug substance information is adequate to control the identity, purity, strength, and quality of the drug substance used for manufacturing the drug product. The proposed drug substance specifications are consistent with batches used in clinical, nonclinical, and registration stability studies.

Based on review of the stability information, Dr. Jansen granted a retest period of (b) (4) months for the drug substance when stored (b) (4) (b) (4) Dr. Jensen's recommendation for this application is adequate. For additional details, please refer to Dr. Jensen's CMC review dated 7/16/2020 in Panorama.

Drug Product

Drug Product Formulation: The proposed product is a clear, colorless, sterile, aqueous solution packaged in a 1.5 mL single patient use pre-filled pen. Each mL of Leqvio contains 189 mg of inclisiran (provided as 200 mg of inclisiran sodium) in water for injection. Each pre-filled pen contains 284 mg of inclisiran. The final pH of the formulation is adjusted with sodium hydroxide and phosphoric acid to 7. The composition of the proposed commercial product is the same as the one used during development. The viscosity of the product is 20cP. The osmolality of the product (~300 mOsm/kg).

Initially, a vial presentation was used during development. The final commercial presentation (pre-filled pen) was used for Phase III studies. The primary container closure system consists of (b) (4) 2.25 mL Type (b) (4) glass syringe fitted with a 27G staked needle and (b) (4) on one end and a (b) (4) (b) (4) plunger on the other end as the. The final product is provided in a carton. (b) (4) The container closure components proposed for use in the product are known for use in approved products and the applicant provided leachable study assessment to assure the safety of the proposed container closure system.

Dr. Ted Carver reviewed the drug product information including drug product composition, drug product specification, excipient information, analytical methods, container closure system, compatibility information, and stability data. The compatibility of the active ingredient with excipients and the container closure components is supported by drug product stability data.

The drug product is tested for visual clarity, color, appearance, identity, particulate matter, pH, osmolality, assay, purity and impurities, deliverable volume, uniformity of dosage units, closure integrity, gliding and break loose force, endotoxin content, and

sterility. The drug product conforms to USP <1> injection, USP <788> particulate matter, US<71> sterility, USP <785> osmolality, USP <697> container content for injections, and USP <85> bacterial endotoxin. Dr. Carver concluded that the proposed specification is adequate to support the quality of the proposed product. Please refer to the drug product review dated 9/4/20 for additional information.

Manufacturing Process and Controls: The manufacturing process involves (b) (4) (b) (4)

The applicant is using batch record instructions and the following in-process control tests to control quality of the product: (b) (4) (b) (4) container closure integrity, and visual inspection of the product.

The composition of the drug product, the drug product manufacturing process, and the packaging system used to manufacture the drug product used in phase 3 clinical and registration stability studies are the same as that proposed for commercial use. The batch size of registration stability batches ranged from (b) (4) The proposed commercial batch size is (b) (4).

No overage is used. No reprocessing will be employed in the manufacture of this drug product. For detailed information on the manufacturing process and process control, refer to Dr. Janoria' s process review in Panorama dated 9/30/2020. The process review concluded that the proposed drug product manufacturing process controls are adequate to support the NDA.

Microbiological control information: Microbiology reviewer, Dr. Shimanovich reviewed the microbiological controls used in the drug product manufacturing process including (b) (4), drug product specifications for sterility, container closure integrity, endotoxin, (b) (4), media fill studies, hold times, and post-approval stability commitment. Dr. Shimanovich also reviewed the (b) (4) and validation information provided in Type V DMF (b) (4) (b) (4). Her review concluded that the proposed microbiological controls are adequate to support the NDA. Refer to CMC (Microbiology) review by Dr. Shimanovich dated 6/12/2020 in Panorama.

Control Strategy: The critical quality attributes of the product are controlled through batch records instructions, process design, excipient specifications including bioburden and endotoxin control, drug substance specification, component specifications for incoming container closure components, (b) (4) techniques, in-process controls for the (b) (4), bioburden,

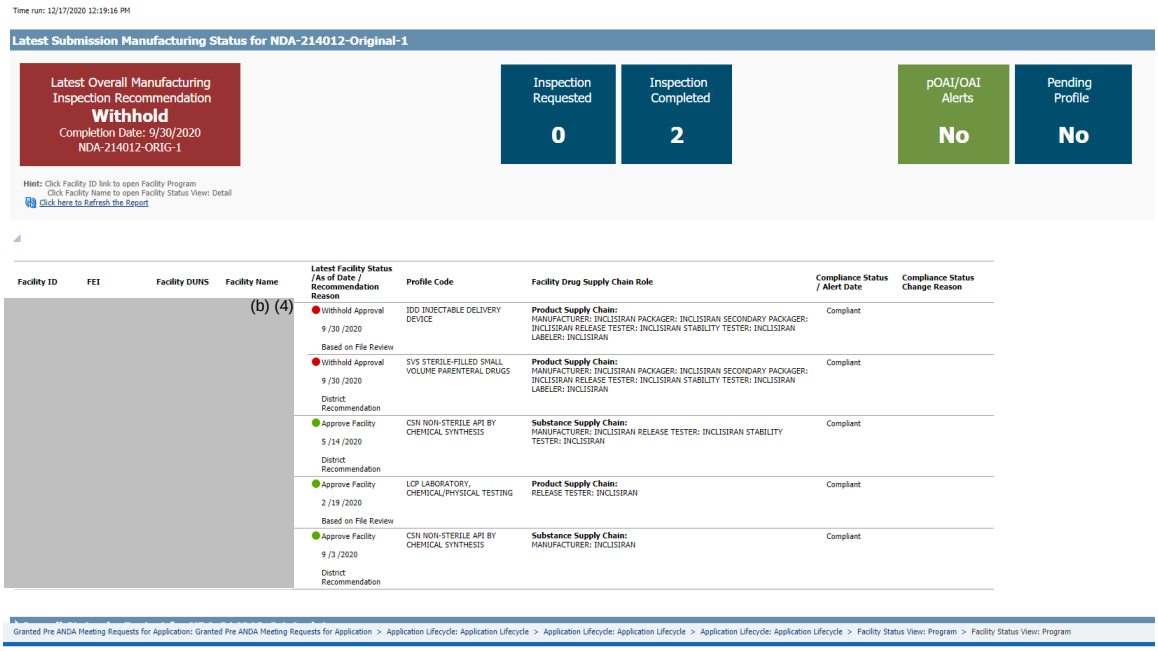
visual verification (b) (4), product pH verification, (b) (4), and adequate finished product specification.

The proposed control strategy is adequate to assure the quality of the product. For additional information, please refer to the following CMC reviews in Panorama: Dr. Jansen's drug substance review dated 7/16/2020, Dr. Shimanovich 's microbiology review dated 6/12/2020, Dr. Carver's drug product review dated 9/4/2020 and Dr. Janoria' s process reviews dated 9/30/2020.

Risk Assessment: CMC Reviewer performed a risk assessment for critical attributes and concluded that the final risk is low for the proposed product.

Facility compliance information: Facility compliance information for the drug product and drug substance manufacturing and testing facilities was reviewed by Dr. Kumar Janoria. His facility review concluded that with the exception of drug product manufacturing facility located (b) (4) the manufacturing facilities associated with this application are acceptable (b) (4) (b) (4) (b) (4)

The drug product manufacturing facility was inspected by FDA in (b) (4). Due to concerns related to the operations and (b) (4) used for manufacturing the proposed product, a pre-approval inspection of the drug product manufacturing facility was recommended by the facility review team. This is to confirm the (b) (4) and to verify the corrective actions. Due to travel restrictions, a 704(a)4 records review was initiated in lieu of a PAI. The 704(a)4 records request and review process identified several deficiencies related to (b) (4) (b) (4) The facility review concluded that a pre-approval inspection and/or adequate facility responses addressing these conditions is needed before this application may be approved. Thus, the overall manufacturing inspection recommendation (OMIR) from the Office of Process Manufacturing Assessment (OPMA) for this NDA is withhold approval. The Panorama screen shot facility assessment recorded on 12/17/2020 is shown below. For additional details, please refer to Dr. Janoria' s facility review in Panorama dated 9/30/2020 and 12/17/20.



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Latest Submission Manufacturing Status for NDA-214012-Original-1

Latest Overall Manufacturing Inspection Recommendation: **Withhold**
Completion Date: 9/30/2020
NDA-214012-ORIG-1

Inspection Requested	Inspection Completed	pOAI/OAI Alerts	Pending Profile
0	2	No	No

Hint: Click Facility ID link to open Facility Program
Click Facility Name to open Facility Status View: Detail
Click here to Refresh the Report

Facility ID	FEI	Facility DUNS	Facility Name	Latest Facility Status /As of Date / Recommendation Reason	Profile Code	Facility Drug Supply Chain Role	Compliance Status / Alert Date	Compliance Status Change Reason
(b) (4)				Withhold Approval 9/30/2020 Based on File Review	IDD INJECTABLE DELIVERY DEVICE	**Product Supply Chain:** MANUFACTURER: INCLISIRAN PACKAGER: INCLISIRAN SECONDARY PACKAGER: INCLISIRAN RELEASE TESTER: INCLISIRAN STABILITY TESTER: INCLISIRAN LABELER: INCLISIRAN	Compliant	
				Withhold Approval 9/30/2020 District Recommendation	SVS STERILE-FILLED SMALL VOLUME PARENTERAL DRUGS	**Product Supply Chain:** MANUFACTURER: INCLISIRAN PACKAGER: INCLISIRAN SECONDARY PACKAGER: INCLISIRAN RELEASE TESTER: INCLISIRAN STABILITY TESTER: INCLISIRAN LABELER: INCLISIRAN	Compliant	
				Approve Facility 5/14/2020 District Recommendation	CSN NON-STERILE API BY CHEMICAL SYNTHESIS	**Substance Supply Chain:** MANUFACTURER: INCLISIRAN RELEASE TESTER: INCLISIRAN STABILITY TESTER: INCLISIRAN	Compliant	
				Approve Facility 2/19/2020 Based on File Review	LCP LABORATORY, CHEMICAL/PHYSICAL TESTING	**Product Supply Chain:** RELEASE TESTER: INCLISIRAN	Compliant	
				Approve Facility 9/3/2020 District Recommendation	CSN NON-STERILE API BY CHEMICAL SYNTHESIS	**Substance Supply Chain:** MANUFACTURER: INCLISIRAN	Compliant	

Granted Pre ANDA Meeting Requests for Application: Granted Pre ANDA Meeting Requests for Application > Application Lifecycle: Application Lifecycle > Application Lifecycle: Application Lifecycle > Application Lifecycle: Application Lifecycle > Facility Status View: Program > Facility Status View: Program

Environmental assessment: The applicant sought exemption from environmental impact analysis per 21CFR 25.31(b) as the action on this NDA may not significantly affect the quality of the human environment. The estimated concentration of the drug substance at the point of entry into the aquatic environment would be below 1 part per billion (1 ppb). Dr. Ted Carver granted categorical exclusion from submitting environmental assessment. Please refer to drug product review dated 9/4/20 for additional information.

Expiration Date & Storage Conditions: The application contains 24 months of long-term stability data (25°C/60% RH), 12 month accelerated stability (40°C/65% RH), for 3 primary stability batches manufactured at (b) (4) scale. Dr. Ted Carver reviewed the stability information. The product is (b) (4) *Based on available stability data, an expiration period of 24 months is granted when stored at 20°C to 25°C (68°F to 77°F) in commercial packaging.* Please refer to the drug product review dated 9/4/20 in Panorama for additional information.

Container and Carton Label Review: The drug product reviewer completed review of container and carton label. The dosage form description, strength, established name, NDC #, Lot #/Expiry, and storage conditions are adequately described in the carton and container label, which meets relevant regulatory requirements for labeling. Refer to the Dr. Carver's labeling review dated 9/4/2020 for additional information.

OVERALL ASSESSMENT AND SIGNATURES:

At present, there are no outstanding deficiencies related to the drug substance, drug product, process, microbiology, environmental analysis, and container/ carton label sections of the NDA. Due to outstanding manufacturing facility related deficiencies, the OPQ overall recommendation for NDA *214012 is complete response.*

Muthukumar Ramaswamy, Ph.D. 12/17/2020

Application Technical Lead Name and Date:

18 Page(s) have been Withheld in Full as b4 (CCI/TS) immediately following this page

Vidya Pai

Digitally signed by Vidya Pai
Date: 12/17/2020 01:10:46PM
GUID: 53b581d20000464509a65e37ec9ad4a2

Kumar Janoria

Digitally signed by Kumar Janoria
Date: 12/17/2020 01:08:27PM
GUID: 53160a1a0000844db16f58dfff6949ed

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/

MUTHUKUMAR RAMASWAMY
12/17/2020 02:43:33 PM
This review is an addendum to the previous integrated quality review dated 10/1/20 for NDA 214012 in DARRTS

QUALITY ASSESSMENT

Recommendation: Complete Response

NDA 214012
Review 1

Drug Name/Dosage Form	Leqvio (inclisiran) injection
Strength	284 mg/1.5 mL
Route of Administration	Subcutaneous injection
Rx/OTC Dispensed	Rx
Applicant	Novartis Pharmaceuticals
US agent, if applicable	-

SUBMISSION(S) REVIEWED	DOCUMENT DATE	DISCIPLINE(S) AFFECTED
Pre-submission, Original submission, and amendments	Original submission: 12/23/2019 Amendments: 2/20/2020, 3/16/2020 and 4/14/2020 and 4/24/2020 (Stability), 6/03/2020 (Microbiology), 6/19/2020, 6/24/20 (Label), 8/7/20 (drug product), 8/14/2020 (Facility) and 9/29/20.	Quality modules 3, 1.14, and 1.11

Quality Review Team

Discipline	Reviewer/Secondary	Branch/division
Drug Substance	Daniel Jansen/ Suong Tran	Branch III/Division of New Drug Active pharmaceutical
Drug Product	Theodore Carver / David J Claffey	Branch V/New Drug Products III
Process/ Facility	Kumar Janoria/ Vidya Pai	Branch II/ Office of Pharmaceutical Manufacturing Assessment (OPMA)
Microbiology	Avital Shimanovich/ John Metcalfe	OPMA
Regulatory Business Process Manager	Leeza Rahimi/ Hamet Toure	Branch I/Regulatory Business Process Management I
Application Technical Lead	Muthukumar Ramaswamy	Branch V/New Drug Products III
Environmental Analysis (EA)	Theodore Carver/ David J Claffey	Branch V/New Drug Products III of New Drug Products

Quality Review Data Sheet

1. RELATED/SUPPORTING DOCUMENTS

A. DMFs:

DMF #	Type	Holder	Item Referenced	Status	Date Assessment Completed	Comments
(b) (4)	III		(b) (4)	Active	9/24/2019	LOA dated 4/10/2019
	III			Active	*	LOA dated 5/6/2019
	V			Active	5/03/2019	LOA dated 5/6/2019
	V			Active	1/02/2020	LOA dated 4/10/2019
	III			Active	*	LOA dated 4/10/2019

*Sufficient information provided in the NA

B. Other Documents: *IND, RLD, or sister applications*

DOCUMENT	APPLICATION NUMBER	DESCRIPTION
IND	127589	Inclisiran injection

2. CONSULTS: None

DISCIPLINE	STATUS	RECOMMENDATION	DATE	REVIEWER

Executive Summary

I. Recommendations and Conclusion on Approvability

The Office of Pharmaceutical Quality (OPQ) recommends a **complete response** action due to OPMA's 'withhold approval' recommendation because of deficiencies related to the (b) (4) drug product manufacturing site.

Summary of Complete Response Issues
The drug product is manufactured at (b) (4) located at (b) (4) This facility was inspected by FDA in (b) (4) and a 6-item 483 was issued. A pre-approval inspection of this facility was recommended by the facility review team to verify the corrective actions and confirm the required (b) (4). Due to travel restrictions, a 704(a)(4) records review was initiated in lieu of a PAI. The 704(a)4 records request and review process identified several deficiencies related to (b) (4) (b) (4) Therefore, facility review concluded that a pre-approval inspection and/or adequate facility responses addressing these conditions is needed before this application may be approved. The overall manufacturing inspection recommendation (OMIR) from the Office of Process Manufacturing Assessment (OPMA) for this NDA in Panorama is "withhold approval'. Thus, the overall facility assessment recommendation from OPMA for this NDA is inadequate.

Action Letter Approvability Deficiencies:

During a review of records requested under section 704(a)(4) of the Federal Food, Drug, and Cosmetic Act, and provided by (b) (4) manufacturing facility, the FDA noted objectionable conditions. These objectionable conditions will be conveyed to the representative of the facility within 10 business days of this Complete Response Letter. Satisfactory resolution of these objectionable conditions is required (e.g., preapproval inspection and/or adequate facility responses addressing these conditions) before this application may be approved.

If it is determined that an inspection is needed to approve your application, please note that FDA continues to monitor the public health situation as well as travel restrictions. We are actively working to define an approach for scheduling outstanding inspections, once safe travel may resume and based on public health need and other factors.
For more information, please see the FDA guidances related to COVID 19. These guidances can be found at https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-related-guidance-documents-industry-fda-staff-and-other-stakeholders

Additional comments (Not approvability deficiency)
Include an identification (ID) test in the drug product specification that has higher specificity for inclisiran. Although the ID tests in the current specification, duplex retention time by IPRP-HPLC and ID by infrared spectrum, are orthogonal tests, they may not provide an adequate level of discrimination with respect to similar siRNA products that may be manufactured at the same facility. Similar siRNA products may also have similar IR spectra and duplex retention times. The test for identity by single strand molecular weight, which is used as an ID test for the inclisiran drug substance, has more discriminating power and would be appropriate for use as an additional ID test for the drug product.

II. Summary of Quality Assessments

A. Product Overview

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Leqvio is for adults with primary hyperlipidemia (including heterozygous familial hypercholesterolemia (HeFH) or clinical atherosclerotic cardiovascular disease (CVD), who require additional lowering of to reduce low-density lipoprotein cholesterol (LDL-C). Leqvio is for subcutaneous administration by healthcare professional. After initial dosing, the proposed product is for dosing once every 6 months.

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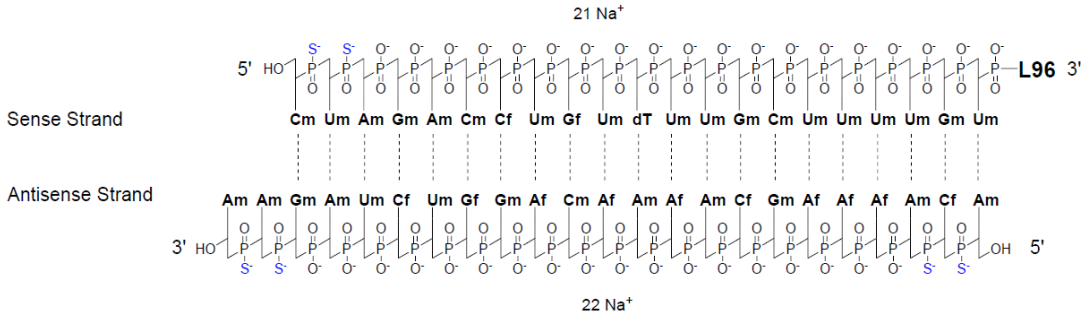

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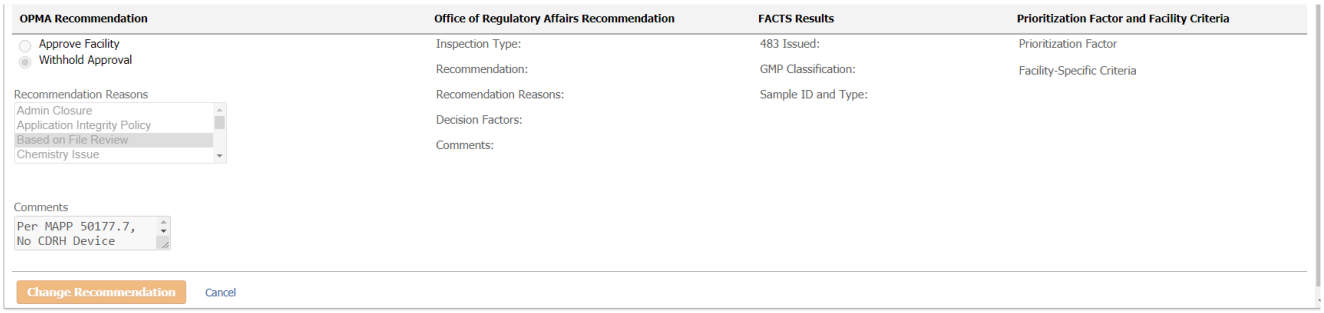
The proposed control strategy is adequate to assure the quality of the product. For additional information, please refer to the following CMC reviews in Panorama: Dr. Jansen's drug substance review dated 7/16/2020, Dr. Shimanovich 's microbiology review dated 6/12/2020, Dr. Carver's drug product review dated 9/4/2020 and Dr. Janoria' s process reviews dated 9/30/2020.

Risk Assessment: CMC Reviewer performed a risk assessment for critical attributes and concluded that the final risk is low for the proposed product.

Facility compliance information: Facility compliance information for the drug product and drug substance manufacturing and testing facilities was reviewed by Dr. Kumar Janoria. His facility review concluded that with the exception of drug product manufacturing facility located in (b) (4), the manufacturing facilities associated with this application are acceptable (b) (4) (b) (4) (b) (4)

The drug product manufacturing facility was inspected by FDA in (b) (4). Due to concerns related to the operations and (b) (4) used for manufacturing the proposed product, a pre-approval inspection of the drug product manufacturing facility was recommended by the facility review team. This is to confirm the (b) (4) and to verify the corrective actions. Due to travel restrictions, a 704(a)4 records review was initiated in lieu of a PAI. The 704(a)4 records request and review process identified several deficiencies related to (b) (4) (b) (4) The facility review concluded that a pre-approval inspection and/or adequate facility responses addressing these conditions is needed before this application may be approved. Thus, the overall manufacturing inspection recommendation (OMIR) from the Office of Process Manufacturing Assessment (OPMA) for this NDA is withhold approval. The Panorama screen shot facility assessment recorded on 10/1/2020 is shown below. For additional details, please refer to Dr. Janoria' s facility review in Panorama dated 9/30/2020.

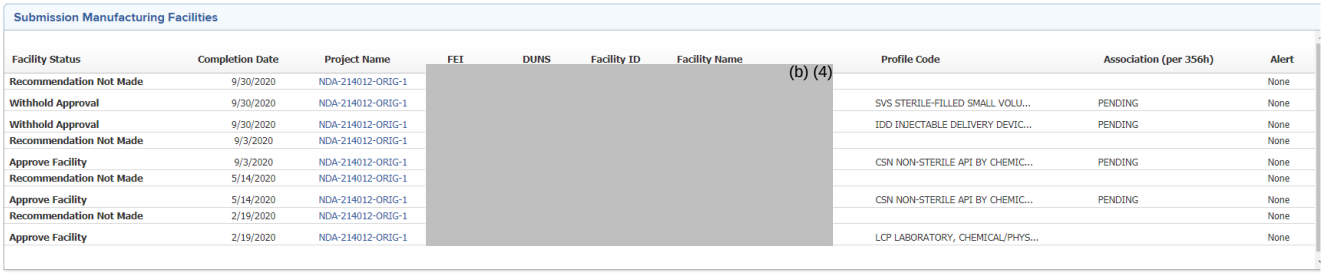


OPMA Recommendation	Office of Regulatory Affairs Recommendation	FACTS Results	Prioritization Factor and Facility Criteria
○ Approve Facility ◉ Withhold Approval	Inspection Type: Recommendation: Recomendation Reasons: Decision Factors: Comments:	483 Issued: GMP Classification: Sample ID and Type:	Prioritization Factor Facility-Specific Criteria

Recommendation Reasons: Admin Closure; Application Integrity Policy; Based on File Review; Chemistry Issue

Comments: Per MAPP 50177.7, No CDRH Device

Change Recommendation | Cancel



Submission Manufacturing Facilities

Facility Status	Completion Date	Project Name	FEI	DUNS	Facility ID	Facility Name	Profile Code	Association (per 356h)	Alert
Recommendation Not Made	9/30/2020	NDA-214012-ORIG-1				(b) (4)			None
Withhold Approval	9/30/2020	NDA-214012-ORIG-1					SVS STERILE-FILLED SMALL VOLU...	PENDING	None
Withhold Approval	9/30/2020	NDA-214012-ORIG-1					IDD INJECTABLE DELIVERY DEVIC...	PENDING	None
Recommendation Not Made	9/3/2020	NDA-214012-ORIG-1							None
Approve Facility	9/3/2020	NDA-214012-ORIG-1					CSN NON-STERILE API BY CHEMIC...	PENDING	None
Recommendation Not Made	5/14/2020	NDA-214012-ORIG-1							None
Approve Facility	5/14/2020	NDA-214012-ORIG-1					CSN NON-STERILE API BY CHEMIC...	PENDING	None
Recommendation Not Made	2/19/2020	NDA-214012-ORIG-1							None
Approve Facility	2/19/2020	NDA-214012-ORIG-1					LCP LABORATORY, CHEMICAL/PHYS...		None

<u>Environmental assessment</u>: The applicant sought exemption from environmental impact analysis per 21CFR 25.31(b) as the action on this NDA may not significantly affect the quality of the human environment. The estimated concentration of the drug substance at the point of entry into the aquatic environment would be below 1 part per billion (1 ppb). Dr. Ted Carver granted categorical exclusion from submitting environmental assessment. Please refer to drug product review dated 9/4/20 for additional information.

<u>Expiration Date & Storage Conditions</u>: The application contains 24 months of long-term stability data (25°C/60% RH), 12 month accelerated stability (40°C/65% RH), for 3 primary stability batches manufactured at 5.5L to 12L scale. Dr. Ted Carver reviewed the stability information. The product is (b) (4). *Based on available stability data, an expiration period of 24 months is granted when stored at 20°C to 25°C (68°F to 77°F) in commercial packaging.* Please refer to the drug product review dated 9/4/20 in Panorama for additional information.

<u>Container and Carton Label Review</u>: The drug product reviewer completed review of container and carton label. The dosage form description, strength, established name, NDC #, Lot #/Expiry, and storage conditions are adequately described in the carton and container label, which meets relevant regulatory requirements for labeling. Refer to the Dr. Carver's labeling review dated 9/4/2020 for additional information.

OVERALL ASSESSMENT AND SIGNATURES:

At present, there are no outstanding deficiencies related to the drug substance, drug product, process, microbiology, environmental analysis, and container/ carton label sections of the NDA. Due to outstanding manufacturing facility related deficiencies, OPQ overall recommendation for NDA *214012 is complete response.*

Muthukumar Ramaswamy, Ph.D. 10/1/2020

Application Technical Lead Name and Date:

Muthukumar Ramaswamy

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David Claffey

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CHAPTER VII: MICROBIOLOGY

IQA NDA Assessment Guide Reference

Product Information	
NDA Number	214012
Assessment Cycle Number	01
Drug Product Name/ Strength	Inclisiran, 300 mg/dose
Route of Administration	Subcutaneous
Applicant Name	The Medicines Company
Therapeutic Classification/ OND Division	Division of Metabolism and Endocrinology Products
Manufacturing Site	(b) (4)
Method of Sterilization	(b) (4)

Assessment Recommendation: **Adequate**

Assessment Summary:

Document(s) Assessed	Date Received
Initial submission	12/23/2019
CMC IR responses	04/24/2020
CMC IR response	06/03/2020

List Submissions being assessed: See table

Highlight Key Issues from Last Cycle and Their Resolution: N/A

Remarks: The drug product is a single dose pre-filled syringe for treatment of hypercholesterolemia. The API is a siRNA.

Concise Description of Outstanding Issues: None

Supporting Documents:

- (b) (4)

- (b) (4)
-

S DRUG SUBSTANCE

The drug substance is a chemically modified double stranded 21-23mer small interfering RNA conjugated to N-GalNAc. The drug substance is not-sterile and, therefore, the drug substance will not be assessed for sterility assurance.

P.1 DESCRIPTION OF THE COMPOSITION OF THE DRUG PRODUCT

- **Description of the drug product –** The drug product is a clear, sterile solution in a pre-filled syringe (PFS).

- **Composition of the drug product –** The following table provides the composition of the drug product:

Ingredient	Function	Amount per PFS
Inclisiran Sodium, in-house	300 mg	API
WFI, USP	q.s. to 1.5 mL	Diluent
Sodium Hydroxide, NF	q.s. to pH	pH adjustor
Phosphoric Acid, NF	q.s. to pH	pH adjustor

- **Description of the container closure system –** The following table provides a description of the container closure system (CCS):

	Description	Supplier
Syringe	(b) (4)	
Needle		
(b) (4)		
Plunger		

Assessment: *Adequate*

The applicant provided a description of the drug product (DP) and the container closure system (CCS).

P.2 PHARMACEUTICAL DEVELOPMENT
P.2.5 MICROBIOLOGICAL ATTRIBUTES
Container/Closure and Package Integrity

Section 3.2.P.5.1, Specifications, Section 3.2.P.5.3, REP 1.1631 Container Closure Validation Report, Section 3.2.P.5.4, Batch Analyses

(b) (4)

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John Metcalfe

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Comments: I concur with the primary reviewer's assessment.

CHAPTER IV: LABELING

IQA NDA Assessment Guide Reference

1.0 PRESCRIBING INFORMATION

Assessment of Product Quality Related Aspects of the Prescribing Information: ***Adequate with revisions as noted below.***

1.1 HIGHLIGHTS OF PRESCRIBING INFORMATION

Item	Information Provided in the NDA	Assessor's Comments
Product Title in Highlights		
Proprietary name	INCLISIRAN.INCLISIRAN (inclisiran) injection, for subcutaneous use	Replace highlighted with proprietary name: LEQVIO (inclisiran) injection, for subcutaneous use Also include proprietary name throughout.
Established name(s)		Adequate.
Route(s) of administration		Adequate.
Dosage Forms and Strengths Heading in Highlights		
Summary of the dosage form(s) and strength(s) in metric system.	Injection: 284 mg/1.5 mL in a single-dose prefilled syringe	Change to include unit concentration: Injection: 284 mg/1.5 mL (189 mg/mL) in a single-dose prefilled syringe
Assess if the tablet is scored. If product meets guidelines and criteria for a scored tablet, state "functionally scored"		N/A

For injectable drug products for parental administration, use appropriate package type term (e.g., single-dose, multiple-dose, single-patient use). Other package terms include pharmacy bulk package and imaging bulk package.		Adequate

1.2 FULL PRESCRIBING INFORMATION

1.2.1 Section 2 (DOSAGE AND ADMINISTRATION)

Item	Information Provided in the NDA	Assessor's Comments
DOSAGE AND ADMINISTRATION section		

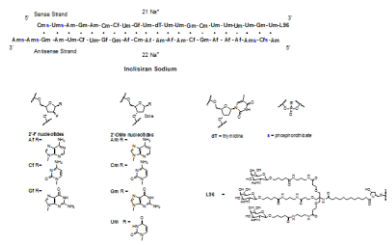
Special instructions for product preparation (e.g., reconstitution and resulting concentration, dilution, compatible diluents, storage conditions needed to maintain the stability of the reconstituted or diluted product)	**2.2 Important Administration Instructions** I (b) (4) (b) (4) into the abdomen. (b) (4) in areas of active skin disease or injury, such as sunburns, skin rashes, inflammation, or skin infections. (b) (4) INCLISIRAN should be administered by a healthcare professional. (b) (4)	Revise first highlighted section as follows: (b) (4) (b) (4) into the abdomen. (b) (4) in areas of active skin disease or injury, such as sunburns, skin rashes, inflammation, or skin infections. (b) (4) Inspect visually for particulate matter and discoloration. Do not use if discoloration or foreign particles are present. LEQVIO is a clear colorless to pale yellow solution." Revise second highlighted to use metric units: (b) (4)

1.2.2 Section 3 (DOSAGE FORMS AND STRENGTHS)

Item	Information Provided in the NDA	Assessor's Comments

DOSAGE FORMS AND STRENGTHS section		
Available dosage form(s)	INCLISIRAN (b) (4) clear, and colorless to pale yellow solution in a single-dose prefilled syringe containing 284 mg/1.5 mL for injection.	Revise to the following: "Injection: 284 mg/1.5 mL (189 mg/mL) of inclisiran as a clear, and colorless to pale yellow solution in a single-dose prefilled syringe."
Strength(s) in metric system		Revise to express units in metric form as shown in revision above.
If the active ingredient is a salt, apply the USP Salt Policy per FDA Guidance		Revise to include active moiety as shown in revised text above.
A description of the identifying characteristics of the dosage forms, including shape, color, coating, scoring, and imprinting		Adequate.
Assess if the tablet is scored. If product meets guidelines and criteria for a scored tablet, state "functionally scored"		N/A
For injectable drug products for parental administration, use appropriate labeling term (e.g., single-dose, multiple-dose, single-patient-use). Other package type terms include pharmacy bulk package and imaging bulk package.		Adequate.

1.2.3 Section 11 (DESCRIPTION)

Item	Information Provided in the NDA	Assessor's Comments
DESCRIPTION section		
Proprietary and established name(s)	INCLISIRAN contains inclisiran sodium (b) (4)	Revise to include proprietary name, as shown in revision below.
Dosage form(s) and route(s) of administration	small interfering ribonucleic acid (siRNA) covalently linked to a ligand containing three N-acetylgalactosamine (GalNAc) residues (b) (4) (b) (4) delivery to hepatocytes.	Adequate.
If the active ingredient is a salt, apply the USP Salt Policy and include the equivalency statement per FDA Guidance.	The molecular formula of inclisiran sodium is $C_{529}H_{664}F_{12}N_{176}Na_{43}O_{316}P_{43}S_6$ and its molecular weight is 17,284.72 g/mol. It has the following structural formula:  (b) (4) INCLISIRAN is a sterile, preservative-free, clear, and colorless to pale yellow solution for subcutaneous (b) (4) in a prefilled syringe (PFS). Each PFS contains 1.5 mL of solution containing 284 mg inclisiran (equivalent to 300 mg inclisiran sodium salt). INCLISIRAN is formulated in Water for Injection and may include sodium hydroxide	1st highlighted section: revise to the following: LEQVIO contains inclisiran, a Proprotein convertase subtilisin/kexin type 9-directed, (b) (4) small interfering ribonucleic acid (siRNA) covalently linked to a ligand containing three N-acetylgalactosamine (GalNAc) residues (b) (4) (b) (4) delivery to hepatocytes. 3rd highlighted section: revise as follows: "LEQVIO is a sterile, preservative-free, clear, and colorless to pale yellow solution for subcutaneous (b) (4) in a prefilled syringe (PFS). Each 1 mL of solution contains 189 mg inclisiran, provided as 200 mg inclisiran sodium salt. LEQVIO is formulated in Water for Injection and may also contain sodium hydroxide and/or phosphoric acid for pH adjustment to a target pH of 7.0."

List names of all inactive ingredients. Use USP/NF names. Avoid Brand names.	and/or phosphoric acid for pH adjustment to target 7.0.	Adequate.
For parenteral injectable dosage forms, include the name and quantities of all inactive ingredients. For ingredients added to adjust the pH or make isotonic, include the name and statement of effect.		Adequate
If alcohol is present, must provide the amount of alcohol in terms of percent volume of absolute alcohol		N/A
Statement of being sterile (if applicable)		Adequate.
Pharmacological/ therapeutic class		See revised version of 1st highlighted section above to contain "…Proprotein convertase subtilisin/kexin type 9-directed-…"
Chemical name, structural formula, molecular weight	(b) (4)	(b) (4)
If radioactive, statement of important nuclear characteristics.		N/A

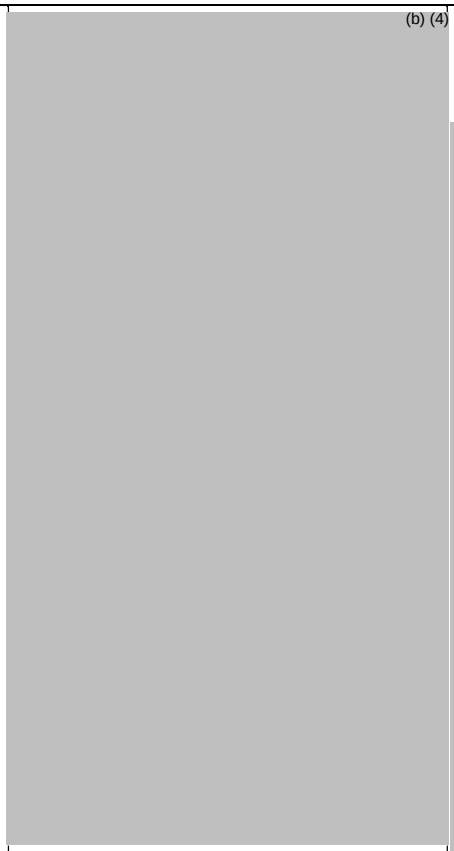
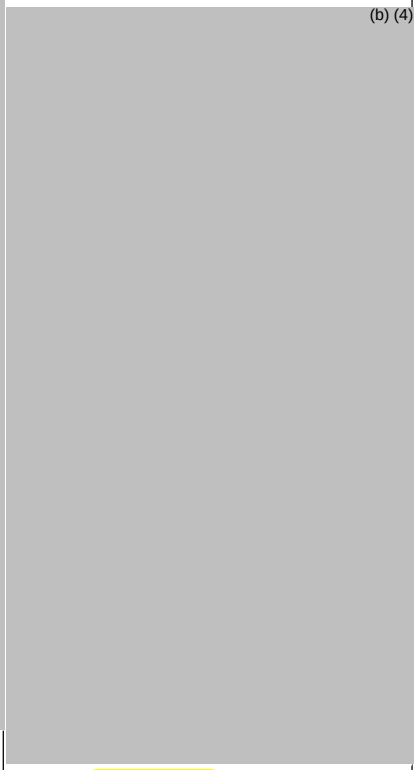
Other important chemical or physical properties (such as pKa or pH)		Adequate

Section 11 (DESCRIPTION) Continued

Item	Information Provided in the NDA	Assessor's Comments
For oral prescription drug products, include gluten statement if applicable	None.	N/A
Remove statements that may be misleading or promotional (e.g., "synthesized and developed by Drug Company X," "structurally unique molecular entity"	None.	Adequate.

1.2.4 Section 16 (HOW SUPPLIED/STORAGE AND HANDLING)

Item	Information Provided in the NDA	Assessor's Comments
HOW SUPPLIED/STORAGE AND HANDLING section		

Available dosage form(s)	(b) (4)  Store INCLISIRAN prefilled syringes at controlled room temperature 20°C to 25°C (68°F to 77°F) with allowable excursions between 15°C and 30°C (59°F and 86°F) [see USP, Controlled Room Temperature].	Revise as follows: (b) (4)  Store LEQVIO prefilled syringes at 20°C to 25°C (68°F to 77°F) with allowable excursions between 15°C and 30°C (59°F and 86°F).
Strength(s) in metric system		Adequate.
Available units (e.g., bottles of 100 tablets)		Adequate.
Identification of dosage forms, e.g., shape, color, coating, scoring, imprinting, NDC number		Adequate.

Assess if the tablet is scored. If product meets guidelines and criteria for a scored tablet, state "functionally scored"	N/A	N/A
For injectable drug products for parental administration, use appropriate package type term (e.g., single-dose, multiple-dose, single-patient-use). Other package terms include pharmacy bulk package and imaging bulk package.	See above.	Change "single-use" to "single-dose" as shown in revision above.

Section 16 (HOW SUPPLIED/STORAGE AND HANDLING) (Continued)

Item	Information Provided in the NDA	Assessor's Comments
Special handling about the supplied product (e.g., protect from light, refrigerate). If there is a statement to "Dispense in original container," provide reason why (e.g. to protect from light or moisture, to maintain stability, etc.)		Adequate. The PFS drug product (b) (4) (b) (4) Excursions above 25 degrees supported by stability data.

If the product contains a desiccant, ensure the size and shape differ from the dosage form and desiccant has a warning such as “Do not eat.”	N/A	N/A
Storage conditions. Where applicable, use USP storage range rather than storage at a single temperature.	See above.	Adequate.
Latex: If product does not contain latex and manufacturing of product and container did not include use of natural rubber latex or synthetic derivatives of natural rubber latex, state: “Not made with natural rubber latex. Avoid statements such as “latex-free.”	No information included.	N/A
Include information about child-resistant packaging	No Information included.	Not applicable

1.2.5 Other Sections of Labeling

N/A

1.2.6 Manufacturing Information After Section 17 (for drug products)

Item	Information Provided in the NDA	Assessor’s Comments
Manufacturing Information After Section 17		
Name and location of business (street address, city, state and zip code) of the manufacturer, distributor, and/or packer	None.	Inadequate – no manufacturing information provided. Recommendation: (b) (4)

2.0 PATIENT LABELING

Assessment of Product Quality Related Aspects of Patient Labeling (e.g., Medication Guide, Patient Information, Instructions for Use):
Not provided/ not applicable (clinical overview states that all instructions are contained in the labeling).

3.0 CARTON AND CONTAINER LABELING

Assessment of Container and Carton labeling:
For the single-dose syringe label, make the following changes:

- Change the label strength from "284 mg/1.5 mL" to "284 mg/1.5 mL (189 mg/ml)"
- Delete the statement of equivalence, i.e. "~~Equivalent to 300 mg inclisiran sodium~~".

For the syringe tray and carton labels, make the following changes:

- In the side panel list of ingredients change "284 mg inclisiran (equivalent to 300 mg inclisiran sodium)" to "284 mg inclisiran (provided as 300 mg inclisiran sodium)".

3.1 Container Label

Single-dose syringe label:

Syringe tray label:

3.2 Carton Labeling

(b) (4)

ITEMS FOR ADDITIONAL ASSESSMENT

N/A

Overall Assessment and Recommendation:

The labeling/labels will be adequate from a quality perspective after the recommended changes have been made.

Theodore Carver

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David Claffey

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MUTHUKUMAR RAMASWAMY
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Immunogenicity Assay Validation Assessment

Submission Tracking Number (STN):	NDA 214012
Subject:	Review on the immunogenicity assay validation report for ELISA method for the detection of anti-inclisiran-reactive IgG/IgM antibodies in human serum
Date Received:	January 27, 2020
Assessment/Revision Date:	August 13, 2020
Primary Assessor:	Mayumi Takahashi, Ph.D. (CDER/OPQ/OBP/DBRR IV)
Secondary Assessor:	Frederick Mills, Ph.D. (CDER/OPQ/OBP/DBRR IV)
RPM:	Kati Johnson
Applicant:	Novartis
Product:	Inclisiran
Indication:	Treatment of hypercholesterolemia
Clinical Division	CDER/ODEII/DMEP
Recommended Regulatory Action	Approval with PMRs

Table of Contents

1. Recommendation

An ELISA method was developed and used for the detection of anti-inclisiran-reactive immunoglobulin (Ig)G/IgM antibodies in clinical serum samples. All the validation parameters are appropriately evaluated except the following deficiencies;

Two separately prepared unpurified positive controls were used in the assay validation; rabbit anti-KLH-TTRSC serum (RAKS or Ab19151 bleed Day 110) for the sensitivity assessment, and rabbit anti-KLH-TTRSC-2017 serum (PC) for assessments of all other validation parameters.
The sensitivity assessment for both screening and confirmatory assays were performed using the unpurified rabbit anti-KLH-TTRSC serum (RAKS or Ab19151 bleed Day 110).

The assay sensitivity and all other assay validation parameters of the ELISA method for the detection of anti-inclisiran-reactive IgG/IgM antibodies in human serum except the cut-points using an affinity-purified anti-inclisiran antibody positive control (e.g. Rabbit Anti-KLH-TTRSC-2017 antiserum) should be re-determined to ensure the assay is a sensitive, specific, and selective assay to measure ADA responses. All existing clinical samples should be re-analyzed using the appropriately validated assay. We recommend post-marketing requirements (PMR) to address these issues.

2. Justification

Inclisiran is a chemically modified double-stranded 21-23mer small interfering RNA, conjugated on the sense strand with triantennary N-Acetylgalactosamine (GalNAc) to facilitate uptake by hepatocytes. The immunogenic potential of inclisiran was evaluated and is considered to be low. Appropriate assessments were conducted throughout the development program to determine its immunogenic potential.

The semi-quantitative ELISA for the detection of anti-inclisiran antibodies was developed and optimized. The assay validation report ((b) (4) Study No. 3000701) was submitted to the Agency for review. The sensitivity, specificity, selectivity, precision, and dynamic range of the method were evaluated. False positive rates of 5% and 1% were used for the screening and confirmatory cut-points, respectively. The Agency concluded that although no assay robustness was determined, the ADA assay was appropriately validated under (b) (4) Study No. 3000701. The assay was used in Phase I and Phase II clinical studies.

The method was re-optimized using a new system suitability positive control, thus, the method was re-validated in (b) (4) Study No. 3001805. The applicant demonstrated the ELISA method is selective, precise and robust for the detection of anti-inclisiran-reactive IgG/IgM antibodies in human serum in clinical samples. In addition, the method exhibits sufficient drug tolerance and no hook effect. However, the assay sensitivity was assessed using unpurified anti-drug polyclonal antiserum (Rabbit Anti-KLH-TTRSC Serum, RAKS) while a different unpurified anti-drug polyclonal antiserum (Rabbit Anti-KLH-TTRSC-2017 Serum, PC) was used in the validation assays for all other parameters. The 2019 FDA Guidance recommends using affinity-purified antiserum to evaluate the assay sensitivity to avoid a misinterpretation of sensitivity assessment results. Therefore, the assay sensitivity should be reassessed using an affinity purified Rabbit Anti-KLH-TTRSC-2017 antiserum (PC).

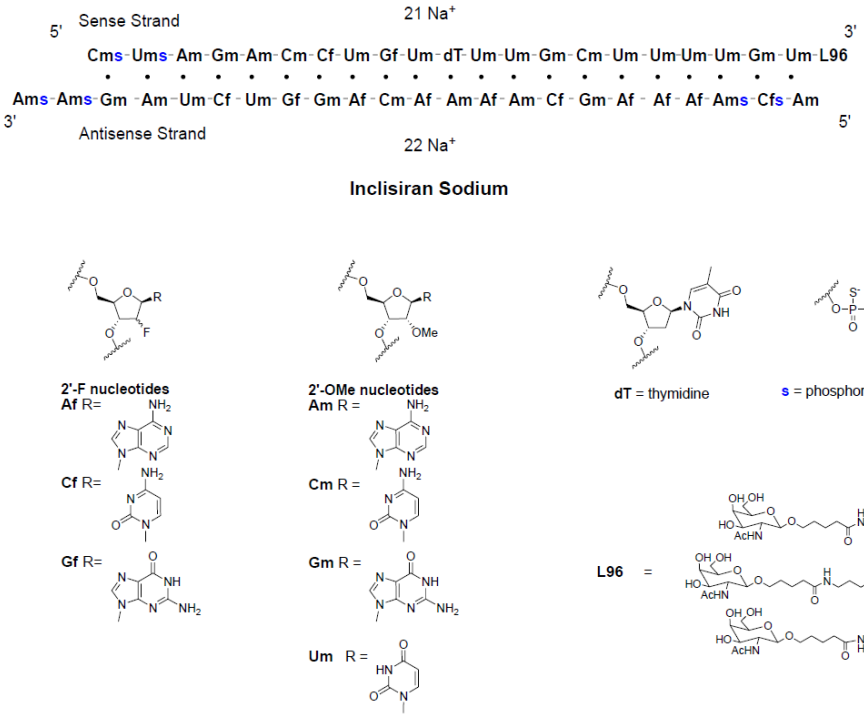
APPEARS THIS WAY ON ORIGINAL

3. Review

3.1. Introduction

Inclisiran is a chemically modified double-stranded 21-23mer small interfering RNA, covalently linked to a ligand containing three N-acetylgalactosamine (GalNAc) residues for selective delivery to hepatocytes. The sequence of inclisiran is designed to match the human messenger ribonucleic acid (mRNA) transcripts for proprotein convertase subtilisin kexin type 9 (PCSK9). In hepatocytes, the antisense strand is incorporated in the RNA-induced silencing complex (RISC) and directs catalytic breakdown of mRNA for PCSK9, resulting in inhibition of the translation of PCSK9 protein. Reduced intrahepatic PCSK9 increases LDL-C receptor recycling and expression on the hepatocyte cell surface, thereby increasing LDL-C uptake and lowering LDL-C levels in the circulation.

The molecular formula of inclisiran sodium is $C_{529}H_{664}F_{12}N_{176}Na_{43}O_{316}P_{43}S_6$ and its molecular weight is 17,284.72 g/mol. It has the following structural formula:



Inclisiran Injection is a sterile, preservative-free, clear, and colorless to pale yellow solution for subcutaneous injection (SC). It is supplied in a prefilled syringe (PFS). Each PFS contains 1.5 mL of solution containing 284 mg inclisiran (equivalent to 300 mg inclisiran sodium salt). Inclisiran injection is formulated in Water for Injection and may include sodium hydroxide and/or phosphoric acid for pH adjustment to target 7.

Inclisiran is intended for use as an adjunct to diet and maximal tolerated statin therapy for the treatment of adults with heterozygous familial hypercholesterolemia (HeFH), atherosclerotic cardiovascular disease

(ASCVD), (b) (4) who require additional lowering of their low-density lipoprotein cholesterol (LDL-C).

The program of clinical development for inclisiran including ongoing studies is summarized in Table 1 below.

Table 1: Summary of clinical studies

Study (Countries)	Population or design	Inclisiran doses	Control group	Total subjects	Treatment period
Phase III Studies					
ORION-9 (US, EU, SA, CA)	HeFH	300 mg	Placebo	482	18 months
ORION-10 (US)	ASCVD	300 mg	Placebo	1561	18 months
ORION-11 (EU, SA, UKR)	ASCVD and ASCVD risk equivalents†	300 mg	Placebo	1617	18 months
ORION-8†	Phase III Extension	300 mg	None	>3000	~36 months
ORION-4††	ASCVD – CVOT	300 mg	Placebo	~15,000	48-60 months
ORION-5††	HoFH	300 mg	Placebo /None	56	6/18 months
Phase II Studies					
ORION-1 (US, EU, CA)	ASCVD, Dose-finding	Day 1: 200|300|500 mg Days 1, 90: 100|200|300 mg	Placebo	501	12 months
ORION-3 (US, EU, CA)	Phase II Extension	300 mg	Evolocumab* /None	371	36 months
ORION-2 (US, EU, SA)	HoFH pilot	300 mg	None	4	6 months
Phase I Studies					
ORION-6 (US)	Hepatic impairment	300 mg	None	28	6 months
ORION-7 (NZ)	Renal impairment	300 mg	None	31	6 months
ORION-12 (US)	Thorough QT/QTc	900 mg	Moxifloxacin /Placebo	48	6 months
ALN-PCSSC-001 (UK)	SAD/MD	SAD: 25|100|300|500|800 mg MD: 125| 250|300|500 mg	Placebo	69	6 months

† Preliminary data from ORION-8 will be presented in the 4-month safety update to the NDA (US only)
†† Study is ongoing at this time and not included in the application
* Subjects on placebo from ORION-1 were given evolocumab for the first year in ORION-3
Abbreviations: ASCVD=atherosclerotic cardiovascular disease; CA=Canada; CVOT=Cardiovascular Outcomes Trial; EU=European Union; HeFH=heterozygous familial hypercholesterolemia; HoFH=homozygous familial hypercholesterolemia; MAD=multiple ascending dose; NZ=New Zealand; QTc=corrected QT; SA=South Africa; SAD=single ascending dose; UK=United Kingdom; UKR=Ukraine; US=United States

The Phase III studies (ORION-9, ORION-10 and ORION-11) were randomized, global, multicenter, placebo-controlled, 18-month studies. In each study, inclisiran sodium 300 mg or placebo was administered as a SC injection on Day 1 with a second dose on Day 90, followed by 6-month dosing intervals at Days 270 and 450 and follow-up until Day 540. The Phase III dose and dosage regimen for inclisiran was selected based on data from the ALNPCSSC-001 Phase I and ORION-1 Phase II studies.

Treatment with inclisiran sodium 300 mg resulted in 50-58% lowering of LDL-C relative to placebo at Day 510 and a time-averaged 44-54% lowering of LDL-C after Day 90 through Day 540. The most common treatment-emergent adverse events (TEAEs) were diabetes mellitus, nasopharyngitis, hypertension, upper respiratory tract infection, arthralgia, and injection site reaction. TEAEs at the injection site were the only adverse events (AEs) associated more frequently with inclisiran injections. No other safety risks were identified with inclisiran. The immunogenic potential of inclisiran is considered to be low and appropriate assessments were conducted throughout the development program to determine its immunogenic potential. Immunogenicity risk assessment and risk mitigation strategies for inclisiran are summarized in Table 1 below.

Table 1: Summary of Risk Evaluation and Risk Mitigation Strategies for Inclisiran

Potential Consequence	Risk Evaluation	Risk Mitigation
ADA	Nonclinical: • Evaluate ADA and potential impact on exposure and PCSK9 levels in repeat-dose studies in monkeys Clinical: • Serum samples for ADA detection collected in all clinical studies, with Phase III samples analyzed using a validated high-sensitivity assay to exclude false negatives	• Designed to minimize ADA (ie, chemically synthesized with modified nucleotides and GalNAc residues to limit systemic exposure and target inclisiran to the liver) • Sporadic incidences of monkeys with low-titer ADA at or near the end of the dosing periods. ADA were nonneutralizing as evidenced by not having an effect on PK (plasma or tissues) or PD (PCSK9 and lipid lowering) parameters • ADA detected at a low incidence and low titer in confirmatory ADA assay, with no apparent effect on PD (PCSK9 levels), efficacy (LDL-C levels), or AEs
Immunologic response	Nonclinical: • Evaluate immunostimulatory potential of inclisiran following single-dose administration to mice (Study PCS-NCD13-002), repeat-dose administration once weekly for 5 weeks to cynomolgus monkeys (Study 8297013), and repeat-dose inclisiran administration with atorvastatin (once every 28 days and daily, respectively) for up to 85 days to cynomolgus monkeys and to evaluate the potential reversibility or delayed occurrence of any findings after a 13-week recovery period (Study 20110258) • Evaluate the potential of inclisiran to activate complement following repeat-dose administration of inclisiran to cynomolgus monkeys in Study 8297013 Clinical: • Evaluate the potential for inclisiran to induce an inflammatory response by assessing hsCRP levels in the Phase III studies • Blood samples were collected and stored for all subject in order to determine if there were any cytokine elevations (ie,IL-6, IFN-γ, TNF-α) if the subject had a treatment-emergent immunologic AE	• Inclisiran did not stimulate pro-inflammatory cytokines in mice or monkeys or activate complement in monkeys • No immunostimulatory activity was seen with inclisiran in mice or monkeys. • No inflammatory response observed in any subject based on routine assessment of hsCRP in Phase III studies.
Allergic-type hypersensitivity/ anaphylaxis	Clinical: • Applied Sampson criteria to identify potential anaphylactic reactions in the clinical studies • If Sampson criteria were positive, assess tryptase levels from a blood sample collected within 30 minutes of the onset of symptoms • Conducted SMQ search for the analysis of hypersensitivity in the Controlled Phase III Safety Pool	• No subject in the clinical studies developed anaphylaxis • No differences in the incidence of AEs related to hypersensitivity for inclisiran versus placebo in the Controlled Phase III Safety Pool

ADA=antidrug antibody; AE=adverse event; GalNac=N-acetylgalactosamine; HCP=host cell proteins; hsCRP= high sensitivity C-reactive protein; IL6=interleukin 6; IFN-γ=interferon-gamma; LDL-C=low-density lipoprotein cholesterol; PCSK9=proprotein convertase subtilisin/kexin type 9; PD=pharmacodynamic; PK=pharmacokinetic; TNF-α= tumor necrosis factor-alpha.

The enzyme-linked immunosorbent assay (ELISA) used for the detection of anti-inclisiran-reactive immunoglobulin (Ig)G/IgM antibodies in clinical serum samples was developed and validated in 2 studies ((b) (4) validation reports 3000701 and 3001805) during clinical studies conducted under IND 127589. The assay was first validated and reported in 3000701. Following unavailability of the positive control (PC), the method was re-optimized using a new system suitability PC and some aspects of the method (e.g., commercial supplier of blocker and type of plate washer) were modified; therefore, the assay was re-validated in 3001805. The assay validated under 3000701 was used in Clinical Studies ALN-PCSSC-001 and ORION-1. The assay re-validated under 3001805 was used in clinical Studies ORION-2, ORION-3, ORION-6, ORION-9, ORION-10, ORION-11, and ORION-12 (Table 2 below).

Table 2: Summary of Methods Applied by Clinical Study

Clinical Study	Method	Sample Analysis Study Reference	Validation Study Reference
ALN-PCSSC-001	Validation of a semi-quantitative ELISA for the detection of inclisiran-reactive IgG/IgM antibodies in human serum	(b) (4) Study No. 3000702	(b) (4) Study No. 3000701
MDCO-PCS-15-01 (ORION-1)		(b) (4) Study No. 3001304	
MDCO-PCS-16-02 (ORION-2)	Validation of an ELISA method for the detection of anti-inclisiran antibodies in human serum*	(b) (4) Study No. 3001366	(b) (4) Study No. 3001805
MDCO-PCS-16-01 (ORION-3)		(b) (4) Study No. 3001534	
MDCO-PCS-18-02 (ORION-6)		(b) (4) Study No. 3001738	
MDCO-PCS-17-03 (ORION-9)		(b) (4) Study No. 3001515	
MDCO-PCS-17-04 (ORION-10)		(b) (4) Study No. 3001516	
MDCO-PCS-17-08 (ORION-11)		(b) (4) Study No. 3001517	
MDCO-PCS-17-09 (ORION-12)		(b) (4) Study No. 3001709	

*Re-optimized direct ELISA, re-validated in compliance with Final FDA Guidance from January 2019.
(b) (4) ELISA=enzyme-linked immunosorbent assay; IgG=immunoglobulin G; /IgM=immunoglobulin M.

3.2. History of communications

On March 28, 2017, the initial validation report for the ELISA assay to detect the anti-inclisiran antibodies (ADAs) in human serum ((b) (4) Study No. 300701) was submitted to the Agency. The validation report was reviewed by Rong Wang, Ph.D. (CDER/OPQ/OBP/DBRR III), and comments/recommendations were sent to the applicant (Novartis, previously The Medicines Company) with the FDA letter issued on July 25, 2017. The study report amendment was submitted on September 21, 2017, which was reviewed by Dr. Wang. The FDA letter to the applicant issued on February 8, 2018, states that the ELISA assay was appropriately validated. Following unavailability of the PC, the method was re-optimized using a new system suitability PC and some aspects of the method (e.g., commercial supplier of blocker and type of plate washer) were modified. The applicant re-validated the assay under (b) (4) Study No. 3001805 and the final study report for Study No. 3001805, Validation of a Qualitative ELISA for the Detection of Anti-Inclisiran-reactive IgG/IgM Antibodies in Human Serum ((b) (4) Study No. 3001805) was submitted to the FDA for review on May 15, 2019. In the validation report for Study No. 3001805, the applicant demonstrated drug-tolerance, precision, selectivity, dynamic range of the method, and titer precision. The titration cut point was calculated using a 0.1% false positive rate. However, the assay validation exercises had some deficiencies that need to be addressed. For more detailed information, see the review memo STN_127589_0048 (IND 127589) created by Dr. Rong Wang. The FDA provided feedback to the report on July 26, 2019. On September 18, 2019, the applicant

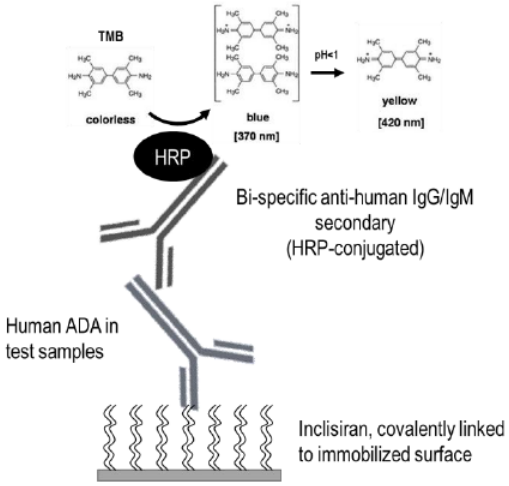
provided a response to the FDA feedback. On May 29, 2020, the applicant submitted a final validation report amendment No. 5 for (b) (4) Study No. 3001805.

The following review addresses the assessment of the applicant's response and the amended final validation of a qualitative ELISA for the detection of anti-inclisiran-reactive IgG/IgM antibodies in human serum ((b) (4) Study No. 3001805).

Reviewer's Comment: the assay validation report for (b) (4) Study No. 300701 has been reviewed by Dr. Rong Wang and confirmed that the assay is appropriately validated. See review memos for IND127589, SDN 50 (eCTD 0048) and SDN 77 (eCTD 0075).

3.3. Assay format

Schematic of the ADA assay format is shown below.



ADA present in the human serum test samples bind to immobilized inclisiran. The ADA are detected with a bi-specific goat anti-human IgG and IgM antibody coupled to HRP. A suitable substrate, TMB, is added that generates a chromophore in the presence of the HRP. The absorbance (A_{450nm}) of the chromophore correlates with the level of IgG and IgM ADA in test samples. To monitor the performance of the assay, a surrogate rabbit positive control with specificity for inclisiran is used. The positive controls used were: a) the rabbit anti-KLH-TTRSC serum, bleed day 110: 20Aug2013 (also referred to as Ab-19151); b) the purified rabbit Anti-KLH-TTRSC serum, Day 98; and c) the rabbit Anti-KLH-ALN-TTRSC-2017 anti-serum. Since the positive control is not detectable by the same secondary antibody as the test samples, whole molecule human IgG and IgM isotype controls were used as positive controls in the assay to monitor the performance of the detection reagent used for the test samples.

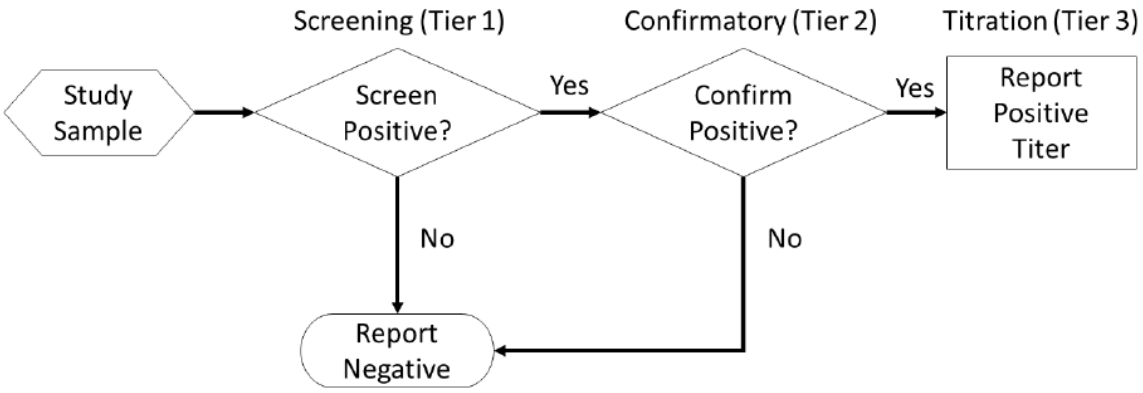
Reviewers Comments:

- *Studies No. 300701 and No. 3001805 use the same assay format.*
- *The Rabbit Anti-KLH-TTRSC serum, Day 98 is an affinity purified positive control anti-drug antibody used for the sensitivity assessment under Study No. 300701. The Rabbit anti-KLH-TTRSC serum, bleed day 110 is an unpurified anti-drug polyclonal antiserum used as the system*

suitability control in the method validated under Study No. 300701, and for sensitivity assessment in the method validated under Study No. 3001805. The rabbit Anti-KLH-ALN-TTRSC-2017 anti-serum is an alternate unpurified anti-drug polyclonal antiserum used as a positive control to assess all other validation characteristics in the method validated under Study No. 3001805 and as the system suitability control to monitor the assay performance in support of Phase III sample analysis. Different positive control antibodies can be used to develop, validate, and monitor system suitability during routine assessment of assay performance. However, using different positive controls in an assay validation is not recommended. This issue was addressed in the previous communication with the applicant. The details are discussed in Section 3.2 Instruments and Reagents below.

3.4. Testing Scheme

Schematic diagram of hierarchical testing scheme is shown below.



Reviewer's Comments:

- *The multi-tiered testing approach is consistent with recommendations in the FDA guidance and is acceptable.*
- *Assessments to determine immunogenic potential of inclisiran were conducted throughout the drug development program. In nonclinical toxicology studies, there were occasional incidences of the ADA development, but they were generally low titer responses in a small number of animals. No association between the presence of ADA and changes in either systemic exposure or PCSK9 levels were observed in repeat-dose studies in monkeys. In clinical studies including 18 months Phase III trials (>9000 ADA assays), no adverse events or loss of effect in LDL-C and PSCK9 levels associated with ADA were observed. In addition, the frequency and the low titer of ADA positive samples was similar in pre- and post-dose timepoints. Therefore, further characterization (e.g. neutralizing antibodies) of ADA positive samples were not conducted. This is acceptable.*

3.5. Method Validation

3.5.1. Summary of method validation performance for ADA assay (Study No. 3001805)

METHOD VALIDATION SUMMARY FOR IMMUNOGENICITY ASSAYS (version 4)

Study/Reference number: 3001805

Analyte: Anti-Inclisiran IgG/IgM antibodies

Sponsor name: The Medicines Company

Matrix: Human serum

Validation Parameter	Validation Parameter Acceptance Criteria	Validation Result	Reference
Minimum required dilution	Descriptive	1/50	N/Ap
Positive Control ranges	Report result (signal-to-noise ratios)	LPC lower limit: 0.916 HPC lower limit: 7.848	Val-01 to Val-16 (Val-15 accepted in deviation), Val-25 to Val-28, Val-33 to Val-48, Val-50 to Val-55 and Val-57 to Val-62
Negative Control range	Report result	NC Upper limit: 0.240	Val-01 to Val-16 (Val-15 accepted in deviation), Val-25 to Val-28, Val-33 to Val-48, Val-50 to Val-55 and Val-57 to Val-62
Screening cut-point factor (multiplicative)	Report result	SCPF = 1.370	Val-01 to Val-16 (Val-15 accepted in deviation), Val-25 to Val-28 and Val-37 to Val-40
Confirmatory cut-point	Report result	31.03% (at 50.0 µg/mL of Inclisiran)	Val-01 to Val-16 (Val-15 accepted in deviation), Val-25 to Val-28 and Val-37 to Val-40
Titration cut-point factor (multiplicative)	Report result	TCPF = 1.994 Based on the precision of titration results, the SCPF will be used to calculate the titration threshold for the method.	Val-01 to Val-16 (Val-15 accepted in deviation), Val-25 to Val-28 and Val-37 to Val-40
Sensitivity	Report result	Screening: 459.55 ng/mL Confirmatory: 78.13 ng/mL	Val-33, Val-35, Val-41, Val-43, Val-45, Val-47

METHOD VALIDATION SUMMARY FOR IMMUNOGENICITY ASSAYS (version 4)

Study/Reference number: 3001805

Screening LPC dilution	Report result	LPC = 1/490 (rounded to 1/500 to facilitate preparation)	Val-34, Val-36, Val-42, Val-44, Val-46 and Val-48
Confirmatory LPC dilution	Report result	LPC = 1/1920 (statistically defined) Screening LPC will be used in the Confirmatory assay	Val-34, Val-36, Val-42, Val-44, Val-46 and Val-48
Intra-assay Precision (screening)	1. The % CV of the mean A_{450nm} values must be ≤ 20% at all PC levels in each run. 2. The mean A_{450nm} values of PCs, for at least 5 out of 6 replicates tested at each level, must be ≥ PSCP 3. LPC A_{450nm} values must be < global mean A_{450nm} value of the MPC and the MPC A_{450nm} values must be < global mean A_{450nm} values of the HPC	Pass: LPC : 2.3% and 4.3% MPC : 15.0% and 6.4% HPC : 2.0% and 3.7% All PC ≥ PSCP in each run. In each run LPC < MPC < HPC	Val-55, Val-57, Val-58 and Val-62
Inter-assay Precision (screening)	1. The % CV of the global mean (i.e. intra-assay mean) A_{450nm} values at all PC levels must be ≤ 20%. 2. The mean A_{450nm} value must be ≥ PSCP for at least 67% of PCs tested in each plate, with a minimum of 50% at each level. 3. In each run, the LPC A_{450nm} values must be < global mean A_{450nm} value of the HPC. When the MPC is included, the LPC values must be < global mean A_{450nm} value of the MPC and the MPC A_{450nm} values must be < global mean A_{450nm} value of the HPC.	Pass: LPC : 12.9% MPC : 14.1% HPC : 12.2% All PC ≥ PSCP in each run. In each run LPC < MPC < HPC	Val-01 to Val-16 (Val-15 accepted in deviation), Val-25 to Val-28, Val-33 to Val-48, Val-50 to Val-55 and Val-57 to Val-62, Val-67, Val-68, Val-70 to Val-72, Val-74, Val-75 and Val-78 to Val-80
Intra-assay Precision (confirmatory)	1. The % CV of the mean % signal inhibition values must be ≤ 20% at all PC levels in each run. 2. The % signal inhibition of PCs, for at least 5 out of 6 replicates tested at each level, must be ≥ CCP. 3. The % inhibition of minimally 2 out of 3 NCs (67%) must be < CCP in each run.	Pass: LPC : 0.1% to 3.3% MPC : 0.1% to 5.8% HPC : 0.1% to 0.6% All PC ≥ CCP in each run. At least 67% of NC-TS % inhibition < CCP in each run.	Val-55, Val-57, Val-58 and Val-62

METHOD VALIDATION SUMMARY FOR IMMUNOGENICITY ASSAYS (version 4)

Study/Reference number: 3001805

Inter-assay Precision (confirmatory)	1. The % CV of the global mean (intra-assay mean) % signal inhibition values of all PC samples at all PC levels must be ≤ 20%. 2. The % signal inhibition must be ≥ CCP for at least 67% of PCs tested in each plate, with a minimum of 50% at each level. 3. The % signal inhibition of at least 50% NCs must be < CCP in each run.	Pass: LPC : 6.2% MPC : 5.7% HPC : 3.1% All PC ≥ CCP in each run. At least 50% of NC-TS % inhibition < CCP in each run	Val-52, Val-53, Val-57, Val-58, Val-59, Val-61 and Val-62, Val-67, Val-68, Val-70 to Val-72, Val-74, Val-75, Val-78, Val-79
Drug tolerance	Report result	Screening: LPC = 0.01 µg/mL HPC = 2.0 µg/mL Confirmatory: LPC = 2.0 µg/mL HPC = 2.0 µg/mL	Val-50 and Val-53
Selectivity (screening)	At least 80% of the unspiked normal human serum lots (blanks) should be < PSCP. For the PC spiked samples, the % CV between duplicate wells must be ≤ 20% and the mean A_{450nm} value ≥ PSCP for at least 80% of the individual samples analyzed.	Unspiked: 80% < PSCP. LPC: 100% ≥ PSCP % CV between duplicate < 20% for all PC	Val-50 and Val-51
Selectivity (confirmatory)	At least 80% of the unspiked normal human serum lots (not spiked with PC) tested in the confirmatory assay should be < CCP. For the PC spiked samples, the % CV between duplicate wells must be ≤ 20% and the % signal inhibition ≥ CCP for at least 80% of the individual samples analyzed.	Unspiked: Pass: 90% < CCP. The lot that had a % signal inhibition ≥ CCP had a unspiked value ≥ PSCP. LPC: Pass: 100% ≥ CCP.	Val-50 and Val-51
Selectivity (hemolysis)	Screening: The samples without PC should yield A_{450nm} values < the PSCP. For the PC spiked samples, the % CV between duplicate wells must be ≤ 20% and the mean A_{450nm} value ≥ PSCP. Confirmatory: The samples without PC should yield % signal inhibition < the CCP. For the PC spiked samples, the % CV between duplicate wells must be ≤ 20% and the % signal inhibition ≥ CCP.	Screening: Unspiked: Pass LPC: Pass Confirmatory: Unspiked: Pass LPC: Pass	Val-59
Selectivity (lipemia)	Screening: The samples without PC should yield A_{450nm} values < the PSCP. For the PC spiked samples, the % CV between duplicate wells must be	Screening: Unspiked: Failed: 40% < PSCP LPC: Pass: 100% ≥ PSCP	Val-59 to Val-61

METHOD VALIDATION SUMMARY FOR IMMUNOGENICITY ASSAYS (version 4)

Study/Reference number: 3001805

	≤ 20% and the mean A_{450nm} value ≥ PSCP. Confirmatory: The samples without PC should yield % signal inhibition < the CCP. For the PC spiked samples, the % CV between duplicate wells must be ≤ 20% and the % signal inhibition ≥ CCP.	Confirmatory: Unspiked: Failed: 90% < CCP LPC: Pass: 100% ≥ CCP	
Prozone	Descriptive	Prozone sample was >PSCP No prozone effect was observed	Val-52
Precision of Titration	For the titration results to be acceptable, the titer of each sample should remain within ± one 2-fold dilutions for all analyses (e.g.: 50, 100, 200).	Pass Titer for all analyses: 400 SCPF will be used to calculate the titration threshold for the method.	Val-54, Val-58, Val-60 and Val-62
Robustness	Robustness was assessed using the overall inter-assay precision and will be considered acceptable if the signal of the NC and PC samples are in the following order: HPC > MPC > LPC > NC PC and LPC ≥ PSCP. Additionally, robustness will be considered acceptable if mean A_{450nm} values of all PCs (LPC, MPC, HPC), and NC are within the acceptance ranges.	Fail, the median NC from Val-62 was above the upper NC limit.	Val-01 to Val-16 (Val-15 accepted in deviation), Val-25 to Val-28, Val-33 to Val-48, Val-50 to Val-55 and Val-57 to Val-62
Robustness (using the Biomek FX liquid handler)	The Biomek Fx performance will be considered acceptable if 90% of the assays met the acceptance criteria mentioned in the robustness section. In addition: Intra-assay precision: Minimum 5 /6 mean A_{450nm} PC must meet the acceptance criteria described in the intra-assay section. Inter-assay precision: All reportable results will be included in the inter-assay precision assessment.	Intra-assay: Pass at all PC level Inter-assay: Pass (refer to inter-assay precision for screening and confirmatory) Fail for the use of the Biomek FX to perform screening and confirmatory assay, since 69.2% of the assay met the assay acceptance criteria. Assessment for the use of the Biomek FX to perform titration assays is ongoing.	Val-67 to Val-79
Stability	Descriptive	As per Study no 3000698: stable up to 6 FT combined with 24 hrs and 5 minutes at ambient RT after storage at -80°C Additional stability assessments are ongoing Long term stability is supported by literature.	N/Ap

Reviewer's Comments:

- *The screening assay sensitivity of Study No. 3001805, 459.55 ng/mL appears excessively high. Traditionally, FDA has recommended sensitivity of at least 250 to 500 ng/mL. However, recent data suggest that concentrations as low as 100 ng/mL may be associated with clinical events. The applicant justified the appropriateness of the sensitivity of the screening assay by describing as follows; "this method uses a surrogate positive control (rabbit antibodies) and therefore a dual detection system. The sensitivity values are based on the positive control and may not be representative of the sensitivity of the detection in study samples. During selectivity assessment performed using human serum the false positive rate was 10% (1/10) and therefore the sensitivity of the screening assay was considered appropriate." Nevertheless, the sensitivity was assessed using the unpurified anti-drug polyclonal antiserum (Rabbit Anti-KLH-TTRSC Serum, RAKS or Ab19151 bleed Day 110) which is not appropriate because using unpurified antibody could lead to a misinterpretation of sensitivity assessment results. The sensitivity for screening and confirmatory assay should be reevaluated using an affinity purified positive control.*
- *In the final study report amendment 5 for Study No. 3001805, the applicant updated the inter-assay precision results for the screening and confirmatory assay and included the utilization of the liquid handler Biomek Fx for the robustness assessment. These updates are discussed in later sections.*

3.5.2. Instruments and Reagents

In this section, the following deficiency was identified in the previous submission.

The positive controls used for method development and validation are;

- An unpurified anti-drug polyclonal antiserum (Rabbit Anti-KLH-TTRSC Serum, RAKS or Ab19151 bleed Day 110) was used as the system suitability control in the method validated under (b) (4) Study 3000701, and for the sensitivity assessment in the method validated under (b) (4) Study 3001805. The concentration (9.2 mg/mL) of the Ab19151 bleed Day 110 was determined by a direct binding ELISA method using ALN-TTRSC-coated plates, and the affinity purified Ab19151 material was used to generate the calibration curve (Alnylam Pharmaceuticals Inc).

- An alternate unpurified anti-drug polyclonal antiserum (Rabbit Anti-KLH-TTRSC-2017 serum, PC) was generated to be used as the system suitability control to monitor the assay performance in support of Phase III sample analysis due to volume limitations with the RAKS material.

Using unpurified antibodies to determine the assay sensitivity is generally not acceptable. In addition, a different unpurified anti-drug serum (Rabbit Anti-KLH-TTRSC-2017 Serum, PC) was used to assess all other validation characteristics which is also not appropriate. The following comments were communicated to the applicant:

FDA Comment #1:

You used an unpurified anti-drug polyclonal antiserum (Rabbit Anti-KLH-TTRSC Serum, RAKS) for assessing the assay sensitivity. However, you used a different unpurified anti-drug polyclonal antiserum (Rabbit Anti-KLH-TTRSC-2017 Serum, PC) for all other parameters except the assay sensitivity in your validation exercises. This is not appropriate. When unpurified antibodies are used to assess sensitivity then only a small percentage of the antibody pool is antigen specific and the assay appears very insensitive. A purified preparation of antibodies specific to the drug should be used as the positive control to determine the sensitivity of the assay so that assay sensitivity can be reported in mass units/mL of matrix. Positive control antibodies used to assess sensitivity can take the form of polyclonal preparations affinity purified against the therapeutic protein product or monoclonal antibodies (mAb). Re-evaluate the assay sensitivity using the affinity-purified Rabbit Anti-KLHTTRSC-2017 Serum (PC). Re-evaluate the appropriateness of the chosen LPC concentration or dilution factor after you re-assess the assay sensitivity using an affinity purified positive control antibody.

The applicant responded to the comments on September 18, 2019:

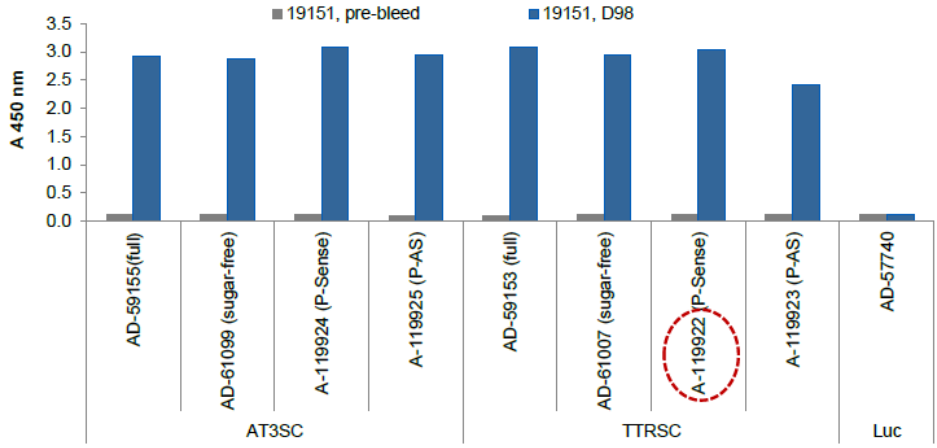
Novartis response:
An affinity-purified antibody specific to inclisiran was no longer available to be used in the method re-validation. The unpurified anti-drug polyclonal antiserum (Rabbit Anti-KLH-TTRSC Serum, RAKS or Ab19151 bleed Day 110) was the only material available to reliably determine a sensitivity value. As per the memorandum provided by the supplier, Alnylam Pharmaceuticals Inc., the concentration (9.2mg/mL) of the Ab19151 bleed Day 110 was determined by a direct binding ELISA method using ALN-TTRSC-coated plates, and the affinity purified Ab19151 material was used to generate the calibration curve. The memorandum detailing the determination of the Ab19151 bleed Day 110 polyclonal antibody concentration will be appended to the amended method validation report. The amended method validation report will be submitted as part of the licensing application.
Since the volume of the RAKS PC was insufficient to support the Phase III sample analysis studies, the unpurified Rabbit Anti-KLH-TTRSC-2017 Serum was generated to be used as the system suitability control to monitor the assay performance in sample analysis. A final low level of positive control (LPC) was therefore established to monitor assay sensitivity by diluting the Rabbit Anti-KLH-TTRSC-2017 Serum using at least eight (8) 2-fold serial dilutions in neat pooled human serum. Each dilution was run as one duplicate set (2 wells) diluted to the minimum required dilution in assay buffer (i.e. blocking buffer). The samples were assayed in replicate of at least 1 (n=1), in a total of six runs performed by two analysts using a balanced design. In order to statistically determine an LPC that predictably fails at least 1% of the time, it was calculated as the 99% upper limit using the PC dilution curves interpolated with the screening cut-point (SCP) as per the following:
PC level at SCP in each run = MRD / (PC dilution at SCP in each run)
Screening LPC = MRD/ {Mean PC level at SCP + ($t_{0.01,df}$ × SD)}
Where if N=6, $t_{0.01,df}$ = 3.365

In this NDA submission, the applicant provided bioanalytical memoranda prepared by Alnylam Pharmaceuticals Inc. for RAKS (Ab19151 bleed Day 110) polyclonal antibody and Rabbit Anti-KLH-TTRSC-2017 Serum.

The memo for RAKS polyclonal antibody details the generation, purification of the antibody and quantification of its concentration:

Generation

The RAKS polyclonal antibodies (Ab19151) were generated by immunizing rabbit with keyhole limpet hemocyanin (KLH) conjugated ALN-TTRC (KHL0ALN-TTRC). Two batches of production bleeds were collected (day 98 and day 110). All bleeds were subjected to titer determination by ELISA using the ovalbumin-conjugated ALN-TTRSC. The specificity of Ab19151 was tested using plates coated with different compounds (Figure below). Preimmune serum was included as a negative control. Results show that Ab19151 recognized/reacted with ALN-AT3SC and ALN-TTRSC or other derivatives (duplex or single strand), all of which carry phosphorothioate (PS) (except the ALN-TTRSC sense strand, highlighted with a red circle in the figure), 2-*O*-methyl, 2'-fluoro (F) modification. The Luc-siRNA control (AD-57740, carrying PS and 2'-*O*-methyl but ligand-free (no linker or no GalNAc) showed negative results. These data indicated that Ab19151 recognizes/reacts specifically oligonucleotides containing 2'-F modification.



Quantification of Ab19151 concentration in production bleeds

The concentration of unpurified Ab19151 (RKAS polyclonal antibody) was determined using the ELISA plates coated with phosphorylated ALN-TTRSC. A standard curve was generated with the affinity purified Ab19151 (bleed day 98) (Figure 5). The Ab19151 concentration in the day 98 bleed was determined to be 10.5 mg/mL and in day 110 bleed to be 9.2 mg/mL (Table 1).

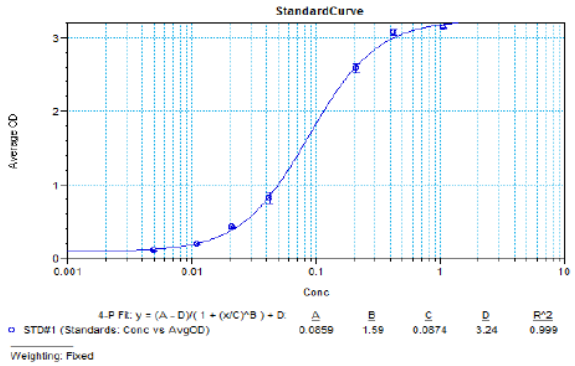


Figure 5 *The Standard curve generated using the affinity purified Ab19151-3 (stock at 207.3 µg/mL).*

Table I *Data of Quantification by ELISA*

Sample	Dilution (fold)	OD 450 nm	Concentration (µg/mL)	Average Concentration (µg/mL)	Deviation (Avedev)	%CV	Final Concentration (mg/mL)
Day 98	100,000	1.818	0.099	0.105	0.009	8.6	10.5
		1.967	0.112				
Day 110	1,000,000	0.162	0.009	0.009	0.001	11.1	9.2
		0.183	0.01				

A summary of the bioanalytical memo for Rabbit Anti-KLH-TTRSC-2017 Serum is as follows:

Rabbit Anti-KLH-TTRSC-2017 Serum was generated by immunizing rabbit with keyhole limpet hemocyanin (KLH) conjugated ALN-TTRC (KHL0ALN-TTRC). Two batches of production bleeds were collected (day 98 and day 113 terminal bleed). The pre-bleed (7NOV2017) and the terminal bleed (28FEB2018) were subjected to titer determination by ELISA using NH plates coated with AD-62912 (phosphorylated ALN-PCSSC). The assay data showed that the pre-bleed has extremely low signal, while the terminal bleed has a high signal, indicating that the Rabbit Anti-KLH-TTRSC-2017 Serum (Ab25963) recognizes ALN-PCSSC (Figure 3).

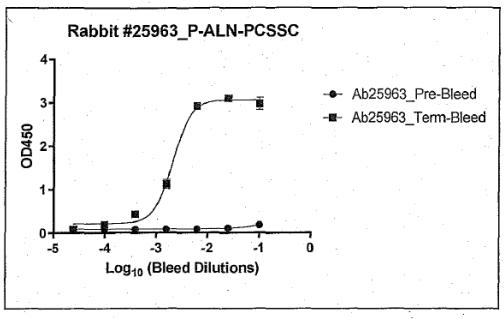


Figure 3 *ADA assay showed that Ab25963 can recognize ALN-PCSSC.*

Reviewer's Comments:

- *In the response to the FDA's comments, the applicant failed to re-evaluate the assay sensitivity using an affinity-purified positive control. Using the unpurified positive control in the sensitivity assessment is not appropriate. Unpurified polyclonal antibodies are likely to have less affinity,*

avidity, specificities and concentration compared to those of purified polyclonal antibodies, and consequently this could lead to a misinterpretation of sensitivity assessment results. Therefore, a purified preparation of antibodies specific to the therapeutic product should be used as the positive control to determine the sensitivity of the assay. The positive control antibodies can be affinity purified using the therapeutic product (inclisiran) to enrich the polyclonal antibody preparation for ADA.

- *The applicant used two different separately prepared unpurified positive controls in the assay validation; rabbit anti-KLH-TTRSC serum (RAKS or Ab19151 bleed Day 110) for the sensitivity assessment, and rabbit anti-KLH-TTRSC-2017 serum (PC) for assessments of all other validation characteristics. This is not appropriate. Different preparation generates polyclonal antibodies with different characteristics (e.g. affinity, specificities and concentrations). Therefore, when used as positive controls, these polyclonal antibodies could yield different assay sensitivities.*
- *The PC (unpurified Rabbit anti-KLH-TTRSC-2017 serum) was used for all validation runs for the detection of ADA in pooled human serum except for sensitivity assessment. The mean LPC (1/500) A_{450} from 12 validation runs is 0.378 (n=32, SD=0.056, CV(%)=14.9%), and the mean NC A_{450} from the same 12 runs is 0.198 (n=12, SD=0.034, CV(%)=17.2%). Thus, the mean LPC/NC is 1.880 (n=32, SD=0.393, CV(%)=20.9%) (the values are calculated using data from Table 9-11 in the validation report). Overall, data from both the LPC and NC show low variability in pooled human serum, and the LPC/NC values appear to be reproducible.*
- *As was also communicated in previous FDA comments, the applicant should affinity purify the rabbit anti-KLH-TTRSC-2017 serum and reassess the assay sensitivity using the purified polyclonal antibodies. In addition, although the applicant's approach to determine the final LPC level appears to be reasonable, the chosen LPC level should be justified based on the assay sensitivity determined using the purified positive control to ensure that the sensitivity of the assay is acceptable across assay runs.*

3.5.3. Acceptance Criteria

This part was previously reviewed by Dr. Wong (see the review memo for IND 127589_SDN205_eCTD 0203 [Reference ID: 4461526]). The system suitability acceptance criteria are reasonable and acceptable.

3.5.4. Minimum Required Dilution

This part was previously reviewed by Dr. Wong (see the review memo for IND 127589_SDN205_eCTD 0203 [Reference ID: 4461526]) and is acceptable.

3.5.5. Screening Cut Points (SCP), Titration Cut Point (TCP), Normalization Factor (NF), and Titration Normalization Factor (TNF), Confirmatory Cut Point (CCP)

The SPC and CCP were established by analyzing 56 samples of normal human serum (28 males and 28 females) on 6 runs (n=1 in duplicate) by two different analysts using a balanced design. All evaluations were based on log transformed signal-to-noise (S/N) values, where each subject's sample result (S) is

divided by the median instrument response of the negative control (N) in the same run. In order to identify the outliers and evaluate various assay characteristics, a mixed-effects model was fit to the log transformed S/N values. In this model, the analyst, plate order and the interaction of analyst by plate order were included as fixed effects, while subject, run number nested within analyst, and plate ID were included as random effects.

In this section, the following deficiency was identified in the previous submission.

- The SPC factor, 1.370 (multiplicative) was calculated using the lower confidence limit formula provided in a reference. However, the formula and the false positive rate used in the screening cut point factor calculation was not provided

- The CCP, 31.03% (fixed) was calculated using the lower confidence limit formula provided in a reference. However, the formula and the false positive rate used in the confirmatory cut point calculation was not provided.

No information regarding the statistical analysis for the identification of the analytical and biological outliers.

The followings are communication with the applicants regarding these concerns (FDA comments #2 and #3).

FDA Comment #2:
You did not specify what false positive rates were used in the screening and confirmatory cut point calculation. FDA guidance recommends calculate the screening cut point using a 5% false positive rate and the confirmatory cut point using a 1% false positive rate to help reduce the false negative results. In your licensing application, specify the false positive rates used in the calculation of the screening and the confirmatory cut points. Recalculate the screening and the confirmatory cut points if the false positive rates used were not consistent with the FDA guidance recommendations.

Novartis response:
In line with the 2019 FDA Guidance, the SCP and CCP were calculated with 5% and 1% false-positive rates, respectively. The final validation report will be amended to include a description of the formulas used in the calculation of the screening and the confirmatory cut points which further support this claim (refer to the response to comment #3). The amended method validation report will be submitted as part of the licensing application.

Reviewer's Comments:
- *Using 5% and 1% false-positive rates for calculation of the SCP and CCP appears to be consistent with the recommendations of the 2019 FDA guidance. The corresponding section is amended in the current validation report. This is acceptable.*
- *The applicant observed in the three Phase III studies that the false positive rate at the screening level was between 12%-22% which was slightly above the expected false positive rate of 2 to 11% for study sample testing. However, since a confirmatory assay was in place to discriminate*

between true positives and false positives, this was considered to be conservative and appropriate.

FDA Comment #3:
You stated that the screening cut point factor and the confirmatory cut point were calculated using the lower confidence limit formula provided in a reference. However, you did not provide the formula in the validation report. In addition, you stated that the statistical analysis for the outlier identification was provided in (b) (4). *However, you did not provide the information for the identification of the analytical and biological outliers in the validation report. In your licensing application:*
Provide the formulas used in the calculation for the screening and the confirmatory cut points.
Provide the information for the identification of the analytical and biological outliers.

Novartis response:
After excluding all the analytical and biological outliers (refer to the response to point 3b below for further details), the SCP Factor (SCPF) for signal-to-noise (S/N) ratios was evaluated with the remaining data using the formula recommended in the 2019 FDA Guidance and method #4 of Shen et al (2015). This normal approximation formula corresponds to a one-sided 90% lower confidence limit for the 95th percentile, and thus assures at least a 5% false positive rate with 90% confidence. Similar to the SCPF evaluation, the CCP calculation was based on the 2019 FDA Guidance and method #4 of Shen et al (2015). This normal approximation formula corresponds to a one-sided 80% lower confidence limit for the 99th percentile, and thus assures at least a 1% false positive with 80% confidence. Outliers were identified using a two-phase approach. First, the analytical outliers were identified using conditional residuals from this model and excluded. Then this model was refit to the remaining data to identify the biological outliers using the subject best linear unbiased predictors (BLUPs). Tukey's outlier criteria with box-plots was used on the model's conditional residuals to identify the analytical (technical) outliers. Per this criterion, samples that fell below Q1-1.5x(Q3-Q1) or above Q3+1 .5x(Q3-Q1) were considered as outliers, where Q1 and Q3 represent the 25th and 75th percentiles, respectively. The same criteria were applied on the subject BLUPs to identify the biological (subject-level) outliers. The process to remove outliers was similar for both SCP and CCP datasets, with the one difference that the analysis was performed using log-transformed S/N values for the SCP, and using %inhibition values for the CCP. The final validation report will be amended to include a description of the formulas used in the calculation for the screening and the confirmatory cut points and to provide the information for the identification of the analytical and biological outliers. The amended method validation report will be submitted as part of the licensing application.

Reviewer's Comments:

- *The normal approximation formula described by Shen et al. (2015) was used to evaluate the SCPF. This formula corresponds to a one-sided 90% lower confidence limit for the 95th percentile and thus, assures at least a 5% false positive rate with 90% confidence. This is consistent with the recommendations of the 2019 FDA guidance and is acceptable.*
- *The normal approximation formula described by Shen et al. (2015) was used to evaluate the CCP using one-sided 80% lower confidence limit for the 99th percentile which assures at least a 1% false positive rate with 80% confidence. This is consistent with the recommendations of the 2019 FDA guidance and is acceptable.*

- *Tukey's outlier criteria with box-plots was used to identify the analytical outliers. After these analytical outliers are excluded from the dataset, the mixed-effects model was used to refit the remaining data to obtain the BLUP values to identify biological outliers. The applicant's approach for identifying the analytical and biological outliers appears acceptable.*
- *The corresponding sections are amended in the current validation report.*

3.5.6. Assay Sensitivity

The unpurified RAKS stock (2500 ng/mL) was diluted using 2-fold serial dilutions in neat pooled human serum to yield a curve that crossed the PSCP. Each dilution was run as one duplicate set (2 wells) diluted to the MRD (1/50) in the assay buffer (un-spiked) and one duplicate set diluted to the MRD with a solution of 50 µg/mL inclisiran prepared in the assay buffer (spiked) on a total of 6 runs performed by two analysts using a balanced design.

To determine the sensitivity of the screening assay on each run, a regression was performed by plotting the instrument response against the concentration using the PC dilution curves fit with a point-to-point regression model. The RAKS PC concentrations interpolated with the PSCP were defined as the sensitivity for each run. The screening assay sensitivity was reported for 95% consistency using the equation mean PC concentration at PSCP + (t0.05,df × SD). The screening assay sensitivity was determined to be 459.55 ng/mL in neat human serum.

To determine the sensitivity of the confirmatory assay, the % inhibition of the mean instrument response in the presence of inclisiran was calculated at each PC concentration and the confirmatory assay sensitivity was reported as the lowest PC concentration tested that consistently confirmed positive in all runs. The confirmatory assay sensitivity was determined to be 78.13 ng/mL in neat human serum.

Reviewer's Comments:

- *The applicant approach for the evaluation of the sensitivity for the screening and confirmatory assays appears to be appropriate.*
- *The screening assay sensitivity of Study No. 3001805, 459.55 ng/mL appears excessively high. Traditionally, FDA has recommended sensitivity of at least 250 to 500 ng/mL. However, recent data suggest that concentrations as low as 100 ng/mL may be associated with clinical events. The applicant justified the appropriateness of the sensitivity of the screening assay by describing as follows; "this method uses a surrogate positive control (rabbit antibodies) and therefore a dual detection system. The sensitivity values are based on the positive control and may not be representative of the sensitivity of the detection in study samples. During selectivity assessment performed using human serum the false positive rate was 10% (1/10) and therefore the sensitivity of the screening assay was considered appropriate."*
- *The sensitivity assessment for both screening and confirmatory assays were performed using the unpurified rabbit anti-KLH-TTRSC serum (RAKS or Ab19151 bleed Day 110). This is not appropriate. The sensitivity should be reevaluated using affinity purified polyclonal antibodies (e.g., affinity purified rabbit anti-KLH-TTRSC-2017 serum used for all other validation parameters except the assay sensitivity).*

3.5.7. Assay Precision

The applicant updated the inter-assay precision results for the screening and confirmatory assay in the amendment No. 5 after taking into account the result from the additional assays Val-67 to Val-80.

Inter-assay Precision of the Screening and Confirmatory Assays

PC samples (LPC, MPC and HPC) were assayed in replicates of at least 2 (n=2), each one in duplicate, on 6 runs performed by at least 2 analysts. At least 1 duplicate set (2 wells) of NC samples was tested in the confirmatory assays. The median value from the 8 singlicates of NC used to determine the PSCP, was used as the unspiked value for each spiked replicate. The global mean, SD and % CV of all intra-assay mean instrument responses and % signal
inhibition values obtained for each PC level over all runs were calculated and used to determine the inter-assay precision. Acceptance criteria are listed in the method validation summary table.

In the screening assay, the % CV of the global mean (i.e. intra-assay mean) A_{450}nm values are; LPC: 12.9%, MPC: 14.1%, HPC: 12.2%.

In the confirmatory assay, the % CV of the global mean (intra-assay mean) % signal inhibition values of PC samples are; LPC: 6.2%, MPC: 5.7%, HPC: 3.1%

Reviewer's Comments:

- *The results are provided in Table 9 and Table 2 for the screening assay and confirmatory assay, respectively, in the validation report.*
- *Inter-assay precision as expressed by % CV values is expected to be lower than 20%. The % CV values of all PC levels in the screening assay are ≤20% and met the acceptance criteria. Likewise, the % CV values of all PC levels in the confirmatory assay are ≤20% and met the acceptance criteria. This is acceptable.*

Intra-assay precision results remain unchanged for both the screening and confirmatory assays. This part was previously reviewed by Dr. Wong (see the review memo for IND 127589_SDN205_eCTD 0203 [Reference ID: 4461526]). The inter- and intra-assay precision for screening and
confirmatory assays are acceptable.

3.5.8. Drug Tolerance

This part was previously reviewed by Dr. Wong (see the review memo for IND 127589_SDN205_eCTD 0203 [Reference ID: 4461526]). The drug tolerance results are acceptable. The drug tolerance assessment appears appropriate.

3.5.9. Selectivity: Interference from Matrix and Drug

The selectivity of the method was tested in normal healthy human serum. At least 10 individual lots of the respective matrix were tested unspiked and spiked with PC at the LPC level and analyzed in the

screening and confirmatory assay. Eight of the 10 healthy human serum samples screened negative and 9 of 10 lots were confirmed negative. When spiked with the PC at the LPC level, all 10 healthy human samples were screened and confirmed positive, demonstrating that the method was capable of detecting a positive ADA response in the presence of serum components.

The selectivity of the method was also tested in a hyperlipidemic human matrix, defined as human serum samples with triglyceride levels in excess of 300 mg/dL. The selectivity assessment performed using unspiked hyperlipidemic human serum failed to meet the acceptance criteria since 6 out of 10 samples (60.0%) screened positive. The selectivity of the confirmatory assay was considered acceptable with 9 of 10 samples (90.0%) confirming negative, which was within the predetermined acceptance criteria. When spiked with the PC at the LPC level, all lipemic samples analyzed were screened and confirmed positive.

Reviewer's Comments: A higher false positive rate was observed in hyperlipidemic human serum samples, which could potentially impact the screening assay results. However, since the confirmatory assay can discriminate between true and false positive in lipemic samples, the applicant believes that the method is considered suitable for use.

The selectivity in hemolyzed matrix (normal healthy human serum with 2% hemolyzed whole blood) was also tested and no interference was noted. All samples without PC tested negative in screening and confirmatory assays, while hemolyzed human serum spiked with the PC at the LPC level screened and confirmed positive.

This part was previously reviewed by Dr. Wong (see the review memo for IND 127589_SDN205_eCTD 0203 [Reference ID: 4461526]) and is acceptable.

3.5.10. Prozone (Hook) Effect

In order to assess the prozone (hook) effect, a sample with a PC concentration five times greater than the HCP in neat human serum was prepared. This sample was then diluted to the MRD (1/50) using an assay buffer (I-Block) before being diluted using ten (10) 2-fold serial dilutions in pooled normal human serum diluted 1/50 in I-Block buffer. The PC curve of the mean A_{450nm} showed a steady increase with the increase of PC concentration. No prozone effect was observed in the PC range tested.

This part was previously reviewed by Dr. Wong (see the review memo for IND 127589_SDN205_eCTD 0203 [Reference ID: 4461526])

3.5.11. Precision of Titers

This part was previously reviewed by Dr. Wong (see the review memo for IND 127589_SDN205_eCTD 0203 [Reference ID: 4461526]) and is acceptable.

3.5.12. Sample Stability

Additional freeze-thaw and bench-top stability assessments using positive controls (LPC and HPC) are ongoing to cover the storage conditions of the study samples.

In the freeze-thaw stability study, stability PC samples are stored in a freezer set to maintain -80°C for at least 24 hours and thawed unassisted at ambient room temperature. When completely thawed (at least 4 hours), the samples will be re-frozen for at least 12 hours. This cycle of freezing and thawing will be done at least 8 times and the samples will be analyzed after the last cycle. Stability results will be considered acceptable if the PC mean A_{450}nm values are > PSCP and have % CV ≤ 20% between duplicate wells.

Bench top stability is assessed as follows; bench-top stability samples are stored in a freezer set to maintain -80°C for at least 24 hours and thawed unassisted at ambient room temperature. These samples are stored at the same temperature for at least 32 hours. After the completion of the stability treatment, samples will be re-frozen until analysis.

Reviewer's Comments:

- *The sample stability assessment was performed in other studies using a surrogate PC (Rabbit anti-KLH-ALNTTRSC serum, RAKS) in monkey serum or human serum. The long-term stability of clinical samples at -20ºC and -80ºC is supported by previous studies performed by other groups. This was reviewed by Dr. Wong (see the review memo for IND 127589_SDN50_eCTD 0048 [Reference ID: 4128063])*
- *Because multiple freeze-thaw cycles of antibody samples (e.g. PCs and subject samples) may affect assay results, information from studies evaluating short-term stability such as freeze-thaw cycles may be useful. Therefore, the applicant's approach for the assessment of freeze-thaw and bench-top stability appears acceptable.*

3.5.13. Robustness

In the previous submission, the assay robustness was assessed by varying the incubation times. However, no information about the tested incubation times were provided. In addition, it was not clear whether other conditions such as buffer, plates, incubation temperature were tested. Therefore, the following comments were communicated to the applicant.

FDA Comment #4:
You assessed the assay robustness for the variations in incubation times. However, it is unclear from the report what incubation times were tested. In addition, you did not evaluate the assay robustness for other changes, such as blocking buffer lots, plates lots, and incubation temperature etc.
Provide a summary of all the conditions tested as a part of robustness assessment.
Provide additional data evaluating incubation temperature, plate shaking speeds, plate lots, reagent concentration and lots.

Novartis response:
The direct ELISA method was demonstrated to be robust to variations in incubation times at the coating, blocking, confirmatory, loading, and detection steps throughout the method validation within the framework of the final analytical procedure included as an appendix in the Final Method Validation report.

The method was considered robust to changes in plate lot, coating material, and reagent lots or batches of wash buffer, blocking buffer, assay buffer, and stop solution. In addition, the methods ruggedness to operator and general laboratory equipment (pipets, plate washers, plate shakers and incubators) were also demonstrated during the method validation.
The method was however not considered robust for changes in incubation temperature, beyond expected variation in ambient room temperature at the coating and blocking steps, reagent concentration, and plate shaking speeds. As a result, deviations to these parameters were not considered acceptable throughout the conduct of the sample analysis.
The final validation report will be amended to include a summary of incubation time variance observed at the coating, blocking, loading, and detection steps during the validation. In addition, variations in lots of critical and non-critical reagents, laboratory equipment, and operator will be described in the robustness section of the amended report and included in a table detailing the additional robustness evaluations. The amended method validation report will be submitted as part of the licensing application.

Reviewer's Comments: Robustness was assessed by varying the incubation times at the coating, blocking, confirmatory, loading and detection steps. In addition to the incubation times, different plate lot, coating material, and reagent lots or batches of wash buffer, blocking buffer, assay buffer, and stop solution were evaluated as part of robustness assessment. A summary of the tested conditions is provided in Table 17 in the amended validation report (amendment No.1). This is acceptable.

During the review cycle, the applicant submitted the amendment for immunogenicity assay validation report (amendment No. 5) on May 29, 2020. In the assay validation report amendment No. 5, the robustness assessment was updated to include the utilization of the liquid handler Biomek Fx (Val-67 to Val-79) for plate coating. The use of the liquid handler Biomek was assessed for the performance of both the coating step and the assay.

PC samples (LPC, MPC and HPC) were assessed in replicates of 6 (n = 6), each one in duplicate on at least 3 occasions in the screening and confirmatory assays using the Biomek. These assays were performed using plates coated using the Biomek on at least 2 different occasions.

Reviewer's Comments: The results were provided in the amended Table 17. In these assays, only 9 out of 13 assays (69.2%) met the acceptance criteria. This is lower than the 90% required for the Biomek Fx performance to be considered acceptable. The applicant considers this is acceptable following investigation which indicated that this high failure rate was due to a specific lot of anti-human IgG/IgM reagent. However, no further information is provided to support this justification. Therefore, the following information request (IR) was sent to the applicant on July 6, 2020. Note that in manually handled assays Val-1-Val-62, 14 out of 62 assays are also rejected by various reasons. On July 20, 2020, the applicant submitted the following response to the IR.

FDA Comment #1
In your immunogenicity assay validation report (3001805), we note that the failure rate of the robustness assessment is excessively high. You stated in the report that a specific lot of goat anti-human IgG+IgM (H+L) used in the assays Val-67 to Val-77 and the assays that failed to meet the acceptance criteria with LPC<PSPC (Val-69, Val-73, Val-76 and Val-77) is linked to the high failure rate. However, no further

details are provided. Please provide the investigation report on these failed assays to support your statement.

Novartis response to FDA Comment #1:
With this response, we provide the requested investigation documentation (Appendix 1). Please note that the investigation documentation refers to the method development study (3001742) that supports the linked validation (3001805).
It should be taken into consideration that the low positive control (LPC) in the method is reported for 99% consistency based on positive control dilution curves (refer to Section 11.3 in the Final Validation Report Amendment 5). This method of statistical determination accounting for a 1% failure rate with respect to the plate-specific cut-point (PSCP) complies with the Final FDA Guidance on the Validation of Anti-Drug Antibody Assays (January 2019). This assessment predicts a failure rate based on a relatively small sample population (positive controls dilution curves from six runs). Therefore, it can be reasonably expected that the actual failure rate may deviate from the predicted value during the assay's life cycle as new critical reagents are qualified in the method and new operators perform the method. Given that the PSCP is determined using the median value of the test sample negative control (NC-TS), and that the NC-TS is detected using the goat anti-human IgG+IgM (H+L) HRP detection antibody, the variability in the performance of any specific lot of this secondary antibody, in addition to variability at the low positive control level, will contribute to the variability in the failure rate observed.
As described in the validation report, 4 out of 13 assays performed during the robustness assessment (Val-69, Val-73, Val-76, and Val-77) failed as a result of the LPC signal < PSCP. Following the investigation into the failure rate, no single cause was identified for the overall increase in assay failure. However, the variability in performance of different lots of the goat anti-human IgG+IgM (H+L) HRP detection antibody were found to contribute to higher variability in NC-TS signal, therefore, impacting the assay threshold (PSCP) and failure rate of the LPC in the method. Specifically, lot 140654 was shown to exhibit higher variability in the method, which was the same lot of goat anti-human IgG+IgM (H+L) HRP detection antibody used in Val-67 to Val-77, and was suspected to be a contributing factor to the observed variability. Importantly, the goat anti-human IgG+IgM (H+L) HRP detection antibody is identified as a critical reagent in the method, in accordance with the analytical procedure, new lots of the secondary antibody are being qualified prior to use in the method. Moreover, the failure rate of the assay is monitored in sample analysis to understand the impact of the performance of newly qualified reagents and the failure rate is reported in the respective study phase reports. Throughout the sample analysis studies, all reported assays met the acceptance criteria and therefore the failure rate did not impact the integrity of the reported results. The immunogenicity assay is considered robust based on the overall performance throughout the sample analysis studies and continues to perform in compliance with the January 2019 FDA Guidance "Immunogenicity Testing of Therapeutic Protein Products —Developing and Validating Assays for Anti-Drug Antibody Detection".

Reviewer's Comments:

- *The applicant provided an investigation report for the high assay failure rate (Appendix 1). This high assay failure rate was due to a high and variable signal in the positive controls and the negative controls. During the investigation, assays were also performed to assess the impact of the I-Block buffer and the wash buffer preparation, along with their critical component reagents (Tropix I-Block and Tween-20), and different batches of TMB from the same lot on the variability of the signal. In addition, the impact of the different lots and different shipment of coating*

material was also assessed. No reproducible trend was observed that could link the buffers, reagents or coating materials to the cause of the variability in signal. However, during the investigation, an assay variability was observed between the goat anti-human lgG+lgM HRP batches prepared using lot 140654 which was greater than the variability observed between the batches prepared using lot 142598. The use of Biomek FX was examined in Study 3001742 (method development study). When compared to Study 3001738 in which manual coating method was used, Study 3001742 showed almost no difference in the assay failure rate. Although a synergistic interaction between multiple factors could cause the observed variability, the applicant concluded that the high failure rate observed in Val-67 to Val-77 was suspected to be caused by a specific lot of goat anti-human lgG+lgM HRP. The applicant stated that to minimize this inter-batch variability moving forward, when preparing a new batch of goat anti-human lgG+IgM HRP, when feasible several vials will be reconstituted simultaneously and will be pooled together before being aliquoted, and the signal obtained in future assays will be closely monitored. The assay failure rate observed in the assays on patient samples from Phase III studies (ORION-9: 20.0%, ORION-10: 13.9%, ORION-11: 14.2%) appear acceptable, and are consistent with or slightly better than the failure rate observed in the validation studies (23.7%). Therefore, the failure rate of the robustness assessment does not seem to have significant impact on the clinical assay performance, and this is acceptable. Nevertheless, the applicant will need to repeat the robustness assessment using the purified PC.

- *The applicant appropriately performed the investigation into the high assay failure rate and provided a reasonable justification for the cause of the failure. This is acceptable.*

FDA Comment #2

We also note that Table 17 of your validation report indicates runs Val-17 through Val-24 and Val-29 through Val-32 were rejected. Please explain why these runs were rejected, and describe how these runs were used to support assay validation.

Novartis response to FDA Comment #2:

Val-17 to Val-24 and Val-29 to Val-32 were rejected since they did not meet the assay acceptance criteria. In all these cases, the immunoglobulin positive control IgM (IPC-IgM) signal in the assay was below the median test sample negative control (NC-TS). Following these repeated failures, an investigation was conducted, leading to the increase of the IPC-IgM concentration from 500ng/mL to 2000ng/mL in order to appropriately monitor the performance of the test sample detection system (i.e. goat anti-human IgG+IgM (H+L) HRP).

Reviewer's Comments:

- *The applicant provided an investigation report for the assay failures in Val-17 to Val-24 and Val-29 to Val-32 (Appendix 2).*
- *The applicant was not able to identify a reagent that possibly cause the variability in signal for the IPC-lgM. However, the issue appears to be resolved by increasing the IPC-IgM concentration from 500ng/mL to 2000ng/mL. The increase in IPC-lgM concentration does not impact on the results obtained for the validation samples.*
- *The applicant appropriately performed the investigation into the repeated assay rejections caused by higher NC-TS and lower IPC-lgM.*

4. Assessment Conclusions

In the immunogenicity assay validation report (b) (4) Study No. 3001805, the applicant demonstrated the ELISA method is selective, precise and robust for the detection of anti-inclisiran-reactive IgG/IgM antibodies in human serum in clinical samples. In addition, the method exhibits sufficient drug tolerance and no hook effect.

All the validation parameters are appropriately evaluated except;

- Two different separately prepared unpurified positive controls were used in the assay validation; rabbit anti-KLH-TTRSC serum (RAKS or Ab19151 bleed Day 110) for the sensitivity assessment, and rabbit anti-KLH-TTRSC-2017 serum (PC) for assessments of all other validation characteristics. This is not appropriate.
- The sensitivity assessment for both screening and confirmatory assays were performed using the unpurified rabbit anti-KLH-TTRSC serum (RAKS or Ab19151 bleed Day 110). This is not appropriate. The sensitivity should be re-evaluated using affinity purified polyclonal antibodies (e.g., affinity purified rabbit anti-KLH-TTRSC-2017 serum used for all other validation parameters except the assay sensitivity).

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/s/

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08/13/2020 04:33:59 PM

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