

CBER CMC BLA Review Memorandum

BLA STN 125835/0

MNEXSPIKE

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1. **BLA#:** STN 125835/0

2. **Applicant Name and License Number:** ModernaTX, Inc. (# 2256)

3. PRODUCT NAME/PRODUCT TYPE

Proprietary name: MNEXSPIKE

Non-proprietary name: COVID-19 Vaccine, mRNA-1283

Product Type: Human Coronavirus mRNA vaccine expressing the RBD and NTD domains of Spike protein of SARS-CoV-2 formulated with lipids SM-102, PEG2000-DMG, DSPC, and cholesterol to form RNA-encapsulating lipid nanoparticles (LNPs).

4. GENERAL DESCRIPTION OF THE FINAL PRODUCT

The COVID-19 Vaccine, mRNA-1283 (MNEXSPIKE) is an mRNA-based vaccine indicated for active immunization for the prevention of coronavirus disease 2019 (COVID-19) in individuals 12 years of age and older. The active ingredient of MNEXSPIKE is a nucleoside-modified (b) (4) mRNA encoding the receptor-binding domain (RBD) and N-terminal domain (NTD) of the SARS-CoV-2 Spike protein, with both domains containing the immunodominant epitopes for protective immune responses. The mRNA is encapsulated in lipid nanoparticles composed of four lipids: SM-102 (ionizable lipid), mPEG2000-DMG, cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), all of which protect mRNA and facilitate its uptake by cells after administration.

The vaccine is provided as a sterile, clear, (b) (4) suspension for intramuscular injection and supplied as a single-dose 1-mL COC prefilled syringe (PFS). Each 0.2 mL vaccine dose is targeted to contain 10 µg of mRNA, 200 µg of total lipids, 0.09 mg tromethamine, 0.51 mg tromethamine hydrochloride, and 17 mg sucrose. The vaccine does not contain preservatives, antibiotics, adjuvants, and human- or animal-derived materials.

The vaccine is stored frozen between -40°C to -15°C but can be stored refrigerated between 2° to 8°C for up to 90 days prior to first use.

5. MAJOR MILESTONES

Regulatory Events / Milestones	Date
Application Received	09/30/2024
Committee Assignment	10/14/2024
First Committee Meeting	10/21/2024 (by email)
Filing Meeting	11/14/2024
Filing Action	11/29/2024
Mid-Cycle Meeting (internal)	01/14/2025

Late Cycle Meeting	03/16/2025
PeRC Meeting	03/18/2025
PMR Study Target	04/19/2025
First Action Due	05/31/2025

6. CMC/NON-CLINICAL/CLINICAL ASSAYS REVIEW TEAM

Reviewer/Affiliation	Section/Subject Matter
Alena Dabrazhynetskaya, OVRD/DVP	<p>Primary product reviewer (CMC/Non-clinical)</p> <p>MODULE 1:</p> <p>1.14 Labeling</p> <p>MODULE 2:</p> <p>2.2 Introduction</p> <p>2.3 Quality Overall Summary</p> <p>2.4 Non-clinical Overview</p> <p>2.6 Non-clinical Summaries</p> <p>MODULE 3: Quality</p> <p>3.2.S [mRNA-1283 (b) (4)] – all sections</p> <p>3.2.S [(b) (4)] – all sections</p> <p>3.2.S [mRNA-1283 (b) (4)] – all sections</p> <p>3.2.P [mRNA-1283 DP] – all sections</p> <p>3.2.A Appendices – Adventitious Agents Safety Evaluation</p> <p>MODULE 4: Non-clinical Study Reports – all sections</p>
Swati Verma, OVRD/DVP	<p>Product reviewer (Clinical Assays)</p> <p>MODULE 5: Clinical Study Reports</p> <p>5.3.1 Reports of Biopharmaceutical Studies</p>

7. INTER-CENTER CONSULTS REQUESTED: None.

8. SUBMISSIONS REVIEWED

Date Received	Submission	Comments/ Status
September 30, 2024	STN 125835/0	BLA submission for all quality-related information included in Modules 1-3, non-clinical study Reports in Module 4, and Clinical Study Report in Module 5.
November 1, 2024	STN 125835/0.2	Response to the DBSQC IR regarding compendial analytical methods.
January 9, 2025	STN 125835/0.10	Response to the OBPV IR#8 regarding the statistical analysis of the analytical procedure validations.
January 9, 2025	STN 125835/0.11	Response to the DBSQC IR#9 regarding the Lot-Release Protocol (LRP) template.

Date Received	Submission	Comments/ Status
February 5, 2025	STN 125835/0.19	Response to the CMC IR#10 regarding mRNA-1283 RNA manufacturing process and controls, SOPs, and method validation results.
February 7, 2025	STN 125835/0.20	Response to the DBSQC IR#14 regarding samples, reagents, and CoAs of mRNA-1283.815 PFS. The SOPs for non-compendial analytical methods are attached to the response.
February 7, 2025	STN 125835/0.21	Response to the DBSQC IR#15 regarding SOP-2460, SOP-1000, SOP-1337, and mRNA-1283 DP testing facilities.
February 14, 2025	STN 125835/0.22	Response to the DBSQC IR#18 regarding the Endotoxin test.
March 3, 2025	STN 125835/0.24	Response to the CMC IR#16, providing information regarding the (b) (4) assay and an overview of SOPs and MVRs.
March 3, 2025	STN 125835/0.25	Response to the IR#19 regarding diagnostic assay verification reports (RT-PCR).
March 11, 2025	STN 125835/0.29	Response to the biostatistician's IR#21 regarding justification of comparability acceptance criteria for mRNA-1283 (b) (4) DP.
March 11, 2025	STN 125835/0.30	Response to the biostatistician's IR#26 regarding the raw data for the validation of analytical methods.
March 14, 2025	STN 125835/0.32	Response to the CMC IR#24 regarding the validation of (b) (4) assay and (b) (4) (SOP-2187).
March 28, 2025	STN 125835/0.37	Response to substantive review issues identified during the BLA late-cycle meeting regarding the mRNA-1283 (Omicron JN.1).
March 31, 2025	STN 125835/0.38	Response to the CMC IR#33 regarding DP stability.
April 2, 2025	STN 125835/0.39	Response to substantive review issues identified during the BLA late-cycle meeting regarding re-submission of the (b) (4) information.
April 11, 2025	STN 125835/0.41	Response to the IR#36 regarding labeling comments.
April 18, 2025	STN 125835/0.43	Response to the IR#37 regarding PMRS.
April 18, 2025	STN 125835/0.44	Response to the IR#38 regarding labeling comments.
April 18, 2025	STN 125835/0.45	Re-submission of eCTD 3.2.S Drug Substance [(b) (4)].

Date Received	Submission	Comments/ Status
April 25, 2025	STN/125835/0.47	Response to the biostatistician's IR#40 regarding validation of the SOP-2187 method.

9. Referenced REGULATORY SUBMISSIONS (e.g., IND BLA, 510K, Master File, etc.)

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
IND 27196	ModernaTX, Inc.	mRNA-1283	NA	IND for mRNA-1283
MF (b) (4)	ModernaTX, Inc.	Lipid excipients: -SM-102 ionizable lipid (b) (4) (b) (4), -Cholesterol -DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), -mPEG2000-DMG (1,2-dimyristoyl-rac-glycero-3-methoxypropylene glycol-2000)	Yes	MF for lipid excipients
MF (b) (4)	(b) (4)	Elastomeric Formulations, Coating and Films. Piston plunger (b) (4) gray	Yes	Permission to cross reference file section 32P7. No need to review DMF since information pertinent to the plunger is included in the BLA
Type III DMF MF (b) (4)	(b) (4)	(b) (4) syringe	Yes	Permission to cross-reference MF. No need to review DMF since information pertinent to the PFS is included in the BLA

10. REVIEWER SUMMARY AND RECOMMENDATION

A. Executive Summary

In the original BLA 125835 submission, pertinent information supporting the licensure of the mRNA-1283 vaccine based on the Omicron subvariant XBB.1.5 (2023-2024 Formula) was submitted. This review encompasses all CMC-related information provided in Modules 2 and 3 of the original BLA 125835 and additional information submitted in multiple BLA amendments, as listed in the table above. The memorandum also summarizes non-clinical data included in Module 4 that support vaccine

immunogenicity and efficacy. Additional CMC and non-clinical data were subsequently amended to BLA 125835 in support of the Omicron subvariant lineage JN.1 (2024-2025 Formula) of mRNA-1283. This dataset was reviewed by Dr. Christian Sauder and documented in a separate memorandum. The validation results for clinical diagnostic assays used to assess clinical efficacy endpoints (Module 5) were reviewed by Dr. Swati Verma, and her assessment has been incorporated in this memorandum.

Chemistry, Manufacturing, and Controls

The manufacturing process for the mRNA-1283 drug substance (DS) consists of (b) (4)

[REDACTED]. The mRNA-1283 drug product (DP) is manufactured by adjusting the concentration of the (b) (4) to the target RNA dose and formulating with a (b) (4), filling into syringes, labeling, and packaging. To support the BLA, process performance qualification (PPQ) data and results from in-process, release, extended characterization, and stability testing for (b) (4) DP (including unlabeled UDP- (b) (4) and labeled LDP- (b) (4)) were provided for each manufacturing facility.

The manufacture of MNEXSPIKE underwent multiple upgrades during product development to optimize the manufacturing process and scale up production to achieve the intended commercial capacity. Manufacture of the (b) (4)

[REDACTED]

Each manufacturing process, (b) (4), and UDP- (b) (4) /LDP- (b) (4), was successfully validated based on the results obtained from a minimum of 3 consecutive PPQ lots. Comprehensive comparability studies were performed using development, clinical, GMP, and PPQ lots of (b) (4) DP. The analytical, extended characterization, and stability data demonstrated that all

quality attributes were highly comparable across the comparability lots of each mRNA-1283 product analyzed. In summary, the mRNA-1283 manufacturing process is under control and capable of consistently producing a DP that complies with the established specifications.

The analytical procedures developed and used for the release and stability monitoring of (b) (4) DP include tests to ensure vaccine identity, purity, quality, potency, and safety. The appropriate assay methods were established and performed in accordance with the standard operating procedures (SOPs). Each analytical procedure has been adequately validated at each facility proposed for the release and stability testing, either through an analytical method validation protocol or by a method-transfer protocol. The validation results demonstrate acceptable specificity, sensitivity, accuracy, precision, and reproducibility of the analytical assays, indicating that they are suitable for mRNA-1283 quality control.

Stability studies have been conducted using the representative lots of (b) (4) DP to support the licensure of the mRNA-1283 vaccine. All data generated in real-time and end-to-end sequential stability studies were used in statistical analysis and shelf-life modelling, supporting the initial shelf-life of 12 months for the commercial mRNA-1283 DP lots stored in the 1 mL COC PFS at the recommended long-term storage condition of -40°C to -15°C. The proposed shelf life may include up to 90 days of storage at 2°C to 8°C and up to 24 hours at room temperature (15°C to 25°C) to support administration of the vaccine at the point-of-care site.

The date of manufacture for MNEXSPIKE is defined as the date of labeling and packing of PFS.

Non-clinical Pharmacology

The results of non-clinical studies performed in mice and (b) (4) rats demonstrated that MNEXSPIKE is well tolerated, safe, and elicits a robust and effective immune response against SARS-CoV-2.

B. RECOMMENDATION

I. APPROVAL: WE RECOMMEND APPROVAL OF THIS BLA.

II. COMPLETE RESPONSE (CR): NO CMC DEFICIENCIES NOTED.

III. SIGNATURE BLOCK

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Alena Dabrazhynetskaya Biologist/LDNAV/DVP/OVRR	Concur	
Swati Verma Biologist/DVP/OVRR	Concur	
Keith Peden Laboratory Chief/LDNAV/DVP/OVRR	Concur	

Robin Levis Deputy Division Director/DVP/OVRR	Concur	
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Abbreviations Used in the Memorandum

(b) (4)	
(b) (4)	(b) (4)
bAbs	Binding Antibodies
(b) (4)	
CCS	Container Closure System
CIPC	Critical In-process Control
(b) (4)	(b) (4)
(b) (4)	(b) (4)
CoA	Certificate of Analysis
CPD	Cumulative Process Duration
CPP	Critical Process Parameter
CPV	Continuous Process Verification
CQA	Critical Quality Attribute
CRT	Controlled Room Temperature
(b) (4)	
GMP	Good Manufacturing Practice
(b) (4)	
IPC	In-process Control
(b) (4)	
(b) (4)	(b) (4)
LOD	Limit of Detection
LOQ	Limit of Quantification
LNP	Lipid Nanoparticle
(b) (4)	(b) (4)
mAbs	Monoclonal Antibodies
MCB	Master Cell Bank
NTD	N-terminal domain
(b) (4)	(b) (4)
PAR	Proven Acceptable Ranges
(b) (4)	

(b) (4)	(b) (4)
PPQ	Process Performance Qualification
PVU	Personal Vaccine Unit
RBD	Receptor-binding domain
RSD	Relative Standard Deviation
RT-qPCR	Quantitative Reverse Transcription Polymerase Chain Reaction
S-2P	SARS-CoV-2 Spike protein with 2 Proline pre-fusion stabilizing mutations
TAR	Target Acceptable Range
(b) (4)	(b) (4)
(b) (4)	(b) (4)
TOR	Time Out of Refrigeration
(b) (4)	(b) (4)
TSE	Transmissible Spongiform Encephalopathy
USP	United State Pharmacopeia
(b) (4)	(b) (4)
WCB	Working Cell Bank
WFI	Water for Injection

Abbreviations not included in the above list are provided in figure legends and table footnotes.

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MODULE 3

INTRODUCTION

The BLA eCTD includes the following sections:

- mRNA-1283 (b) (4) - Drug Substance Intermediate (DSI)
- (b) (4) - DSI
- mRNA-1283 (b) (4) - Drug Substance (DS), the Drug Product active component
- mRNA-1283 (DP) - Drug Product (DP).

(b) (4) that were referred to as (b) (4) in the original BLA 125752 (Spikevax®) are now defined for MNEXSPIKE as (b) (4) and categorized as a Drug Substance Intermediate (DSI) used in the manufacture of (b) (4) Drug Substance (DS). All four lipids comprising the LNPs are defined as critical raw materials in the manufacture of the (b) (4) and contribute to the mRNA-1283 LNP functional and physicochemical properties. With respect to the mRNA-1283 DP, the lipids are categorized as excipients. Although the lipids do not represent active ingredients in the final mRNA vaccine product, they contribute to LNP-mediated delivery and cellular uptake. The complete CMC information for all raw materials (including four lipids) used in the manufacture of (b) (4) is cross-referenced to MF2 (b) (4). However, since the (b) (4) is classified as the DSI, its information should be included in the BLA submission based on the MF Final Rule. The (b) (4) CMC data package was submitted in Amendment 125835.45 (dated April 19, 2025) to include data in Module 3 Section 3.2.S *Drug Substance* (b) (4)] as per the following Agency's request (dated March 19, 2025):

While we agree that the chemistry, manufacture, and control (CMC) information for the (b) (4) components may be cross-referenced, we request that you submit information pertaining to (b) (4) to STN 125835/0 as required by the MF Final Rule because we consider (b) (4) to be a DSI.

Platform Manufacturing Process and Validation

A platform approach is utilized by Moderna across multiple mRNA-based vaccines that are manufactured using similar process steps with closely aligned process parameters and comparable materials and equipment. Leveraging extensive manufacturing experience with the COVID-19 Vaccine, mRNA-1273 (Spikevax®), the platform "Process (b) (4)" was employed for the manufacture of Respiratory syncytial virus (RSV) vaccine, mRNA-1345 (mRESVIA™), including (b) (4), and UDP-(b) (4)/LDP-(b) (4) products. For the COVID-19 Vaccine, mRNA-1283 (MNEXSPIKE), the (b) (4) manufacturing process was substantially modified (b) (4), resulting in the implementation of a new "Process (b) (4)" for the commercial mRNA (b) (4). The licensed

(b) (4) and UDP-(b) (4)/LDP-(b) (4) processes remain unchanged for the mRNA-1283 and continue to be utilized in the manufacture of the (b) (4) UDP (unlabeled DP)/LDP (labeled DP) commercial lots, respectively. The nomenclature and process code references defined for different Moderna mRNA-based products are provided in the summary table below to facilitate the review.

Table 1. Summary of Nomenclature and Code References for Moderna's mRNA Products

Product	(b) (4)
Spikevax	
mRESVIA	
mRNA-1283 (XBB.1.5 variant)	
(b) (4) UDP – unlabeled Drug product; LDP – labeled Drug Product.	

A new terminology implemented by Moderna for mRNA-1283 products for further harmonization of nomenclature and codes is included in **Table 2**.

Table 2. New Terminology Suggested for mRNA-1283

(b) (4)

Platform Analytical Test Methods and Validation

Moderna utilizes a platform approach for testing quality attributes of mRNA-based products of similar composition and biochemical characteristics. This approach applies only to methods that have not been significantly changed when used for testing different mRNA products from the SM-102 vaccine family, that is, operating conditions, system suitability, and result reporting remain the same or are only slightly adjusted. The platform analytical methods were consistently performed for multiple mRNA vaccines in Moderna's infectious-disease portfolio, including commercial, clinical, and investigational products. These procedures were utilized for analytical testing of mRNA-1283 GMP and registration (PPQ) lots for (b) (4)

(b) (4) (DP) and will continue to be used for testing all future commercial scale lots.

The use of platform methods for testing the mRNA-1283 (b) (4) DP was verified based on a combination of method-validation data obtained using both mRNA-1273- and mRNA-1283-specific materials. These two vaccines share common manufacturing processes and controls, and comparison of the (b) (4) DP characteristics revealed compositional, biochemical, and biophysical consistency of both mRNA-1283 and mRNA-1273. Since the primary differences between these two vaccines are the RNA length and sequence, the mRNA-1283-specific validations were performed only for those methods, for which the product-specific parameters must be confirmed.

The necessity of product-specific validation for each platform method performed on (b) (4) DP release was additionally confirmed based on product-specific reference materials or assay-control materials. Reference materials served as comparators, against which test sample results are calculated, while assay controls were used to assess system performance parameters and assay validity criteria. A summary of the platform analytical methods used for mRNA-1283, including both sequence-agnostic and sequence-specific methods, is provided in **Table 3**.

Table 3. Summary of Platform Analytical Methods Used for Testing the mRNA-1283

(b) (4) DP									
Test Method	RNA agnostic vs. RNA specific assays	ModernaTX SOP#	(b) (4)	DP	Product Specific Reference Material	Product Specific Assay Control	Product Agnostic Assay Control		
Identity by (b) (4)	Specific	1019	(b) (4)		X		X		
Identity by (b) (4)	Specific	1337			X		X		
Total RNA content by (b) (4)	Agnostic	0995					X		
Total RNA Content by (b) (4)	Specific	0999			X				
(b) (4)	Agnostic*	2871					X		
(b) (4)	Agnostic	0994					X		
(b) (4)	Agnostic	1000					X		
mRNA Purity/Impurities by (b) (4)	Specific	1142				X			
Lipid Identity/Content /Impurities by (b) (4)	Agnostic	1001					X		
(b) (4)	Agnostic	0998					X		
(b) (4)	Specific	2460					X		

(b) (4)

√ - Release test method used for specific mRNA-1283 material testing.

(b) (4)

Reviewer's note: The Lipid Identity/Content/Impurities by (b) (4) (SOP-1001) and (b) (4) (SOP-0998) methods used for the (b) (4)

(b) (4) DP testing are not affected by mRNA-1283-specific materials. These methods were qualified for release testing of the licensed vaccines, and, therefore, are not covered in this memo.

Prior to implementation, all non-compendial procedures validated using mRNA-1273 materials and revised for the mRNA-1283 testing were additionally assessed to confirm their compliance with the quality-management system. Based on the assessment, no significant updates were made to these methods, and no impact on method performance or validation was identified.

The status of each platform-analytical method validation across the QC facilities qualified for testing the mRNA-1283 materials is presented in **Table 4**.

Table 4. Summary of Platform-analytical Methods Validation for Release and Stability Testing of mRNA-1283 Materials

Test Method	ModernaTX SOP#	(b) (4)	(b) (4)	(b) (4)	DP	ModernaTX*	(b) (4)	Moderna Spain**
Identity by (b) (4)	1019	(b) (4)				RPT-73182	N/A	N/A
Identity by (b) (4)	1337					RPT-73182	RPT-16816	RPT-15579
Total RNA content by (b) (4)	0995					QC-MVR-0003	N/A	N/A
Total RNA content by (b) (4)	0999					QC-MVR-0008	AST-CMO-0049	MQR-0784
(b) (4)	0994					QC-MVR-0007	N/A	N/A
(b) (4)	2187					RPT-18060	N/A	N/A
RNA purity/Product-related Impurities by (b) (4)	1142					RPT-73353	RPT-17909	RPT-17154
Lipid Identity/ Content/ Impurities by (b) (4)	1001					QC-MVR-0010	AST-CMO-0056	MQR-0758
(b) (4)	0998					QC-MVR-0011	AST-CMO-0052	SOP-0998
(b) (4)	1000					QC-MVR-0009	AST-CMO-0050	MQR-0706
(b) (4)	2460					RPT-73332	RPT-18323	RPT-18157

(b) (4)

N/A – the test is not performed at the testing site.

*- QC laboratory performing analytical testing for the mRNA-1283 (b) (4).

** - QC laboratories performing analytical testing for the mRNA-1283 DP.

The current memo covers method-validation results for non-compendial procedures used for mRNA-1283 and qualification data for method transfer to the additional testing sites. The full description of compendial and non-compendial analytical procedures developed for testing mRNA-1273 and details on their validation can be found in the BLA 125752 CMC review memos from DVP and DBSQC reviewers. The results of re-validation are provided in this memo under the corresponding mRNA-1283 (b) (4) DP sections.

3.2.S DRUG SUBSTANCE INTERMEDIATE { (b) (4) }

(b) (4)

69 pages have been determined to be not releasable: (b)(4)

(b) (4)

3.2.P DRUG PRODUCT {mRNA-1283 DP}

3.2.P.1 Description and Composition of Drug Product {0.05 mg/mL mRNA-1283 DP – 0.2 mL PFS}

The mRNA-1283 Drug Product (DP) is an mRNA-lipid complex consisting of an mRNA encapsulated in lipid nanoparticles ((b) (4)). The mRNA-1283 DP is a sterile, preservative-free, white to off-white suspension containing 0.05 mg/mL RNA- (b) (4) , 1.0 mg/mL (b) (4) , and (b) (4) g/L sucrose in (b) (4) mM Tris buffer (pH (b) (4)). The Final DP is supplied as a sterile, single-dose, ready-to-use liquid solution in a 1-mL COC prefilled syringe (PFS) for intramuscular (IM) administration.

Each PFS is intended to deliver a 0.2 mL dose containing 10 µg of RNA and 200 µg of total lipids. Composition and properties of the mRNA-1283 DP in PFS are shown in **Table 50**.

Table 50. mRNA-1283 DP Composition

Component		Grade	Function	Unit Formula (mg/mL)		Unit Formula (µg/dose) (0.2 mL dose)	
RNA- (b) (4)		Custom	Contains mRNA encoding the linked N-terminal domain and receptor-binding domain of the Spike glycoprotein of the SARS-CoV-2 virus	0.05		10	
Total Lipids	SM-102	Custom, (b) (4)	Individual lipids make up the lipid components of the LNP	1.0	(b) (4)	200	(b) (4)
	Cholesterol	(b) (4)			(b) (4)		
	DSPC	(b) (4)			(b) (4)		
	mPEG2000-DMG	Custom, (b) (4)			(b) (4)		
Tris		(b) (4)	Buffer components in Tris buffer	0.45		90	
Tris-HCl				2.6		510	
Sucrose			Cryoprotection	(b) (4)		17 mg	
Water for injection			Diluent	q.s. to 1.0 mL		q.s. to 0.2 mL	

(b) (4); RNA -
 ibonucleic acid; LNP - lipid nanoparticle; SM-102 -
 (b) (4); DSPC - 1,2-distearoyl-sn-glycero-3-phosphocholine; PEG2000-DMG - 1,2- dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000; q.s. - quantum sufficit.

The PFS presentation is detailed in **Table 51** and discussed in Section 3.2.P.7 Container-Closure System of the memo.

Table 51. mRNA-1283 DP in Pre-filled Syringes

RNA Dose	10 µg
Administration Route	Intramuscular
Syringe	1-mL long COC with halobutyl rubber tip-cap in rigid plastic cover
Plunger Rod	Polypropylene plunger rod
Plunger	1-mL long halobutyl rubber plunger with (b) (4) coating on product contact surface
Nominal doses/syringe	Single dose of 0.2 mL
Long-term storage condition	-40°C to -15°C followed by 2°C to 8°C

COC - cyclic olefin copolymer.

3.2.P.2 Pharmaceutical Development {0.05 mg/mL mRNA-1283 DP – 0.2 mL PFS}

3.2.P.2.1 Drug Product Components

The function of each DP component listed in Table 44 was defined as follows:

RNA- (b) (4) encodes the linked NTD and RBD domains of the Spike glycoprotein of the SARS-CoV-2 sublineage XBB.1.5. The protein is expressed in cells following IM injection and cellular uptake and translation in vivo. The expressed protein is monomeric and serves as an antigenic stimulus for an immune response.

- Excipients:
 - SM-102 (b) (4)

- Cholesterol (b) (4)
- DSPC
- mPEG2000-DMG
- Tris buffer (composed of tromethamine and Tris HCl)
- Sucrose is added as a cryoprotectant.

3.2.P.2.2 Pharmaceutical Development

The clinical formulations tested in the clinical studies and the proposed commercial formulation are summarized in **Table 52**. The process and control changes implemented to manufacture each formulation of mRNA-1283 DP are described below in Section 3.2.P.2.3 Manufacturing History {DP}.

Table 52. Comparison of Clinical and Commercial Formulations of mRNA-1283 DP

(b) (4)

The early clinical DP formulations with target RNA concentrations of 0.5 mg/mL (study mRNA-1283-P101) and 0.4 mg/mL and 0.1 mg/mL (study mRNA-1283-P201) were tested in a range of doses prepared by dilution of the product at time of administration. The DP formulation with 0.05 mg/mL RNA was developed for the clinical study mRNA-1283-P301 as a (b) (4) to support a 10-µg dose commercial product. The commercial presentation was then developed as a 10-µg dose PFS to enable ease of administration.

The regression of target concentrations during pharmaceutical development resulted in differences in (b) (4) content in the final mRNA-1283 DP formulations. In the commercial DP formulation, the (b) (4) has been classified as an impurity as discussed in Section 3.2.P.5.5 {DP} of the memo.

Reviewer's note: The density of the clinical and commercial mRNA-1283 DP formulations listed in Table 46 was not indicated in this submission. However, it is expected to be close to Spikevax density of (b) (4), as both DPs, the licensed mRNA-1273 and the proposed mRNA-1283, are formulated in (b) (4) mM Tris buffer with (b) (4) g/L sucrose.

3.2.P.2.2.1 Development Stability Studies

Development stability studies were executed to evaluate the impact of container closure, exposure to light, transport stress, and in-use conditions on stability of the mRNA-1283 DP.

Container-Closure Impact

Container-closure impact was assessed using (b) (4) development lots of mRNA-1283 DP, each filled in (b) (4) container-closure systems, (b) (4) PFS, and stored at the recommended long-term and accelerated storage conditions as indicated in **Table 3 (Appendix 3)**. For each stability-indicating attribute, RNA purity, (b) (4), lipid impurities, and (b) (4), statistical model was established to assess the differences between slopes in the (b) (4) containers at each temperature storage condition. The results showed that stability trends at all storage conditions are consistent between (b) (4) PFS, and the magnitude of differences is not practically important considering the proposed DP storage duration at each temperature condition.

Photostability

Photostability studies were performed using commercial labeled drug product (LDP) filled in PFS. The DP materials were exposed to different light conditions ((b) (4)) and light intensity either with or without representative secondary packaging. The values of biophysical ((b) (4)) and biochemical (total RNA content, lipid content, and lipid impurities) attributes observed in the PFS placed on light exposure were comparable with those of the corresponding (b) (4) controls at (b) (4) °C, with or without secondary packaging. Decreases in mRNA purity and (b) (4) on (b) (4) light exposure were observed when compared with the (b) (4) control at (b) (4) °C for PFS without secondary packaging. No substantial impact to mRNA purity or (b) (4) on light exposure was observed for PFS in secondary packaging.

Overall, the change in DP attributes on light exposure demonstrated that the product requires protection from light. Thus, a "Protect from Light" precautionary statement was included in the DP label. Furthermore, the results demonstrated that the proposed

commercial secondary packaging suitably protects the product from light.

Stability During Transportation

A shipping simulation study was performed using a development PFS lot representative of the commercial mRNA-1283 LDP in both ten-pack and one-pack carton secondary packaging configurations. The PFS in the frozen (-25°C to -15°C) or liquid (2°C to 8°C) state was subjected to shipping stress in a representative small parcel shipper per (b) (4). The impact of shipping on the product quality was assessed by comparing to a parallel set of controls (stored at -25°C to -15°C or 2°C to 8°C, respectively) that did not undergo shipping stress. All results of simulation in frozen or liquid states met the acceptance criteria for both, the ten-pack and one-pack cartoon configurations.

3.2.P.2.3 Manufacturing Process Development {0.05 mg/mL mRNA-1283 DP – 0.2 mL PFS}

Manufacturing process development for mRNA-1283 DP progressed to support clinical development and commercial registration as shown in the scheme below.

(b) (4)

The Version A is a (b) (4) -batch manufacturing process, developed to supply early-phase clinical studies, mRNA-1283-P101 and mRNA-1283-P201, and pivotal clinical study mRNA-1283-P301 Part 1. For details on (b) (4) -batch manufacture and controls, please see Section 3.2.S.2.6 Manufacturing Process Development {mRNA-1283 LNP} above.

The Version B is a (b) (4) DP process, developed to supply pivotal clinical studies and is representative of the intended commercial-scale process. The commercial process was developed to achieve the target strength, safety, and product quality attributes in a scaled-up production, ensuring comparability with the previously manufactured clinical DP.

A summary of DP product IDs and part numbers used to distinguish between the different process versions, RNA inputs, and SARS-CoV-2 variants used for clinical studies and commercial manufacturing is provided in **Table 53**. Each LDP/UDP ID is unique and comprised of (b) (4) parts encrypting the manufactured final product, process, and mRNA sequence.

Table 53. DP Process Development Overview of mRNA-1283

(b) (4)

(b) (4)

3.2.P.2.3.1 Manufacturing Process Changes

The most significant mRNA-1283 DP process changes introduced from Version A through commercial scale are summarized below.

Table 54. Summary of mRNA-1283 DP Manufacturing Process and Control Changes

(b) (4)

(b) (4)

3.2.P.2.3.2 Comparability Assessment

Comparability assessment was performed to ensure that the quality attributes of the DP, from the Version B process for the pivotal mRNA-1283-P301 clinical studies to the commercial-scale PPQ lots, remain consistent throughout the implemented manufacturing process changes.

(b) (4)

(b) (4)

3.2.P.2.3. Manufacturing Process Characterization Studies {0.05 mg/mL mRNA-1283 DP - 0.2 mL PFS}

Characterization of Critical Parameters and In-Process Controls

Cumulative Process Duration

Cumulative Process Duration (CPD) is defined as a CPP for DP manufacturing process and includes both, duration at (b) (4) and Time out of Refrigeration (TOR) at (b) (4), Controlled Room Temperature (CRT). For mRNA-1283, CPD starts at (b) (4)

The duration of Process (b) (4) at (b) (4) is not included in CPD. The details of CPD validation at (b) (4) are discussed in Section 3.2.P.3.5 Process Validation and/or Evaluation (b) (4) below, and the qualified PARs are summarized in **Table 58**.

Fill volume

The 0.05 mg/mL DP is filled into PFS to enable delivery of a 10-µg dose in a 0.2 mL injection. To determine the appropriate fill volume required to achieve this dose, an (b) (4) was generated taking into consideration (b) (4).

The study results showed that a fill volume of (b) (4) assures that at least 0.2 mL of mRNA-1283 DP is consistently delivered with 99.9% probability with the proposed commercial syringes.

A fill-(b) (4) range for 1 mL COC PFS was established as (b) (4) based on the mRNA-1283 DP (b) (4). This range was used during the manufacture of PPQ batches at the (b) (4) manufacturing site.

(b) (4)

(b) (4)

Filtration

The process parameters for (b) (4), were first defined for the development-scale DP process. Maximum filtration (b) (4) was calculated based on the filter (b) (4). Filter (b) (4) was assessed based on the filter (b) (4) was determined. The filtration (b) (4) limit was monitored throughout the process to ensure that it is below the limit of (b) (4). The defined process parameters were then applied at commercial scale and qualified as part of the PPQ study performed at (b) (4). All parameters established for (b) (4) sterile filtration are included in summary **Table 59**.

Characterization of Non-Critical In-Process Controls

(b) (4)

(b) (4)

3.2.P.2.4 Pharmaceutical Development - Container Closure System {0.05 mg/mL mRNA-1283 DP - 0.2 mL PFS}

The commercial PFS container-closure system consists of a 1-mL long syringe, 1-mL long plunger, and a 1-mL long plunger rod, all described in **Table 51** above. The suitability of the selected primary container-closure system for the mRNA-1283 DP was demonstrated through functionality, compatibility, and extractable/leachable studies, as well as container-closure integrity and plunger placement characterization.

The 1-mL cyclic olefin copolymer (COC) syringes are compliant with applicable (b) (4) requirements. No animal-derived materials are used in the manufacturing of the container closure. The technical features and specifications for COC syringes are described in the report QER-12180 attached to the file. The Certificate of Quality is provided to support the use of (b) (4) Sterile 1-mL Long, Integrated Luer Lock with Tip Cap (b) (4) syringe. Empty syringe barrels, ready-to-use (RTU), are received sterile in plastic tubs with polypropylene nests and (b) (4) lids. Prior to using, (b) (4) sterilization of the syringe barrels (b) (4) is performed at the site to achieve the sterility assurance level of (b) (4).

The 1-mL long (b) (4) -coated plungers are compliant with applicable (b) (4) requirements. The product technical specifications are provided in the report EXT-19448. The CoA attached to the file is supportive of (b) (4) plunger. The 1-mL long plungers are received RTU. The plungers are sterilized via (b) (4) following (b) (4), to achieve the sterility assurance level of (b) (4).

The plunger rods are received RTU. The technical specifications for the polypropylene plunger rod are provided in the report QER-12167 and the CoA for the product is attached to the submission. 3.2.P.2.4 Pharmaceutical Development - Container Closure System

Functionality

The functionality of syringes prefilled with 0.2 mL DP was tested for (b) (4), and deliverable volume. Verification data demonstrated the suitable functionality of the 1-mL long plunger as a closure for the 1-mL long COC syringe in conjunction with the 1-mL long plunger rod.

Compatibility

The DP compatibility with the selected container-closure system was demonstrated through a combination of stability studies and evaluation of the extractable and leachable profile described below.

Extractables and Leachables for Container Closure

The 1 mL COC syringe and 1-mL long plunger were deemed as a high-risk consumable for the DP. The syringe is coated with an (b) (4)-compliant (b) (4) for lubrication and contains a rigid tip-cap closure, in which the inner halobutyl tip-cap contacts the product at the syringe tip; however, the product does not contact the outer, transparent layer of the rigid cap closure. The halobutyl rubber plunger is coated on the product-contact surface with an (b) (4) coating that reduces the risk for leachables entering the product.

Extractable studies were performed by (b) (4) using (b) (4) solvents, e.g., (b) (4)

A simulated leachables study was performed using a representative DP lot of mRNA-(b) (4) vaccine filled at an increased fill volume in syringes, which were then stored (b) (4) at (b) (4) for (b) (4) to ensure contact between product solution and all components of the syringe. Although the study results revealed the presence of several extractables, no leachables were detected above the AET of (b) (4).

Altogether, these data indicate that the syringe container used for the mRNA-1283 DP does not pose any safety risk based on its extractables and leachables profile, and the 1-mL long COC syringe with the 1-mL long plunger container-closure configuration is considered suitable for use with the mRNA-1283 DP.

3.2.P.2.6 Compatibility {0.05 mg/mL mRNA-1283 DP - 0.2 mL PFS}

In-use Stability

Compatibility of the DP with the proposed preparation instructions was demonstrated through in-use stability studies performed using a representative mRNA-1283 DP and by mimicking the handling of the LDP at administration sites. Briefly, the PFSs were thawed in accordance with the proposed administration instructions and equilibrated to room temperature. Following tip-cap removal and needle attachment, the PFSs were held at room temperature under ambient light conditions for up to 24 hours. Samples were collected at varying hold durations, while a control sample was collected immediately without a hold to assess the quality attributes. All results obtained for appearance, identity, total RNA content, RNA purity/product-related impurities, (b) (4), lipid content, lipid impurities, and (b) (4), were within the proposed DP stability specifications effective at the time of testing.

Thaw Duration Study

A development study was conducted to assess the duration of PFS thawing in refrigerator (2°C to 8°C) and at CRT (15°C to 25°C). Briefly, thermocouples were placed in a representative DP filled in PFS prior to freezing and were subsequently used to monitor the change in temperature during the DP thaw event. Notably, the representative DP shares the same formulation and physicochemical properties as the mRNA-1283 DP. The worst-case duration representing the slowest thaw time was captured by monitoring PFS positioned (b) (4). The thaw duration times were (b) (4) interval and are presented in **Table 55** for each temperature range.

Table 55. Summary of Thaw Duration Study Results

Configuration	Thaw in Refrigerator	Thaw Duration (mins)	Thaw at Room Temperature	Thaw Duration (mins)
(b) (4) Carton Containing up to 2 PFS	2°C to 8°C (36°F to 46°F)	100 min	15°C to 25°C (59°F to 77°F)	40 min
Carton Containing 10 PFS	2°C to 8°C (36°F to 46°F)	160 min	15°C to 25°C (59°F to 77°F)	80 min

Reviewer's comment:

The fill volume of representative PFS lots used in the thaw duration studies was not indicated. Please see the sponsor's response to Item1 in the IR#4 on pp 114-115.

3.2.P.3 Manufacture {0.05 mg/mL mRNA-1283 DP - 0.2 mL PFS}**3.2.P.3.1 Manufacturers**

The DP manufacturing and testing operations are performed at the sites listed in **Table 56**.

Table 56. mRNA-1283 DP Manufacturers

Facility	Responsibility
(b) (4)	<ul style="list-style-type: none"> Manufacturing of unlabeled drug product (UDP) In-process testing
(b) (4)	<ul style="list-style-type: none"> (b) (4) Assembly, label, and packaging of drug product In-process testing Release and stability testing
(b) (4)	<ul style="list-style-type: none"> Release and stability testing

Moderna Biotech Spain, SL Calle Julián Camarillo, 31, Planta 4a, Madrid 28037 Madrid, Spain	<ul style="list-style-type: none"> • Batch certification • Release and stability testing
(b) (4)	<ul style="list-style-type: none"> • (b) (4) • Frozen storage (b) (4) long-term)
(b) (4)	<ul style="list-style-type: none"> • Distribution
(b) (4)	<ul style="list-style-type: none"> • Distribution

^a (b) (4) is subcontracted by (b) (4)

3.2.P.3.2 Batch Formula

For the 0.05 mg/mL DP in 0.2 mL PFS, the nominal batch size ranges from (b) (4)

Representative batch size and formula for mRNA-1283 DP are provided in Error!

Reference source not found. 57.

Table 57. mRNA-1283 DP Batch Size and Composition

Component	Amount per (b) (4) DP Batch ^(a)	Amount per (b) (4) DP Batch ^(a)
RNA	(b) (4)	
Total Lipids ^(b)		
Tris ^(c)		
Tris-HCl ^(c)		
Sucrose		
Water for injection		

^a Amounts per batch are calculated based on target LNP input and a nominal RNA content of (b) (4).

^b There are four lipid components: SM-102, cholesterol, DSPC, and PEG2000-DMG.

^c Tris is referred to as Tromethamine in the (b) (4) and as Trometamol in the (b) (4).

3.2.P.3.3 Description of Manufacturing Process and Controls {0.05 mg/mL DP - 0.2 mL PFS}

The 0.05 mg/mL mRNA-1283 DP in a single-dose PFS with a 0.2 mL nominal fill volume is manufactured using the Process-(b) (4) platform as shown in **Figure 5 (Appendix 1)**.

(b) (4) process options, Process (b) (4), are used for the manufacture of mRNA-1283 DP in PFS. (b) (4)

(b) (4)

The description of each unit operation for Process (b) (4) is provided below.

(b) (4)

One page has been determined to be not releasable: (b)(4)

3.2.P.3.4 Controls of Critical Steps and Intermediates {0.05 mg/mL mRNA-1283 DP - 0.2 mL PFS}

All CQAs defined for the commercial 0.05 mg/mL mRNA-1283 DP in 0.2 mL PFS are the same as the release specifications shown in **Table 68**. The CPPs and their PARs established for the 0.05 mg/mL PFS process are summarized in **Table 58**.

Table 58. Critical Process Parameters for the Manufacture of 0.05 mg/mL PFS

Process Step	Critical Process Parameter (CPP)	Proven Acceptable Range (PAR)	Rationale
(b) (4)	(b) (4)	(b) (4)	(b) (4)
Cumulative Processing Duration ^(b)	Cumulative Process Duration (TOR, (b) (4)) Cumulative Process Duration ((b) (4) TOR)	(b) (4) (b) (4)	Longer than specified TOR and CPD processing times may impact a DP CQA ((b) (4)).

^a Established based on process and equipment capability, as discussed in Section 3.2.P.2.3 *Manufacturing Process Development - Process Characterization {DP}*.

^b Cumulative process duration begins at the (b) (4)

durations for Process^{(b) (4)} It does include transport time to (b) (4) .

The process parameters (PPs) and their acceptable ranges/acceptance criteria are summarized in the table below for each unit operation and its associated operation variables.

Table 59. Process Parameters for the Manufacture of 0.05 mg/mL PFS

(b) (4)

(b) (4)

The CIPCs and acceptance ranges established for the 0.05 mg/mL PFS manufacturing process are summarized in **Table 60**.

Table 60. Critical In-Process Controls for the 0.05 mg/mL PFS Process

(b) (4)

The in-process hold conditions (duration and temperature) established for each unit operation, involved in the LDP- (b) (4) manufacture, e.g., (b) (4), are provided in summary **Table 59**.

The limits for microbial controls performed during (b) (4) sterile filtration are listed in **Table 61**.

Table 61. Microbial Controls for the 0.05 mg/mL PFS Process

(b) (4)

(b) (4)

All consumables and materials of construction used to manufacture the 0.05 mg/mL PFS at (b) (4) are single-use, (b) (4) and sourced to meet biocompatibility and BSE/TSE requirements, at a minimum. No consumables used for the commercial DP manufacture are sampled or tested in-house.

For additional information regarding characterization studies performed to assess the process parameter ranges and in-process control limits, please see the details provided in Section 3.2.P.2.3. Manufacturing Process Characterization Studies of the memo.

3.2.P.3.5 Process Validation {0.05 mg/mL mRNA-1283 DP – 0.2 mL; (b) (4) }

The UDP-(b) (4) process was validated in the (b) (4) filling line at (b) (4) in accordance with PPQ protocol PVP-3203011. The UDP (b) (4) study was conducted per the stability protocol at the (b) (4) site. The assembly, labeling, and packaging activities were validated at (b) (4) per the established LDP-(b) (4) process protocol.

Since Process (b) (4) includes (b) (4) and represents the worst-case conditions, the PPQ results for Process (b) (4) are considered sufficient to support the registration of (b) (4) process options. An overview of PPQ lots used for validation is provided in **Table 62**.

Table 62. Overview of PPQ Lots Used for Validation of 0.05 mg/mL PFS Process at (b) (4) Line

(b) (4)

(b) (4)

The validation results were provided in Summary PPQ Report PVR-32803-2. Data from Simulated Media Fills, Filter Validation, and Shipping Validation studies were included in separate reports in eCTD Section 3.2.P.3.5 *Process Validation and/or Evaluation* {DP – 0.05 mg/mL PFS} and are summarized below.

Process Performance Qualification

All CQA data obtained from PPQ lots listed in **Table 62** met their Target Acceptable Ranges (TARs), demonstrating consistent, robust, and well-controlled process performance and product quality for both UDP-(b) (4) and LDP-(b) (4). For batch release results, please see **Table 6 (Appendix 2)**.

The CPP values defined for the commercial 0.05 mg/mL PFS process were confirmed to be within their PARs presented in **Table 58**. The minor excursions observed during validation were investigated; the root causes were determined and found to have no impact on the process performance. Samples taken throughout the fill from all PPQ batches were tested for quality attributes in accordance with the release specifications and additionally analyzed for (b) (4). All results met specifications.

All CIPCs and IPCs results were within the defined acceptance criteria, demonstrating consistent process performance. No CIPC and IPC deviations were recorded during the validation. The reported process yields for all PPQ batches also demonstrate effective manufacturing control and consistency. No microbial control excursions occurred during PPQ.

Following PPQ, a Continued Process Verification (CPV) program is established to ensure robust process control across the lifespan of the manufacturing process. The CPV program is intended to detect variation in CQAs, process parameters and controls such that the process is maintained in a state of control. The outcome of these investigations or actions will be documented in a CPV report.

Simulated Media Fills

The media fill study was performed at (b) (4) filling line to assess maximum hold times, simulate process interventions, and confirm that the filled PFS are negative for microbial growth. (b) (4)

The results met the acceptance criteria showing that no microbial contaminated filled containers were discovered during the final inspection. The review of data for environmental monitoring, cleaning validation, and sterilization validation of process contact parts are deferred to the DMPQ reviewers.

Filter Validation

(b) (4)

(b) (4)

(b) (4)

Shipping Validation

The mRNA-1283 DP is shipped from the Contract Manufacturing Organizations (CMOs) domestically and internationally to the Third-Party Logistics (3PL) partners for commercial distribution. Shipments are performed via (b) (4) using qualified passive shippers or temperature-controlled vehicles (TCVs) to maintain the defined shipping temperature of (b) (4) per the product specification. Secondary distribution, in small parcels at the 3PL level, may be conducted at 2°C to 8°C as required.

Shipping was successfully validated for both, DP testing following distribution and qualification of shipping systems. Distribution data, based on evaluation of product quality and device functionality following exposure to shipping stress, support product transportation at (b) (4) and 2°C to 8°C in the proposed shipping systems. For analysis of DP stability during transportation, please see Section 3.2.P.2.2.1 Development Stability Studies of the memo.

Reviewer's conclusion: Overall, the results of validation studies indicate that the 0.05 mg/mL PFS manufacturing process at (b) (4) is under control and capable of consistently producing drug product that complies with the established specifications and quality attributes.

3.2.P.4 Control of Excipients {mRNA-1283 DP}

3.2.P.4.1 Specifications

The mRNA-1283 DP excipients, including the four lipid constituents of the (b) (4), Tris buffer components, and sucrose, are described in section 3.2.P.2.1 Drug Product Components of the memo.

All excipients are received from the approved, qualified suppliers and released prior to use per material specifications. A full testing panel for (b) (4) unique lots of each excipient is performed by either ModernaTX or a contract laboratory. Release specifications for the custom synthesized lipids (SM-102 and PEG2000-DMG) and (b) (4) materials (DSPC and Tris-HCl) are provided in **Tables 5 – 8 (Appendix 3)**. Specifications for (b) (4) excipients, i.e., Cholesterol, Tris, and sucrose, are cross-referenced to MF2 (b) (4).

There are no novel excipients or excipients of human or animal origin used in the mRNA-1283 DP.

3.2.P.5 Control of Drug Product {0.05 mg/mL mRNA-1283 DP – 0.2 mL PFS}

3.2.P.5.1 Specifications

Release and End of Shelf Life (EoSL) specifications for the UDP and LDP lots of mRNA-1283 DP are provided in **Table 68**.

Table 68. mRNA-1283 DP Specifications

Test Method	Sample	Release Acceptance Criteria	EoSL Acceptance Criteria
Appearance by Visual Inspection	(b) (4)	White to off-white dispersion. May contain visible, white or translucent product-related particulates	White to off-white dispersion. May contain visible, white or translucent product-related particulates
Identity by (b) (4)		(b) (4)	N/A
Total RNA Content by (b) (4)		(b) (4)	
mRNA Purity by (b) (4)			
Product-related Impurities by (b) (4)			
(b) (4)			
(b) (4)			
(b) (4)			

(b) (4)	(b) (4)	(b) (4)	
Lipid Identity by (b) (4) : SM-102 Cholesterol DSPC PEG2000-DMG			
Lipid Content by (b) (4) : SM-102 Cholesterol DSPC PEG2000-DMG			
Lipid-related Impurities by (b) (4) (b) (4)			
Particulate Matter by (b) (4) (b) (4) (b) (4)			
Bacterial Endotoxins by (b) (4) (b) (4)			
Sterility by (b) (4) (b) (4)			
Deliverable Volume by (b) (4) (b) (4) (b) (4)			
Container Closure Integrity			
		No Growth	No Growth
		For each of the (b) (4) syringes: (b) (4) 0.2 mL	For each of the (b) (4) syringes: (b) (4) 0.2 mL
		(b) (4)	(b) (4)
		(b) (4)	(b) (4)
		N/A	PASS
(b) (4)			
JP - Japanese Pharmacopoeia; LDP - Labeled Drug Product; N/A -not applicable; PFS - pre-filled syringe; Ph. Eur. - European Pharmacopoeia; (b) (4)			
UDP - Unlabeled Drug Product; USP - U.S. Pharmacopoeia			

3.2.P.5.6 Justification of Specifications

The DP specifications for mRNA-1283 were established in accordance with (b) (4), considering critical quality attributes (CQAs) outlined in (b) (4). For sequence-agnostic attributes, specification limits were established based on clinical experience, compendial requirements, manufacturing process capability and prior knowledge from mRNA-1273. For sequence-dependent attributes, acceptance criteria were established by estimating statistical 95%/99% tolerance intervals approximated by a normal distribution obtained using data across a minimum of (b) (4) mRNA-1283 DP lots. The release and stability data from a total of (b) (4) development, clinical, supportive, and registration lots of mRNA-1283 DP and the most recent (b) (4) DP lots of mRNA-1273 were statistically analyzed to set the release and shelf-life specifications. For the mathematical equations and details of statistical analysis and assessment, please see the review memo provided by biostatistics reviewers on the file.

Justifications for the release specifications established for the commercial lots of mRNA-1283 DP are provided in **Table 69**.

(b) (4)

One page has been determined to be not releasable: (b)(4)

(b) (4)

3.2.P.5.2 Analytical Procedures and 3.2.P.5.3 Validation of Analytical Procedures {mRNA-1283 DP}

The platform analytical methods for testing the mRNA-1283 DP are listed in **Table 1** among others. All non-compendial analytical procedures described in Section 3.2.S.4.2 Analytical Procedures { (b) (4) } are qualified for release and stability testing of (b) (4) samples. All compendial release tests are performed in accordance with the compendial regulatory recommendations and are reviewed by the DBSQC reviewers.

Three QC laboratories, (b) (4), and Moderna Madrid, were qualified for release and stability testing of the commercial lots of 0.05 mg/mL mRNA-1283 DP in 0.2 mL PFS. Method Validation Reports and Method Transfer Reports summarized in **Table 70** were submitted for each method validated.

Table 70. Summary of Analytical Procedures and QC Laboratories Qualified for Testing the mRNA-1283 DP PFS

Analytical procedure / SOP	(b) (4)	(b) (4)	Moderna Madrid
Appearance by Visual Inspection (SOP-0278)	N/A	RPT-17519 (mRNA-1283)	RPT-17972 (mRNA-1283)
Identity by (b) (4) (SOP-1337)	N/A	RPT-15579 (mRNA-1283)	RPT-16816 (mRNA-1283)
Total RNA Content by (b) (4) (SOP-0999)	N/A	RPT-15291 (mRNA-1273)	RPT-73121 (mRNA-1273)
mRNA Purity/Product Related Impurities by (b) (4) (SOP-1142)	N/A	RPT-17909 (mRNA-1283)	RPT-17154 (mRNA-1283)
(b) (4) (SOP-1000)	N/A	RPT-20361 RPT-20516 (mRNA-1273)	RPT-72926 (mRNA-1273)
(b) (4)	N/A	RPT-20798	RPT-73126

(SOP-0998)		(mRNA-1273)	(mRNA-1273)
Lipid Identity/Lipid Content / Impurities by (SOP1001) (b) (4)	N/A	RPT-21151 (mRNA-1273)	RPT-15216 (mRNA-1273)
(b) (4) (SOP-2460)	N/A	RPT-18323 (mRNA-1283)	RPT-18157 (mRNA-1283)
(b) (4) (SOP-0279)	N/A	RPT-17519 (mRNA-1283)	RPT-17972 (mRNA-1283)
(b) (4) (SOP-0288)	N/A	RPT-17519 (mRNA-1283)	RPT-17972 (mRNA-1283)
Sterility by (SOP-0480) (b) (4)	RPT-18203 (mRNA-1283)	N/A	RPT-18078 (mRNA-1283)
Bacterial Endotoxin by (SOP-0352) (b) (4)	RPT-18528 (mRNA-1283)	N/A	RPT-18080 (mRNA-1283)
Particulate Matter (SOP-0509) (b) (4)	RPT-18766 (mRNA-1283)	N/A	RPT-17972 (mRNA-1283)
Deliverable Volume by (SOP- 5183) (b) (4)	REC-2299 (mRNA-1283)	N/A	RPT-17972 (mRNA-1283)
(b) (4)	RPT-14884 (mRNA-1283)	N/A	N/A
Container Closure Integrity (EXT-25134)	RPT-14887 (mRNA-1283)	N/A	N/A

For additional information regarding the platform analytical method's validation and method's transfer, please see the Introduction to Module 3, pages 2-4.

3.2.P.5.4 Batch Analysis {0.05 mg/mL mRNA-1283 DP – 0.2 mL PDF}

The release results and CoAs were submitted for the following PPQ lots, all produced at (b) (4) in (b) (4) line:

(b) (4)

The release results from all batches met the release specifications as presented in **Table 6 (Appendix 2)**. Data from LDP lots (b) (4) are not included in this table since the Process (b) (4) option is considered representative of Process (b) (4).

Additional batch analysis results were submitted for a total of (b) (4) mRNA-1283 DP lots used in clinical studies and one supportive manufacturing experience lot. All data presented for unlabeled and labeled DP of each lot were within the specifications in place at the time of release.

3.2.P.5.5 Characterization of Impurities {mRNA-1283 DP}

The impurity profile of the mRNA-1283 DP is the same as that of the (b) (4); there are no new impurities anticipated to form or be introduced during DP manufacturing. Leachables and extractables from process or container components are discussed in this memo in Sections 3.2.P.2.3 {DP} and Section 3.2.P.2.4 Pharmaceutical Development - Container Closure System {0.05 mg/mL DP - 0.2 mL PFS}, respectively.

Product-related (b) (4) particles may appear in the (b) (4) DP; however, they are not considered as impurities. These particulates are inherent and associated with (b) (4)

(b) (4)

may also contribute to the particulate formation in DP. The presence of particulate matter is controlled as part of release and stability testing by (b) (4).

3.2.P.6 Reference Standards or Materials {mRNA-1283 DP}

The RNA reference materials used in the mRNA-1283 DP release/stability testing are (b) (4), both described in the corresponding sections above.

The information regarding reference standards for SM-102, Cholesterol, DSPC, and PEG2000-DMG used for testing the lipid content, identity and impurity is provided in (b) (4) Section 3.2.S.5 *Reference Standards or Materials* { (b) (4) }.

3.2.P.7 Container-Closure System

The primary container-closure system for the mRNA-1283 DP is a pre-filled syringe (PFS). The materials of construction for the PFS are listed in **Table 51**, and the information regarding the manufacturers and PFS testing at release is discussed below.

1-mL long COC syringes are manufactured by (b) (4) and sterilized by (b) (4).

Empty, ready-to-use (RTU) syringes are received sterile, in plastic tubs with polypropylene nests and (b) (4) lids. (b) (4) sterilization of the syringe barrels (b) (4) is performed according to (b) (4), to achieve the sterility assurance level of (b) (4). Syringes comply with (b) (4).

Specifications for release include (b) (4). No animal-derived materials are used in the manufacturing of the syringe container-closure system.

1-mL long plungers are manufactured by (b) (4) and sterilized by (b) (4). The plungers are received sterilized and ready-to-use and compliant with (b) (4). Specifications for release include (b) (4). Materials for the plungers are not derived from materials of human or animal origin.

Plunger rods are manufactured by (b) (4) and received non-sterile. Plunger rods meet specifications per (b) (4).

The secondary packaging for LDP- (b) (4) is an assembled and labeled PFS, packed into a carton. One patient information leaflet (PIL) or prescribing information (PI) is also placed in the secondary carton. Cartons are then placed into a case and the cases are closed.

Reviewer's comment:

The definition of secondary packaging is missing (b) (4) configuration and does not correspond to the proposed PFS supply in the PI section 16. How Supplied/Storage and Handling.

Please see the sponsor's response provided to Item 2 in the IR#4 on page 115.

3.2.P.8 Stability {mRNA-1283 DP}

An initial shelf life of 12 months is proposed for the mRNA-1283 DP lots stored in the commercial container-closure system at the recommended long-term storage condition of -40°C to -15°C. The proposed shelf life may include up to 90 days of storage at 2°C to 8°C and up to 24 hours at room temperature (15°C to 25°C) to support administration of the vaccine at the point-of-care site. The date of manufacture (DOM) is defined as the start date of the packaging and labeling operations.

Stability data for each stability-indicating attribute at all storage temperatures specified in the label claim were obtained from multiple characterization and stability studies. The mRNA-1283 stability was evaluated using a dataset comprising a total of (b) (4) DP lots, including 3 PPQ, (b) (4) clinical, (b) (4) supportive, and (b) (4) development lots. For statistical analysis and shelf-life modelling, the sponsor used only lots with at least (b) (4) data points that were stored in the commercial container-closure system (1 mL COC PFS); the clinical DP lots stored in (b) (4) were excluded from the shelf-life analysis. A Summary of all stability lots used in the studies, including lot ID, lot number, RNA content, fill volume, intended temperature and duration, and data availability, is provided in **Table 3 (Appendix 3)**.

The stability-indicating attributes analyzed in these studies include appearance, mRNA purity, (b) (4), and lipid-related impurities. Although all these quality attributes show varying degrees of change over time, mRNA purity remains a key determinant of product stability. Appearance was not included in the analysis as it is a qualitative attribute. For (b) (4), the change over the intended shelf life was determined to be not practically important and, therefore, was excluded from the shelf-life analysis as well.

Real-time stability data presented for the 3 PPQ lots support the 24-hour duration proposed for in-use storage at RT. At the date of submission, the results for storage at - (b) (4) °C and 2°C to 8°C were available for only (b) (4) and, therefore, were excluded from the analysis. For clinical GMP lots, the mRNA-1283 DP stability was demonstrated for up to (b) (4) months of storage at (b) (4) °C and up to (b) (4) months at 2°C to 8°C. However, as all clinical lots were stored in (b) (4), these data were also excluded from the statistical modeling of shelf-life. Stability data for development lots were summarized as follows:

- at (b) (4) °C: up to (b) (4) M (lot (b) (4)) and up to (b) (4) M (lots (b) (4)).
- at -25°C to -15°C: up to (b) (4) M (lot (b) (4)); up to (b) (4) M (lots (b) (4)); and up to (b) (4) M (lot (b) (4)).

- at 2°C to 8°C: up to (b) (4) M (lot (b) (4)); up to (b) (4) M (lots (b) (4)); and up to (b) (4) M (lot (b) (4)).
- at 235°C to (b) (4)°C: up to (b) (4) (lots (b) (4)) and up to (b) (4) (lot (b) (4)).

Stability Modeling of Shelf Life

The statistical analysis was performed to model the mRNA-1283 DP shelf life at the proposed sequential storage conditions, including -40°C to -15°C, followed by 2°C to 8°C, and then 15°C to 25°C. In accordance with the ICH Q1E Guidance for the Evaluation of Stability Results, data were analyzed to fit a regression model and estimate the rate of change over time (slope) and standard errors under each temperature range. The end-of-shelf life (EoSL) specification limits were calculated taking into account the impact of the sequential storage and were assessed using the methodology outlined in (b) (4).

All equations used to calculate the expected values and associated uncertainty, estimated degradation rates, and assay variabilities were verified by the biostatistics reviewers and found adequate. A summary of estimated degradation rates and assay variability for mRNA-1283 DP is provided in **Table 71**.

Table 71. Summary of Estimated Degradation Rates and Assay Variability, mRNA-1283 PFS

Attribute	Temperature	Estimated Rate of Change (per month, b_k)	Standard Error of Rate of Change (per month, s_{b_k})	Combined Assay Variance Estimate from All Temperatures (s^2_{assay})
In(mRNA Purity (%))	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C	(b) (4)	(4)	(4)
(b) (4)	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C			
(b) (4)	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C			
(b) (4)	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C			
(b) (4)	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C			
(b) (4)	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C			
Total Lipid Impurities (%)	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C	(b) (4)	(4)	(4)
	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C			
	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C			
	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C			
	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C			
	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C			

Table 72 provides a summary of the predicted means and 95% confidence level (CL) for each attribute after 12 months of storage at -25°C to -15°C, inclusive of 90 days at 2°C to 8°C and 24 hours at 23°C to (b) (4)°C. The results of analysis confirm that a worst-

case lot with a value equal to the release specification limit will remain within the EoSL specification limit when stored at the long-term storage condition of -40°C to -15°C for 12 months, including up to 90 days at 2°C to 8°C and up to 24 hours at room temperature (up to (b) (4) °C).

Table 72. Predicted Means and 95% CL(s) at Expiry, mRNA-1283 PFS, Stored for 12 Months at -25°C to -15°C, Including 90 Days at 2°C to 8°C and 24 Hours at 23°C to (b) (4) °C

(b) (4)

Reviewer's comment:

As detailed above, the predicted means and 95%CLs at expiry were calculated using limited real-time stability data. No end-to-end stability study results are provided in support of the proposed sequential storage conditions.

For the sponsor response, please see Item 3 a-c in the IR#4 below.

CMC INFORMATION REQUEST #4

The IR#33 (shown in bold) was issued on March 24, 2025, and the sponsor's response (shown in *Italic*) was received in Amendment 38 on March 29, 2025:

- 1. Please indicate the nominal PFS fill volumes for development DP lots used in your thaw-duration characterization studies performed to establish thawing times recommended for the 0.2 mL PFS preparation for administration at the clinical site.**

The studies were designed to bracket the range of fill volumes and carton configurations intended for Moderna's mRNA vaccines supplied in PFS, including mRNA-1283. The studies were performed using PFS with nominal fill volumes ranging from (b) (4) mL (0.2 mL dose, applicable to mRNA-1283) to (b) (4) mL (0.5 mL dose, applicable to all licensed mRNA vaccines) in secondary packaging configurations ranging from carton of 1 PFS to carton of 10 PFS.

Reviewer's conclusion: The response is acceptable.

- 2. To support the mRNA-1283 PFS supply proposed in the PI section 16. How Supplied/Storage and Handling, please revise Section 3.2.P.7.2 Secondary Packaging in Module 3 and include the following:**

- The definition of the secondary packaging for LDP as “an assembled, labeled pre-filled syringe (PFS) (b) (4) packed into a carton”.
- All carton configurations proposed for the mRNA-1283 PFS containing one dose of 0.2 mL:
 Carton of 1 single-dose PFS
 Carton of 2 single-dose PFSs
 Carton of 10 single-dose PFSs

Section 3.2.P.7 Container Closure System {DP} was updated to include the configurations as requested.

Reviewer’s conclusion: The revised Patient Prescribing Information (PPI) was submitted in Amendment 44 dated April 18, 2025. The response is acceptable.

3. To support the proposed DP shelf life of 12 months when stored in PFS at the recommended long-term storage conditions of -40°C to -15°C, which may include up to 90 days at 2°C to 8°C and 24 hours at 23°C to (b) (4) °C please provide the following additional information:
 - a. For each LDP lot placed on stability, please indicate whether Process (b) (4) was used for its manufacture. For lots manufactured with Process (b) (4) please provide the duration of (b) (4)
 - b. To support the proposed sequential storage conditions, please provide the results from end-to-end stability studies performed using the 0.05 mg/mL mRNA-1283 DP in 0.2 mL PFS manufactured with Process (b) (4) (including at least (b) (4) stored for ≥ 9 months at -40°C to -15°C, followed by at least 90 days at 2°C to 8°C and 24 hours at 23°C to (b) (4) °C.
 - c. If the full-size end-to-end stability study is not completed yet, please submit any available stability data from the frozen mRNA-1283 DP transferred for storage at 2°C to 8°C for a minimum of 3 months followed by 24 hours at 23°C to (b) (4) °C.

*The sponsor submitted additional stability data including up to 6 months for PPQ lots and up to (b) (4) months for representative development lots stored in COC PFS. All provided results are within specification over the shelf-life storage conditions and no unexpected trends have been observed. The updated real-time stability summary is shown in **Table 3 (Appendix 3)**.*

The sponsor stated that all LDP lots placed on stability were manufactured following Process (b) (4) including (b) (4) for each lot is presented in the table below.

Table 73. Updated Summary of PPQ Lots Placed on Stability

(b) (4)

(b) (4)

End-to-end stability studies have been initiated for the three PPQ lots (b) (4) (b) (4) manufactured using Process Flow (b) (4) that included (b) (4). The sponsor stated that these studies were conducted for Post-Approval Stability monitoring. The protocols for LDP stability under sequential storage conditions are provided in the updated Section 3.2.P.8.2 Post-approval Stability Protocol and Commitment {DP – 0.05 mg/mL PFS} and included in the tables below. The studies are expected to be completed by the end of 2025.

Table 74. Post-Approval Stability Protocol mRNA-1283 LDP (-25°C to -15°C)

Test Method	0 month	1 month	3 months	6 months	9 months
Appearance by Visual Inspection	X	X	X	X	X
(b) (4)	X	X	X	X	X
mRNA Purity by (b) (4)	X	X	X	X	X
Product-Related Impurities by (b) (4)	X	X	X	X	X
(b) (4)	X	X	X	X	X
(b) (4)	X	X	X	X	X
(b) (4)	X	-	X	-	X
(b) (4)	X	-	X	-	X
Total RNA Content by (b) (4)	X	-	X	-	X
Lipid Content and Lipid Impurities by (b) (4)	X	X	X	X	X
Bacterial Endotoxins by (b) (4)	X	-	X	-	X
Particulate Matter by (b) (4)	X	-	X	-	X
(b) (4)	X	-	-	-	X
(b) (4)	X	-	-	-	X
Container Closure Integrity	X	-	-	-	X
Sterility by (b) (4)	X	-	-	-	-

Table 75. Post-Approval Stability Protocol mRNA-1283 LDP (2°C to 8°C) after 9 Months at -25°C to -15°C

Test Method	0 month ^(a)	1 month	2 months	3 months
Appearance by Visual Inspection	X	X	X	X
(b) (4)	X	X	X	X
mRNA Purity by (b) (4)	X	X	X	X
Product-Related Impurities by (b) (4)	X	X	X	X
(b) (4)	X	X	X	X
(b) (4)	X	X	X	X
(b) (4)	X	-	-	X
(b) (4)	X	-	-	X
Total RNA Content by (b) (4)	X	-	-	X
Lipid Content and Lipid Impurities by (b) (4)	X	X	X	X
Bacterial Endotoxins by (b) (4)	X	-	-	X
Particulate Matter by (b) (4)	X	-	-	X
(b) (4)	X	-	-	X
(b) (4)	X	-	-	X
Container Closure Integrity	X	-	-	X
Sterility by (b) (4)	-	-	-	-

Table 76. Post-Approval Stability Protocol mRNA-1283 LDP (23°C to (b) (4) °C) after 3 Months at 2°C to 8°C

Test Method	Initial ^(a)	24
Appearance by Visual Inspection	X	X
(b) (4)	X	X
mRNA Purity by (b) (4)	X	X
Product-Related Impurities by (b) (4)	X	X
(b) (4)	X	X
(b) (4)	X	X
(b) (4)	X	X
(b) (4)	X	X
Total RNA Content by (b) (4)	X	X
Lipid Content and Lipid Impurities by (b) (4)	X	X
Bacterial Endotoxins by (b) (4)	X	X
Particulate Matter by (b) (4)	X	X
(b) (4)	X	X
(b) (4)	X	X
Container Closure Integrity	X	X
Sterility by (b) (4)	-	X

Additional end-to-end stability study was initiated for a representative development lot (b) (4) stored in the commercial container closure (COC PFS). This lot has been stored at -25°C to -15°C for 3 months to generate subplot (b) (4) and 6 months for subplot (b) (4). Subplots (b) (4) were then transferred and stored at 2°C to 8°C for 3 months for sequential storage followed by 24 hours at 23°C to (b) (4) °C. All available data for these (b) (4) sublots are within specifications under the long-term storage conditions and no unexpected trends have been observed. This data continues to support the proposed shelf life for mRNA-1283 DP. An additional subplot (b) (4) will be stored at -25°C to -15°C for (b) (4) months and will be subsequently transferred to 2°C to 8°C for 3 months followed by 24 hours at 23°C to (b) (4) °C to cover the proposed shelf life for mRNA-1283 DP. This study is expected to be completed by the end of Q3 2025.

Reviewer's conclusion: The response is acceptable.

Overall, data provided in the initial BLA 125752.0 and the updated real-time and sequential stability results submitted in Amendment 38 support the shelf life of 12 months proposed for the mRNA-1283 DP lots stored in the commercial container-closure system at the recommended long-term storage condition of -40°C to -15°C, that may include up to 90 days of storage at 2°C to 8°C and up to 24 hours at room temperature (15°C to 25°C) for administration of the vaccine at the point-of-care site.

3.2.A.2 Adventitious Agents Safety Evaluation

There are no materials of animal or human origin used in the manufacture of the (b) (4) Drug Product intended for registration.

MODULE 4**NONCLINICAL STUDIES**

This section covers the nonclinical pharmacology, pharmacokinetics, and toxicology studies supporting the development of mRNA-1283 vaccine (hereafter referred to as mRNA-1283).

4.1 Pharmacology

Comprehensive nonclinical pharmacology studies were performed to evaluate the expression and immunity derived from mRNA-1283 encoding the NTD and RBD sub-domains of the SARS-CoV-2 S protein linked to the influenza HATM domain (NTD-RBD-HATM) in comparison with the licensed SPIKEVAX™ vaccine (referred to as mRNA-1273) encoding the full-length S protein of the SARS-CoV-2 (S-2P). All immunogenicity studies were performed in mice, following administration of mRNA-1283 as a primary series or as a booster dose in animals previously vaccinated with mRNA-1273.

A summary of *in vitro* and *in vivo* pharmacology studies, including description of test articles, administered doses, and submitted nonclinical reports is provided in **Table 77**. All studies were performed at the ModernaTX, Inc. facility (Cambridge, MA, USA), with the exception of studies from Stewart-Jones et al., 2023, which were conducted at Washington University School of Medicine (Saint Louis, MO, USA). The test materials used in these studies were non-GLP development DP lots formulated using mRNAs encoding Wuhan-Hu-1 (original), Omicron B.1.351 (Beta), Omicron B.1.1.519 (BA.1), Omicron BA.4/BA.5, Omicron XBB.1.5, or Omicron XBB.1.16. For description of each variant DP composition, please see the footnotes in the table below.

Table 77. Summary of Nonclinical Pharmacology Studies Supporting mRNA-1283

Type of Study	Test Article and Dose (µg) ^a	Species or Cell Line, Strain or Derivative	Administration; Immunization Schedule	GLP	Report Number
Evaluation of <i>in vitro</i> and <i>in vivo</i> expression of mRNA-1273 and mRNA-1283 (SARS-CoV-2)	In vitro: mRNA encoding SARS-CoV-2 S-2P or NTD-RBD-HATM: (0.1 µg to 0.003125 µg via TransIT®-mRNA Transfection Kit) In vivo: mRNA-1273: 2 or 10 mRNA-1283: 2 or 10	In vitro: HEK293T cells, ATCC# CRL-11268 In vivo: Mouse (female), BALB/c	IM; single dose (Day 0)	No	MOD-4112
Evaluation of the immunogenicity and dynamic range of mRNA-1283 (SARS-CoV-2)	mRNA-1283: 0.000305, 0.000611, 0.001221, 0.002441, 0.004883, 0.009766, 0.019531, 0.039063, 0.078125, 0.15625, 0.3125, 0.625, 1.25, 2.5, 5, 10, or 20	Mouse (female), BALB/c	IM; prime/boost (Day 1 and Day 22)	No	MOD-4079
Evaluation of the immunogenicity of mRNA-1283 (SARS-CoV-2)	mRNA-1273: 0.1 or 1 mRNA-1283: 0.1 or 1	Mouse (female), BALB/c	IM; prime/boost (Day 1 and Day 22)	No	MOD-3964 MOD-4035 MOD-4101

Evaluation of immunogenicity of mRNA-1283 primary series and matched variant-specific booster dose in mice (Stewart-Jones et al. 2023) ⁶	mRNA-1283: 0.1 or 1 mRNA-1273: 0.1 or 1 mRNA-1273.351 ¹ : 0.1 or 1 mRNA-1283.351: 0.1 or 1	Mouse, BALB/c	IM; primary series (Day 1 and Day 22) followed by boost (Day 57)	No	NA
Evaluation of immunogenicity of mRNA-1283 variant-specific booster dose following mRNA-1273 primary series in mice (Stewart-Jones et al. 2023) ⁶	mRNA-1283: 1.0 mRNA-1273: 1.0 Variant-specific (B.1.351) monovalent or bivalent mRNA-1273: 1.0 Variant-specific (B.1.351) monovalent or bivalent mRNA-1283: 1.0	Mouse, BALB/c	IM; primary series (Day 1 and Day 22) followed by boost (Day 57)	No	NA
(b) (4)					
Evaluation of the protection of monovalent mRNA-1283 against WA1/2020 D614G or B.1.1.529 (BA.1; Omicron) challenge (Stewart-Jones et al. 2023) ⁶	mRNA-1283: 0.1 or 5 mRNA-1273: 0.1 or 5	Mouse, K18-hACE2 C57BL/6 J	IM; prime/boost (Day 1 and Day 22)	No	NA
Evaluation of in vivo immunogenicity of mRNA-1283.222 in BALB/c mice	mRNA-1283.222-v2 ³ : 0.4 mRNA-1283.222 ³ : 0.4	Mouse, BALB/c	IM; prime/boost (Day 1 and Day 22)	No	MOD-5814-1283
(b) (4)					
Evaluation of immunogenicity of monovalent SARS-CoV-2 XBB-containing mRNA-1283 vaccine boosters in mice	Primary series mRNA-1273: 0.5 Booster dose mRNA-1273.116 ⁴ : 1.0 mRNA-1283.815 ⁵ : 1.0 mRNA-1283.116 ⁴ : 1.0 mRNA-1283.222 ³ : 1.0	Mouse, BALB/c	IM primary series (Day 1 and Day 22) followed by boost (Day 106)	No	MOD-5972.1283

GLP - Good Laboratory Practice; HATM - hemagglutinin transmembrane domain; HEK - human embryonic kidney; IM - intramuscular(ly); mRNA - messenger ribonucleic acid; NA - not applicable; NTD - N-terminal domain; PBS - phosphate-buffered saline; RBD - receptor-binding domain; S-2P - spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2.

^a Dose refers to the total dose of mRNA encapsulated in the administered LNP.

1 - mRNA-1283.351 and mRNA-1273.351: Monovalent, Omicron B.1.351 (Beta) variant.

2 - mRNA-1283.214 and mRNA-1273.214: Bivalent (mRNA-1283 + mRNA-1283.529), Original and Omicron B.1.1.519 [BA.1] variant.

3 - mRNA-1283.222 and mRNA-1283.222: Bivalent (mRNA-1283 + mRNA-1283.045), Original and Omicron BA.4/BA.5 variant.

4 - mRNA-1283.116 and mRNA-1273.116: Monovalent (Omicron XBB.1.16 subvariant).

5 - mRNA-1283.815: Monovalent (Omicron XBB.1.5/XBB.1.9.1 subvariant. Note: S protein of XBB.1.9.1 is identical to XBB.1.5).

6 – Stewart-Jones et al. Domain-based mRNA vaccines encoding spike protein N-terminal and receptor binding domains confer protection against SARS-CoV-2. *Sci Transl Med* . 2023 Sep 13;15(713).

The immunological assays used in the non-clinical pharmacology studies include the following:

- Quantitative ELISA for the full-length S protein (S-2P), N-terminal domain (NTD), or receptor-binding domain (RBD) of SARS-CoV-2 (originally qualified for the non-clinical pharmacology studies and then validated for clinical use).
- Intracellular Cytokine Staining (ICS), a flow cytometry-based method for the evaluation of cell-mediated immunity (qualified for the non-clinical pharmacology studies and validated for clinical use).
- Qualitative EliSpot for analysis of IgG levels of S-2P-, RBD-, and NTD-reactive Antibody-Secreting Cells.
- Quantitative Pseudovirus Virus Neutralization Assay (PsVNA) performed with SARS-CoV-2 S-pseudotyped recombinant VSV-ΔG-firefly luciferase for measuring neutralizing antibodies (nAbs) (qualified for the non-clinical studies only). The assay was originally developed and validated for the SARS CoV-2 ancestral Wuhan-Hu-1 strain containing the D614G mutation in the Spike gene sequence (WA1/2020 D614G) and was updated to include S-gene sequence from different SARS CoV-2 variants.

4.1.1 Evaluation of In Vitro and In Vivo Expression (MOD-4112)

The objectives of Study MOD-4112 were to evaluate the in vitro and in vivo expression of mRNA-1283 in comparison with mRNA-1273.

The test articles used in this study were the monovalent mRNA-1283 DP lot DP-014561 (encodes the NTD-RBD-HATM derived from the SARS-CoV-2 S glycoprotein of the Wuhan-Hu-1 (original) isolate) and the monovalent mRNA-1273 DP lot AMPDP-200005 (encodes the full-length S-2P of the SARS-CoV-2, Wuhan-Hu-1 isolate). PBS was used as a negative control.

Study design

In vitro expression was evaluated in HEK293T cells, which were transiently transfected with mRNA-1273 and mRNA-1283 using a TransIT mRNA transfection kit (Mirus Bio, Madison, WI). 24 hours, 48 hours, and 72 hours post transfection (pt), the cells were collected, resuspended in fluorescence-activated cell sorting (FACS) buffer, stained with the proposed dilution of antigen-specific Abs (CR3022; RBD-specific), incubated with Alexa Fluor 647 goat anti-human

IgG, and then assessed for expression of the encoded antigens by flow cytometry. The cell surface expression was calculated by multiplying the frequency of positive cells by the Median Fluorescence Intensity (MFI*frequency), which indicated the level of S-2P or NTD-RBD-HATM antigen expression after a dose titration from 0.1 µg to 0.003125 µg. The frequency and intensity of mRNA-1273 and mRNA-1283 expression were determined in cells transfected with 0.05 µg or 0.2 µg of mRNA.

In vivo expression was evaluated in 2 groups of female BALB/c mice (n=6/group) after a single IM injection with 2 µg or 10 µg of mRNA-1283, 2 µg or 10 µg of mRNA-1273, or PBS. Assessments were performed on immune-cell populations harvested from the spleen and lymph nodes 24, 48, and 72 hours post injection (pi). The plasmacytoid dendritic cells (pDCs) and conventional dendritic cells (cDCs) collected from both tissues were prepared for flow cytometry following a described procedure. *In vivo* expression was analyzed using RBD-specific (CR3022) or NTD-specific (4A8) primary Abs and anti-human Fc IgG (AL647) secondary Abs.

Results

In Vitro: Sustained expression of both proteins, S-2P and NTD-RBD-HATM, was recorded in HEK293T cells at 24, 48, and 72 hours pt. Overall, a higher expression of NTD-RBD-HATM in comparison with S-2P was observed at all 3 timepoints, with the peak expression recorded at 72 hours pt.

Evaluation of cell-surface expression in cells transfected with 0.05 µg and 0.2 µg of mRNA-1283 or mRNA-1273 revealed an overall increase in the frequency of antigen-expressing cells after staining cells with mAbs that bound to the RBD and the NTD. A higher expression of RBD-positive cells was observed in mRNA-1283-transfected cells when compared with the mRNA-1273-transfected cells. Protein expression was dose dependent, showing greater expression with the 0.2 µg dose versus the 0.05 µg dose. A slightly higher frequency of cells expressing both antigens was observed at 48 hours compared with 24 hours in all groups. The MFI of expression was higher in cells transfected with mRNA-1283 as compared with mRNA-1273.

In vivo: In the spleen, the expression levels of mRNA-1283 and mRNA-1273 showed no statistically significant differences in antigen expression measured in cDCs or pDCs. The higher dose resulted in increased antigen expression in immune cells collected from the spleens, and injection with the higher dose (10 µg) demonstrated an overall increase in antigen-positive cells across both cell types, treatment groups, and timepoints. mRNA-1273 appeared to have slightly better RBD expression than mRNA-1283 in the spleen cDCs, but not in pDCs. No significant difference was evident for NTD expression.

In the lymph nodes, *in vivo* expression of mRNA-1283 and mRNA-1273 resulted in no statistically significant differences in the levels of antigen expression measured in cDCs or pDCs targeting either the NTD or the RBD domains. In the

lymph nodes, 10 µg mRNA-1283 induced higher expression compared with the 10 µg mRNA-1273.

Conclusions

- *In vitro* expression of mRNA-1283 and mRNA-1273 in HEK293T cells showed sustained expression of both NTD-RBD-HATM and S-2P in a dose-dependent manner, with mRNA-1283 exhibiting higher expression.
- *In vivo* expression in BALB/c mice showed no significant differences in RBD or NTD epitope expression after administration of single doses of mRNA-1283 or mRNA-1273. At the 10 µg dose, mRNA-1273 revealed higher expression in the spleen compared with mRNA-1283, whereas mRNA-1283 demonstrated higher expression in the lymph nodes.

4.1.2 Evaluation of the Immunogenicity and Dynamic Range (MOD-4079)

The objectives of Study MOD-4079 were to evaluate the immunogenicity and determine the titer dynamic range of mRNA-1283 (Report MOD-4079).

The study was performed in young female BALB/c mice using the monovalent mRNA-1283 DP lot DP-014561 (with NTD-RBD-HATM derived from the S protein of SARS-CoV-2, Wuhan-Hu-1 isolate) as test article and PBS as negative control.

Study design

17 groups of BALB/c mice (n=8/group) received IM injections of multiple dose levels of mRNA-1283 (ranging from 0.00305 to 20 µg/dose) or PBS on a prime (Week 0/Day1) / boost (Week 3/Day 21) schedule. Sera were collected at Week 3 (Day 21; before the second injection) and Week 5 (Day 36) for assessment of S-, RBD-, and NTD-specific IgGs by ELISA.

The direct ELISA was performed following a described procedure, using S-2P (Genscript, # (b) (4)), RBD (Sino Biological, # (b) (4)), or NTD (Moderna, #1) proteins for plate coating and HRP-conjugated goat anti-mouse IgG antibody (Southern Biotech, #1030-05) for detection of the specific antigens.

Results

On Day 21, 3 weeks after the first (prime) dose, S- and RBD-specific IgG titers were detectable at the 0.039063 µg dose level and above; NTD-specific IgG titers were detected at the 0.078125 µg dose level and above. On Day 36, 2 weeks after the second (boost) dose, S- and RBD-specific IgG titers were detectable at the 0.002441 µg dose level and above; NTD-specific IgG titers were detected at the 0.004883 µg dose level and above. The titers of all 3 Abs measured at Day 36 were higher than those measured at Day 21.

Conclusion

Administration of mRNA-1283 as a primary series in BALB/c mice demonstrated a dose-dependent response in anti-SARS CoV-2 S-protein IgG titers. mRNA-1283 also elicited a dose-dependent response in RBD- and NTD-specific IgG titers, with higher doses necessary to detect the NTD-specific Abs. Boosting with mRNA-1283 significantly increased antibody responses.

4.1.3 Evaluation of the Immunogenicity (MOD-3964, MOD-4035, and MOD- 4101)

The objectives of Study MOD-3964 were to evaluate the immunogenicity of mRNA-1283 compared with mRNA-1273 and to address the theoretical concern of enhanced respiratory disease (ERD). This study was repeated in Studies MOD-4035 and MOD-4101 for confirmation of dose responses and for the evaluation of additional immune endpoints.

All three studies were performed in young female BALB/c mice using the monovalent mRNA-1283 DP lot DP-014561 (NTD-RBD-HATM from the original isolate) and monovalent mRNA-1273 DP lot AMPDP-200005 (S-2P from the original isolate). PBS was used as a negative control.

Study design

3 groups of female BALB/c mice (n=8/group) received IM injections of 0.1 µg or 1 µg of mRNA-1283, 0.1 µg or 1 µg of mRNA-1273, or PBS on a prime (Week 0/Day 1) / boost (Week 3/Day 21) schedule. Sera were collected at Week 3 (Day 21; 1 day before the second injection) and Week 5 (Day 36; 2 weeks after the second injection) and analyzed with ELISA for IgG Ab binding to SARS-CoV-2 S protein S protein (Study MOD-3964) or S-2P (Studies MOD-4035 and MOD-4101); IgG Ab binding to RBD- and NTD-specific protein (Studies MOD-3964 and MOD-4035), and S-2P-specific IgG Ab subclasses, IgG2a and IgG1 (Study MOD-4101). For ELISA, plates were coated with either S protein (S1+S2 (b) (4)), S-2P (prepared by the Sponsor), RBD (Sino Biological #40592-V08H0), or NTD protein (prepared by the Sponsor), incubated with serum followed by treatment with either HRP-conjugated goat anti-mouse IgG (Southern Biotech #1030-05), goat anti-mouse IgG1 (Southern Biotech # (b) (4)), or goat anti-mouse IgG2a (Southern Biotech # (b) (4)). Absorbance was measured at optical density (OD) 450 nm using a (b) (4) plate reader.

Additionally, the correlation between IgG Ab titers and nAb activity (Studies MOD-3964) and ratios of IgG Ab subclasses (IgG2a/IgG1) (Study MOD-4101) were calculated as described in the reports. For PsVNA, heat-inactivated serum samples incubated with rVSV-ΔG-based SARS-CoV-2 pseudovirus were used for infection of A549-hACE2-TMPRSS2 cells. After adding One-Glo reagent (Promega #E6120), the luciferase activity was measured using a BMG PHERastar-FS plate reader.

In Studies MOD-4035 and MOD-4101, the splenocytes were isolated from spleen samples that were collected on Day 36. Antigen-reactive IgG antibody-secreting cells (ASCs) were measured by an ELISpot assay using the S-2P, RBD, and NTD proteins listed above and goat anti-mouse Igk Fab'2 Ab (Southern Biotech # (b) (4)). The plates were imaged using an ImmunoSpot Analyzer (Cellular Technologies), and spots manually counted. T-cell analyses were performed following a described FACS procedure after stimulation with peptide pools of the full-length S (S1+ S2), RBD, or NTD (Study MOD-4101 only) protein.

Results

Evaluation of Humoral Immune Responses

Vaccination with 0.1 µg or 1 µg of mRNA-1283 and mRNA-1273 induced dose-dependent increases in S- and S-2P-specific IgG titers; however, the IgG binding titers induced by mRNA-1283 were significantly elevated compared with mRNA-1273 at both dose levels. Statistical significance was observed on Day 21 at 0.1 µg (Study MOD-4035) and on Day 36 at 0.1 µg (Studies MOD-3964 and MOD-4101) and 1 µg (Study MOD-3964). These results demonstrated that equal mass doses of mRNA-1283 drive equivalent or superior specific S-binding Ab titers.

Vaccination with 0.1 µg and 1 µg of mRNA-1283 or mRNA-1273 also induced dose-dependent increases of RBD- and NTD-specific IgG titers. The mRNA-1283 IgG binding titers were significantly elevated compared with mRNA-1273 at the two dose levels. Statistical significance for RBD-specific IgG was observed on Day 21 at 0.1 µg and 1 µg (Studies MOD-3964 and MOD-4035), on Day 36 at 1 µg (Study MOD-3964), and at 0.1 µg (Studies MOD-4035). Statistical significance for NTD-specific IgG was observed on Day 21 at 1 µg (Studies MOD-3964 and MOD-4035) and on Day 36 at 1 µg and 0.1 µg (Studies MOD-3964 and MOD-4035).

In mice, the ratio of IgG Ab subclasses was considered a surrogate marker of the Th1/Th2 response. A ratio of IgG1 subclass with low or no IgG2a is associated with a Th2-directed response, whereas balanced IgG2a/IgG1 Ab subclasses are associated with a Th1-directed response. In the current studies, dose-dependent, high titers of both IgG1 and IgG2a were detected in mice administered with 1 µg and 0.1 µg of mRNA-1283 or mRNA-1273, with mRNA-1283 driving higher levels of IgG2a. A comparison of the IgG2a/IgG1 ratios showed that both mRNA-1283 and mRNA-1273 revealed a balanced IgG2a/IgG1 ratio, indicating a Th1-directed response.

Vaccination with both mRNA-1283 and mRNA-1273 at 0.1 µg and 1 µg induced consistent dose-dependent increases in nAb titers at the same level. In Study MOD-3964, the nAb titers measured on Day 36 were highly correlated with the S-2P and RBD IgG titers indicating that ERD mediated by immune complex deposition (e.g. high levels of bAbs with low levels of nAbs) and complement activation is not likely to be present after vaccination with mRNA-1283.

Evaluation of Cellular Immune Responses

The ELISpot-based evaluation showed the elevated levels of ASCs reactive to both S-2P and RBD in animals administered with mRNA-1283 and mRNA-1273, with no significant difference observed between these two vaccines. However, the level of NTD-reactive ASCs after administration of mRNA-1283 was substantially higher versus the mRNA-1273 comparator, indicating that the mRNA-1283 elicited more efficient B-cell memory response against the NTD.

Cytokine analyses of T cells stimulated with peptide-specific pools were performed in splenocytes isolated after administration of mRNA-1273 and mRNA-1283. The results demonstrated that the mRNA-1283 elicited increased IFN- γ -, IL-2-, and TNF- α -producing CD4+ T cells after stimulation with the Spike S1, RBD, or NTD peptide pools compared with mRNA-1273. Stimulation with the Spike S2 peptide pool did not elicit production of these cytokines in CD4+ T cells. Vaccination with mRNA-1283 also elicited an IFN- γ increase in CD8+ T cells after stimulation with Spike S1 and RBD peptide pools, but not with Spike S2 or NTD peptide pools. Overall, mRNA-1283 elicited a strong Th1-directed CD4+ T-cell response and CD8+ T-cell response, with no Th2-directed response measured. These responses were higher than those elicited by mRNA-1273.

Conclusions

The results of Studies MOD-3964, MOD-4035, and MOD-4101 demonstrated that:

- mRNA-1283 is immunogenic in BALB/c mice, mediating both humoral (bAb and nAb) and cellular (Th1-directed CD4+ and CD8+) immune responses.
- mRNA-1283-elicited CD4+ T cells re-stimulated with S1 or S2 peptide pools exhibited the IFN- γ , IL-2, TNF- α cytokine production, indicating a Th1-dominant response. The induction of a Th1-directed T-cell response was confirmed through Ab-subclass analysis, with doses of mRNA-1283 driving IgG2a Ab subclass differentiation.
- mRNA-1283 induced a robust CD8+ T-cell response to the S1 peptide pool.
- Compared with mRNA-1273, mRNA-1283 vaccination elicited more consistent responses at the same dose levels, with increased NTD bAbs, NTD-specific ASCs, and antigen-specific CD4+ and CD8+ T-cell responses.

4.1.4 Evaluation of Immunogenicity of mRNA-1283 Primary Series and Matched Variant-specific Booster Dose in Mice (Stewart-Jones et al., 2023)

Immunogenicity of mRNA-1273 and mRNA-1283 was evaluated by Stewart-Jones et al. in a primary series in K18-hACE2 C57BL/6 J mice, followed by administration of matched booster doses as described (Stewart-Jones et al., 2023).

Briefly, a 2-dose primary series of either mRNA-1273 or mRNA-1283, both derived from the Wuhan-Hu-1 (original) isolate of SARS-CoV-2, was administered in mice (n=5/ group) in two regimens, 0.1 µg or 1 µg. After primary immunization, each group was administered a booster dose matching the primary series regimen as follows:

- In mRNA-1273-primed mice: 0.1 µg or 1 µg of monovalent mRNA-1273.351 (S-2P derived from the Omicron lineage B.1.351).
- In mRNA-1283-primed mice: 0.1 µg or 1 µg of monovalent mRNA-1283.351 (NTD-RBD-HATM derived from the Omicron lineage B.1.351).

Serum was collected post-primary series on Day 36, pre-boost on Day 212, and post-boost on Day 233, and samples were evaluated for the D614G (original) and B.1.351-specific nAb titers using PsVNA.

The reduction in D614G-specific nAb titers between Day 36 (14 days post-dose 2) and pre-boost (Day 212) was comparable between mRNA-1273- or 1283-primed mice (1.5-fold vs 1.9-fold). Large fold increases in nAb titers against D614G and B.1.351 were observed 21 days post-boost (Day 233) compared with pre-boost in mice immunized with mRNA-1283.351 (1 µg) vs mRNA-1273.351 (1 µg) (D614G: 4.9-fold vs 4.5-fold; B.1.351: 40.6-fold vs 15.3-fold). Similar trends, albeit less pronounced, were observed for the 0.1 µg dose.

Overall, the greatest increases in variant-specific nAb levels were observed in groups that received variant-matched boosters for both mRNA-1273 and mRNA-1283, supporting the use of variant-matched boosters. Fold changes in nAb titers between pre- and post-boost were more pronounced in mice vaccinated with mRNA-1283 than mRNA-1273.

4.1.5 Evaluation of Immunogenicity of mRNA-1283 Variant-specific Booster Dose Following mRNA-1273 Primary Series in Mice (Stewart-Jones et al., 2023)

Immunogenicity of mRNA-1273 or mRNA-1283 using variant-specific booster doses following a 2-dose primary series with mRNA-1273 was evaluated in BALB/c mice by Stewart-Jones et al., 2023 as presented (Stewart-Jones et al., 2023). Booster doses were administered as follows:

- monovalent mRNA-1273 or mRNA-1283, (D614G original);
- monovalent mRNA-1273.351 or mRNA-1283.351(B.1.351);
- bivalent mRNA-1273.211 or mRNA-1283.211 (original + B.1.351).


All vaccines were administered at a total mRNA level of 1.0 µg. Serum samples were collected on Day 21 (3 weeks post-dose 1), Day 36 (2 weeks post-dose 2), Day 56 (pre-boost), and Day 78 (3 weeks post-boost) and evaluated for nAbs

against the D614G (original), B.1.351-, or B.1.617.2-specific SARS-CoV-2 variant via PsVNA.

Large fold increases in nAb titers were observed post-boost (Day 78) compared with pre-boost (Day 56) in mice administered a mRNA-1283-based booster vs those administered mRNA-1273-based boosters (D614G: up to 9.6-fold vs up to 6.5-fold; B.1.351: 19.5-fold vs 9.7-fold; B.1.617.2: 19.0-fold vs 8.3-fold).

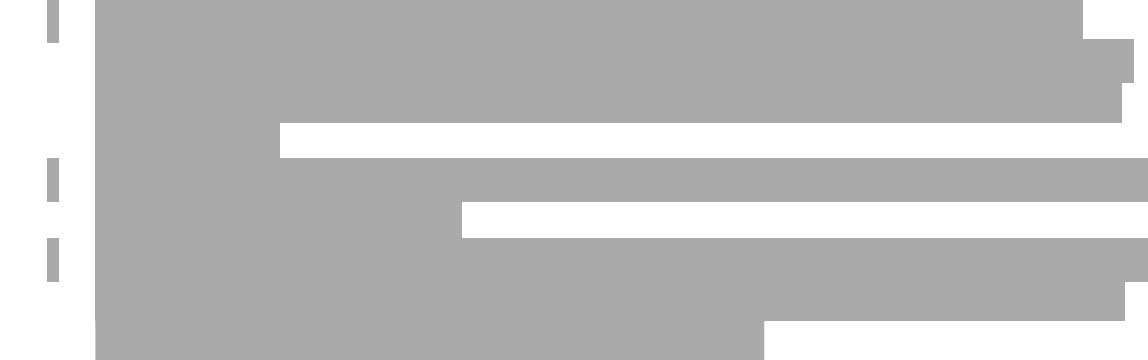
Overall, the greatest variant-specific nAb increases were observed in groups administered variant-matched boosters for both mRNA-1273 and mRNA-1283, supporting the use of variant-matched boosters. Fold changes in nAb titers between pre- and post-boost were more pronounced in mice vaccinated with mRNA-1283 than those administered mRNA-1273.

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4.1.7 Evaluation of the Protection of Monovalent mRNA-1283 Against WA1/2020 D614G or B.1.1.529 (BA.1; Omicron) Challenge (Stewart-Jones et al., 2023)

The ability of a primary mRNA-1283 series to confer protection against WA1/2020 D614G or B.1.1.529 (BA.1; Omicron) was assessed in female K18-hACE2 C57BL/6 J mice as described in Stewart-Jones et. al, 2023 (Stewart-Jones et al., 2023). The test articles evaluated were monovalent mRNA-1283 and mRNA-1273; Fix-DH (random nucleotide mRNA) was used as a control.

K18-hACE2 C57BL/6 J mice were immunized with a 2-dose primary series of either mRNA-1273 or mRNA-1283 administered at the 0.1 µg or 5 µg doses 21 days apart. At Day 42, nAb titers were evaluated by focus reduction neutralization test. Mice were then challenged with 104 focus forming units (FFU) of WA1/2020 D614G or BA.1.1.529 SARS-CoV-2 strains by the intranasal route on Day 56 or 57. Lung and nasal wash were collected for assessment of viral load (viral N copies) following challenge on Day 63.

After primary immunization, nAb titers in mRNA-1283- and mRNA-1273-vaccinated mice were comparable. Higher nAb titers against B.1.1.529 were observed in mice vaccinated with 5 µg of mRNA-1283 than those vaccinated with 0.1 µg of mRNA-1283. The nAb titers against B.1.1.529 at the 5 µg and 0.1 µg doses were ~12-fold and ~29-fold lower, respectively, compared with the nAb titers against WA1/2020 D614G. The immune responses restricted to the RBD and NTD were sufficiently protective, and these observations remained true even at low doses (0.1 µg), which further support dose-sparing properties of mRNA-1283.

After challenge with either WA1/2020 D614G or B.1.1.529, significantly lower viral burden in the lungs, nasal turbinate, and nasal washes were observed in both mRNA-1273- and mRNA-1283-vaccinated mice as compared with the Fix-DH control. When comparing nAb titers with viral N copies in mice vaccinated with mRNA-1283, a strong correlation was observed in lung samples, where higher nAb titers were associated with lower viral N RNA copies for both viruses.

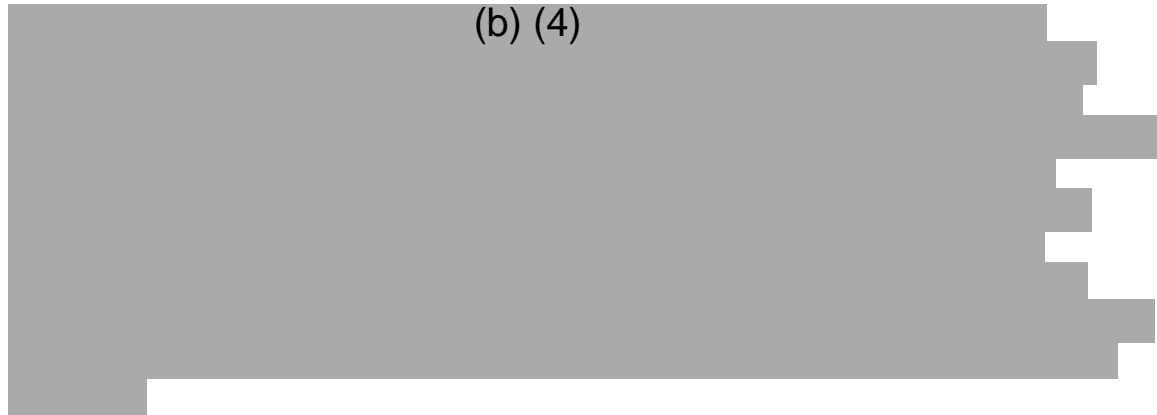
A similar, but less pronounced, effect was observed in the nasal turbinate samples.

Importantly, although mice vaccinated with a 2-dose primary mRNA-1283 series had lower nAb titers against B.1.1.529 than D614G, post-challenge viral titers were significantly lower for both D614G and B.1.1.529 compared with the control groups. In general, nAb titers after immunization and viral copy levels post-challenge were comparable between mRNA-1283- and mRNA-1273-vaccinated mice evaluated in this and previously published studies (Ying et al. Boosting with variant-matched or historical mRNA vaccines protects against Omicron infection in mice. *Cell*. 2022 Apr 28;185(9):1572-1587) as well as in nonhuman primates (Gagne et al. mRNA-1273 or mRNA-Omicron boost in vaccinated macaques elicits similar B cell expansion, neutralizing responses, and protection from Omicron. *Cell*. 2022 Apr 28;185(9):1556-1571).

4.1.8 Evaluation of *In Vivo* Immunogenicity of mRNA-1283.222 (MOD-5814-1283)


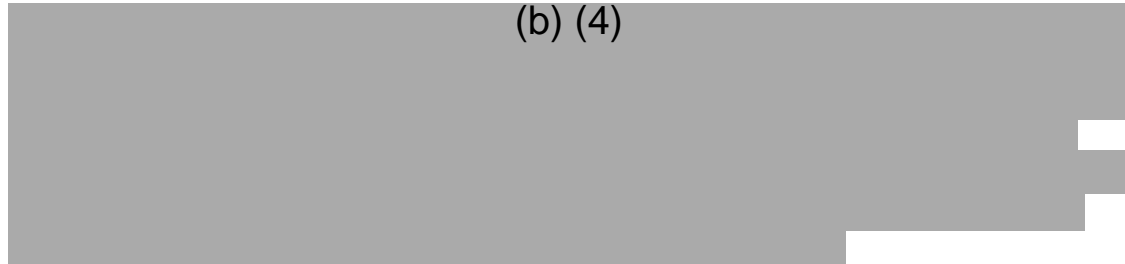
The objective of study MOD-5814-1283 was to evaluate the immunogenicity of mRNA- 1283.222 manufactured with different processes (Report MOD-5814-1283).

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Study Design

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Results

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Conclusion




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4.1.10 Evaluation of Immunogenicity of Monovalent SARS-COV-2 XBB-Containing mRNA-1283 Vaccine Boosters (MOD-5972.1283)

The objective of study MOD-5972.1283 was to evaluate the immunogenicity of a booster dose of monovalent mRNA-1283 vaccines updated to match XBB subfamily strains of SARS-CoV-2 (XBB.1.5 and XBB.16).

The study was performed in BALB/c mice using the following vaccines:

- mRNA-1273 (monovalent; primary series): encodes S-2P of the Wuhan-Hu-1 (original) strain.
- mRNA-1283.815 (monovalent; test article booster): encodes the NTD-RBD-HATM derived from the XBB.1.5/XBB.1.9.1 Omicron subvariant (please note: the Spike protein of XBB.1.9.1 is identical to that of XBB.1.5).
- mRNA-1283.116 (monovalent; test article booster): encodes the NTD-RBD-HATM derived from the XBB.1.16 Omicron subvariant.
- mRNA-1273.116 (monovalent; active control booster): contains S-2P of the XBB.1.16 Omicron subvariant.
- mRNA-1283.222 (bivalent; active control booster): contains mRNA-1283 (NTD-RBD-HATM of original Wuhan-Hu-1 virus) and mRNA-1283.045 (NTD-RBD-HATM of Omicron BA.4/BA.5 lineage).

PBS was used as a negative control.

Study design

5 groups of BALB/c mice (n=8/group) were administered 3 IM doses regimen, including a 2-dose primary series (Doses 1 and 2) plus 1 booster (Dose 3). Each group of animals received 0.5 µg mRNA-1273 on Day 1 and Day 22 (primary series [Doses 1 and 2]). Mice were then boosted on Day 106 (booster dose [Dose 3]) with 1.0 µg of mRNA-1283.815, mRNA-1283.116, mRNA-1283.222, or mRNA-1273.116.

Blood samples were collected on Day 21 (before Dose 2 of the primary series), on Day 36 (2 weeks after Dose 2), on Day 49 (before the booster dose was administered), and on Day 120 (2 weeks after the booster dose).

Samples were analyzed for serum bAbs and nAbs using ELISA and VSV-based PsVNA, respectively.

Results

On Day 120 (2 weeks after Dose 3), all animals boosted with mRNA vaccines had increased bAb (IgG) titers against S-2P compared with PBS controls (fold differences ranged from 4.2- to 6.5-fold), which reflected titers representative of pre-boost levels. S-2P bAb IgG GMTs in animals boosted with mRNA-1283.815 and mRNA-1283.116 (370,582 and 359,676, respectively), were comparable to those in the group boosted with bivalent mRNA-1283.222 (360,977) and numerically higher than those elicited by mRNA-1273.116 (240,493; 1.5-fold difference).

Pre-boost (Day 49) nAb titers against XBB.1.5 or XBB.1.16 strains were at or below the lower limit of quantification for all test groups, indicating substantial immune escape from mRNA-1273 vaccination. Although all mRNA vaccines elicited nAb responses against the XBB.1.5 and XBB.1.16 strains on Day 120, the highest fold increases relative to Day 49 were observed in the groups boosted with mRNA-1283.815, mRNA-1283.116, or mRNA-1273.116 (fold increases ranged from 19- to 28-fold against XBB.1.5 and 38- to 46-fold against XBB.1.16). mRNA-1283.815 and mRNA-1283.116 vaccines elicited higher post-boost nAb titers to XBB.1.5 and XBB.1.16 strains (fold increase ranged from 24- to 38-fold) compared with bivalent mRNA-1283.222 (fold increase ranged from 8.7- to 12-fold). Furthermore, the nAb titer levels against XBB.1.5 and XBB.1.16 strains were comparable between groups immunized with mRNA-1283.815 and mRNA-1283.116 vaccines. In contrast, bivalent mRNA-1283.222 had the highest titers against D614G and BA.4/BA.5 strains compared to all other groups.

Conclusions

The results of study MOD-5972.1283 indicated that all mice that received a booster dose of mRNA-1283.222, mRNA-1283.815, or mRNA-1283.116 after a primary series of mRNA-1273 elicited increased bAb titers and enhanced neutralizing activity. The nAb titers against XBB.1.5 and XBB.1.16 were comparable between groups immunized with mRNA-1283.815 and mRNA-

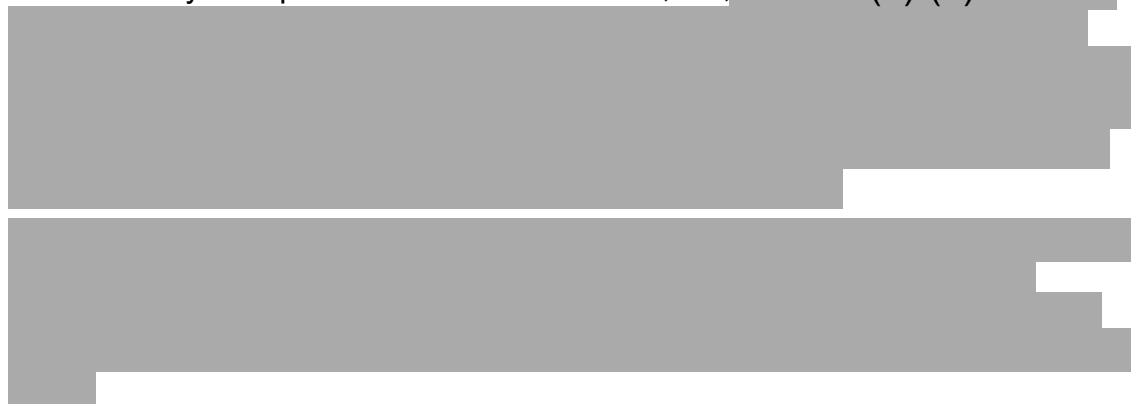
1283.116 vaccines, suggesting cross-recognition of antigenically similar XBB strains.

Pharmacology Summary Conclusion

- The *in vivo* and *in vitro* expression of the mRNA-1283 (NTD-RBD-HATM) vaccine is higher or comparable with that of mRNA-1273 (S-2P) comparator.
- The immune response elicited by the original or variant-containing mRNA-1283 vaccine either as a primary series or as a booster dose in mice previously vaccinated with the licensed mRNA-1273 vaccine is similar or superior to the original or variant-containing mRNA-1273 comparators, suggesting that mRNA-1283 may elicit a more robust immune response at a lower dose.
- mRNA-1283 induced a robust Th1-directed immune response, as evidenced by a balanced IgG2a/IgG1 subclasses response and the predominance of intracellular Th1-cytokine production upon stimulation, excluding the theoretical concern of enhanced respiratory disease (ERD) relative to mRNA-1283.
- Collectively, these findings support the suitability of mRNA-1283 for primary or booster vaccinations as well as seasonal vaccine updates.

4.2 Pharmacokinetics

There are no exclusive non-clinical pharmacokinetics (PK) studies performed with mRNA-1283. The biodistribution, persistence, and clearance studies were conducted by the sponsor on related vaccines, i.e., (b) (4)



Summary of non-clinical pharmacokinetics program supporting mRNA-1283 is provided in **Table 78**.

Table 78. Summary of Non-Clinical Pharmacokinetics Dataset Supporting mRNA-1283

Type of Study	Test Article/Dose	Test System	Method of Administration	Report Number
---------------	-------------------	-------------	--------------------------	---------------

(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Single- or Repeat-dose biodistribution study of mRNA-1273	mRNA-1273: (b) (4) µg/dose ^a	(b) (4) rats	Single- or Repeat IM dose on Days 1 and 28	20456513 ^c
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)

eCTD - electronic common technical document; GLP - Good Laboratory Practice; IV - intravenous; (b) (4) ; (b) (4) .

a - Dose refers to the total dose of mRNA encapsulated in the administered LNP.

b - Calculated with average molecular weight of (b) (4) .

c - Completed by (b) (4) .

d - Completed by (b) (4) .

e - Completed by (b) (4) .

g - Completed by (b) (4) .

The results of these studies were reviewed earlier and are not included in the current memo. However, the most important results collectively demonstrated that:

(b) (4)

(b) (4)

Overall, the available non-clinical pharmacokinetics data are considered sufficient to support the use of mRNA-1283.

4.3 Toxicology

No single-dose toxicity studies were conducted. Toleration after a single IM dose of the mRNA vaccines encapsulated by SM-102-containing LNPs was assessed in repeat-dose toxicity studies.

To support the development of mRNA-1283, three studies, including repeat-dose toxicity study for mRNA-1283.222 (Study 20462697), repeat-dose immunogenicity and toxicity of mRNA-1283 (Study 2308-161), and developmental perinatal/postnatal reproductive toxicity (Study 20346748) were conducted in (b) (4) rats as shown in **Table 79**.

Table 79. Repeat-dose Toxicity Studies Performed to Support mRNA-1283

Type of Study	Test Article	Species and Strain	Method of Admin.; Dose ^a ; Duration of Dosing	GLP	Report Number
4-week repeat-dose toxicity study in rats with a 2-week recovery period	mRNA-1283.222	Rat, (b) (4)	IM; 0, 2, 5, 10 µg/dose; 2 doses over 4 weeks (every 4 weeks)	Yes	20462697 ^a
3-week, repeat-dose immunogenicity and toxicity study	mRNA-1283	Rat, (b) (4)	IM; 0, 30, 60, 100 µg/dose; 2 doses over 3 weeks (every 3 weeks)	No	2308-161 ^a
Combined developmental perinatal/postnatal reproductive toxicity study	mRNA-1283	Rat, (b) (4)	IM; 0, 80 µg/dose; 4 doses over 6 weeks (Study Days 1 and 15 [28 and 14 days prior to mating, respectively] and Gestation Days 1 and 13)	Yes	20346748 ^b Amendment 1

a – Testing facility: (b) (4)

b - Testing facility: (b) (4)

The most important study results are discussed below. For details, please see the memo provided by the toxicology reviewer.

The clinical development of mRNA-1283 was also supported by data from 6 repeat-dose GLP-compliant toxicity studies performed on 5 different vaccines, (b) (4), all of which are comprised of the same lipids as mRNA-1283 but encapsulate mRNA(s) encoding various antigens. This information was reviewed previously and is not included in this memo.

4.3.1 4-Week Repeat-Dose Toxicity Followed by a 2-Week Recovery Period (Report 20462697)

The objectives of this study were to determine the potential toxicity effects of repeat-dose IM injection of mRNA-1283.222 to rats administered once every 4 weeks (2 doses in total) and to evaluate the potential reversibility of any findings following a 2-week recovery period.

In this study, the bivalent mRNA-1283.222 (original and the BA.4/BA.5 Omicron variant) DP lot 8516100102 was used for testing. The same DP lot was further used in the pivotal clinical study mRNA-1283-P301. The vehicle lot (b) (4) (b) (4) mM Tris, (b) (4), (b) (4) g/L sucrose, pH (b) (4)) was used as a negative control.

Study design

4 groups of (b) (4) rats (n=20/group) were administered 2 doses of 2, 5, or 10 µg/dose of mRNA-1283.222 or vehicle control on Days 1 and 29. Mortality, weekly clinical observations, injection-site observations at 6- and 24-hours after each dose, weekly body weights, weekly food consumption were closely monitored. Ophthalmology examinations were performed 6 days prior to dosing and on Day 27, and clinical pathology parameters were assessed on Day 30 (main study animals) and Day 43 (recovery animals), including gross necropsy findings, organ weights (brain, epididymis, adrenal gland, pituitary gland, prostate gland, thyroid/parathyroid, heart, kidney, liver, spleen, testis, thymus, ovary, and uterus/cervix), and histopathologic examinations.

Additionally, anti-SARS-CoV-2 wild-type and BA.4/BA.5 S-2P antibody analyses were evaluated prior to initiation of dosing, on Day 29 (24 hours post-last-dose) and at the end of the 2-week recovery period.

Results and Conclusions

Administration of mRNA-1283.222, by IM injection once every 4 weeks on Days 1 and 29 to rats was clinically tolerated up to 10 µg/dose, the highest dose level examined, with no mortalities, clinical signs, injection site observations, or body weights or food consumptions changes.

At terminal necropsy (main study), minimal to moderate mixed-cell inflammation was observed at Injection Site 2, with secondary infiltration of neutrophils in the iliac lymph node of a few animals. These injection site findings correlated with

clinical pathology changes consistent with an inflammatory and/or acute phase response. At the end of the recovery period, microscopic findings at both injection sites indicated signs of resolution of the inflammatory response (partial recovery) and complete reversibility of the iliac lymph node findings, and thus clinical pathology findings were fully reversible.

Antibody analyses showed robust IgG titers against the original and BA.4/BA.5 S-2P proteins on the last day of dosing, as well as 2 weeks post the last dose. Following the 2-week recovery period, these responses persisted in all dose groups, and there was an increase in the IgG titers against both wild-type and BA.4/5 S2P antigens in the 10 µg/dose group on Day 43 when compared with the Day 29 time point.

The NOAEL for mRNA-1283.222 was determined to be 10 µg/dose (or 33.3 µg/kg based on a rat body weight of 0.3 kg), the highest dose tested, based on recovery from microscopic and clinical pathology changes at the end of the 2-week recovery period.

4.3.2 3-Week Repeat-Dose (2 Doses Every 3 Weeks) Immunogenicity and Toxicity Study (Report 2308-161)

The objective of this study was to determine the potential toxicity effects of mRNA-1283, administered by IM injection to (b) (4) rats in a 2-dose regimen, administered 3 weeks apart.

The test article in this study was the monovalent mRNA-1283 DP lot 8520700101. The vehicle control lot (b) (4) (b) (4) mM Tris, (b) (4) g/L sucrose, pH (b) (4) was used as a negative control.

Study design

4 groups of (b) (4) rats (n=10/ group) were administered 2 doses of 30, 60, or 100 µg/dose mRNA-1283 or control by IM bolus injection into one of the quadriceps muscles (alternating for each dose) on Days 1 and 22. Clinical observations consists of twice per day examinations for mortality and daily detailed examinations, injection site observations immediate and at 6 and 24 hours post-dose, weekly body weight check, clinical pathology parameter evaluation (hematology and clinical chemistry) on Days 23 and 36, immunogenicity assessment on Days 1 and 35, and gross necropsy findings and histopathologic evaluation of liver and spleen.

Results and Conclusions

mRNA-1283 was clinically tolerated up to the highest dose tested (100 µg/dose), with clinical signs limited to transient, dose-dependent edema ≥ 30 µg/dose, with or without hindlimb impairment. Hematology and clinical chemistry changes were consistent with a systemic inflammatory response.

Decreased lymphocyte counts were observed as were decreases in reticulocyte counts which were indicative of diminished erythropoiesis and were sometimes associated with mild effects on mean MCV, MCHC, and/or RDW. These changes were generally resolved or resolving by the end of the 2-week post-last-dose observation period.

mRNA-1283 elicited significant SARS-CoV-2 S-2P binding antibody titers on Day 35 at all doses tested (non-dose-dependent).

At necropsy, minimal or mild mRNA-1283-related increases in extramedullary hematopoiesis were observed in the spleen at doses of ≥ 30 $\mu\text{g}/\text{dose}$.

4.3.3 Combined Developmental Perinatal/Postnatal Reproductive Toxicity Study (Report 20346748)

The objective of this study was to assess the potential effects of mRNA-1283 on reproduction and pre- and postnatal development in pregnant and lactating

(b) (4) rats when administered by IM injection during the premating period (28 and 14 days prior to mating) and on Gestation Days 1 and 13.

The study was conducted using mRNA-1283 DP lot DH-16459 and the negative control vehicle lot (b) (4), formulated with (b) (4) mM Tris, (b) (4) g/L sucrose, pH

(b) (4).

Study design

2 groups of female rats (n=8/group) were administered 4 doses of 80 $\mu\text{g}/\text{dose}$ mRNA-1283 or control on study Days 1 and 15 (28 and 14 days prior to mating, respectively) and on gestation Days 1 and 13, via IM injection.

In each dose group, rats were divided into either a caesarean-sectioning phase cohort (Cohort 1) or a natural delivery phase cohort (Cohort 2). F0 generation rats (dams) in Cohorts 1 and 2 were monitored for clinical observations, body weight, food consumption, estrous cycling, mating, and fertility.

In Cohort 1, F0 rats (dams) were euthanized on Gestation Day 21 for caesarean sectioning, gross pathology, organ weights (gravid uterus), and ovarian and uterine contents examinations; and the F1 generation rats (fetuses) were euthanized for gross pathology and fetal examinations (external abnormalities, visceral examination, skeletal examination, and fetal ossification).

In Cohort 2, F0 rats (dams) were allowed to deliver their litters naturally and were euthanized for gross pathology after completion of the 21-day postpartum period. The F1 rats (pups) were monitored for clinical observations, body weight, and reflex and physical development. F1 rats were euthanized on Postnatal Day 21 for gross pathology.

Serum samples from F0 rats were analyzed on Study Days 1 (pre-dose) and 15 (Cohorts 1 and 2); Gestation Days 1 and 13 (Cohorts 1 and 2); Gestation Day 21 (Cohort 1); and Lactation Days 13 and 21 (Cohort 2) for IgG antibody responses

against the SARS-CoV-2 NTD or RBD; IgG antibodies were also evaluated in F1 fetuses on Gestation Day 21 (Cohort 1), in F1 pups on Postnatal Days 13 and 21 (Cohort 2), and in milk samples from F0 rats on Lactation Days 13 and 21 (Cohort 2). The serum-antibody analyses were not performed with GLP compliance.

Results and Conclusions

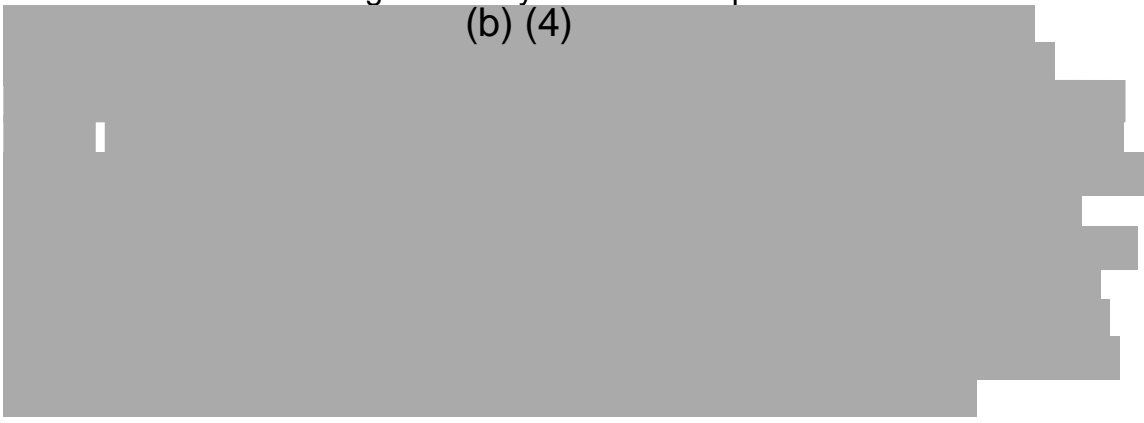
There were no mRNA-1283-related adverse effects on fertility or pre- and postnatal development after administration of mRNA-1283 to female rats during the premating (28 and 14 days prior to mating) and gestation periods (Gestation Days 1 and 13) via IM injection at 80 µg/dose.

Robust IgG antibody responses to the SARS-CoV-2 NTD and RBD regions of the protein were present in the maternal serum samples after dosing and continued into the gestation and lactation periods. Antibodies were also present in maternal milk samples, and in fetal and pup serum during gestation and the postnatal period, respectively, demonstrating effective placental and lactation transfer of anti-SARS-CoV-2 antibodies to offspring when females were immunized prior to and after mating. The NOAEL was determined to be 80 µg/dose, the only dose tested in the study.

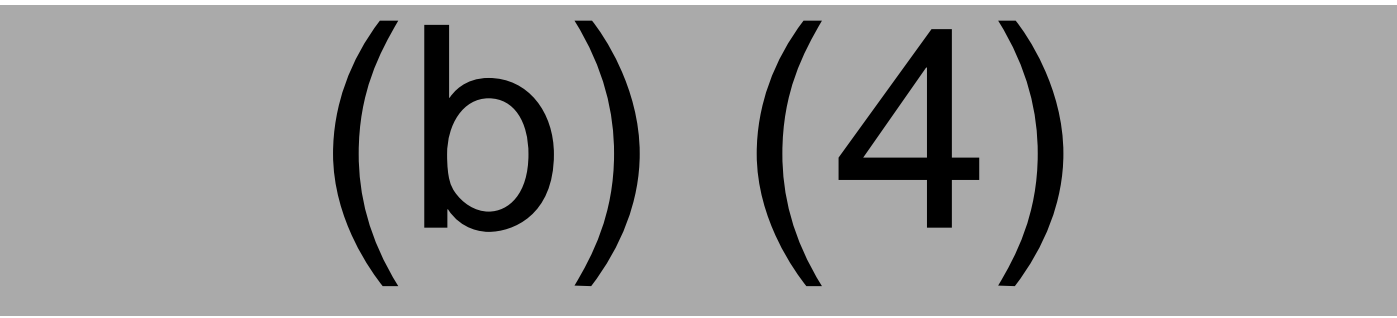
4.4. Genotoxicity

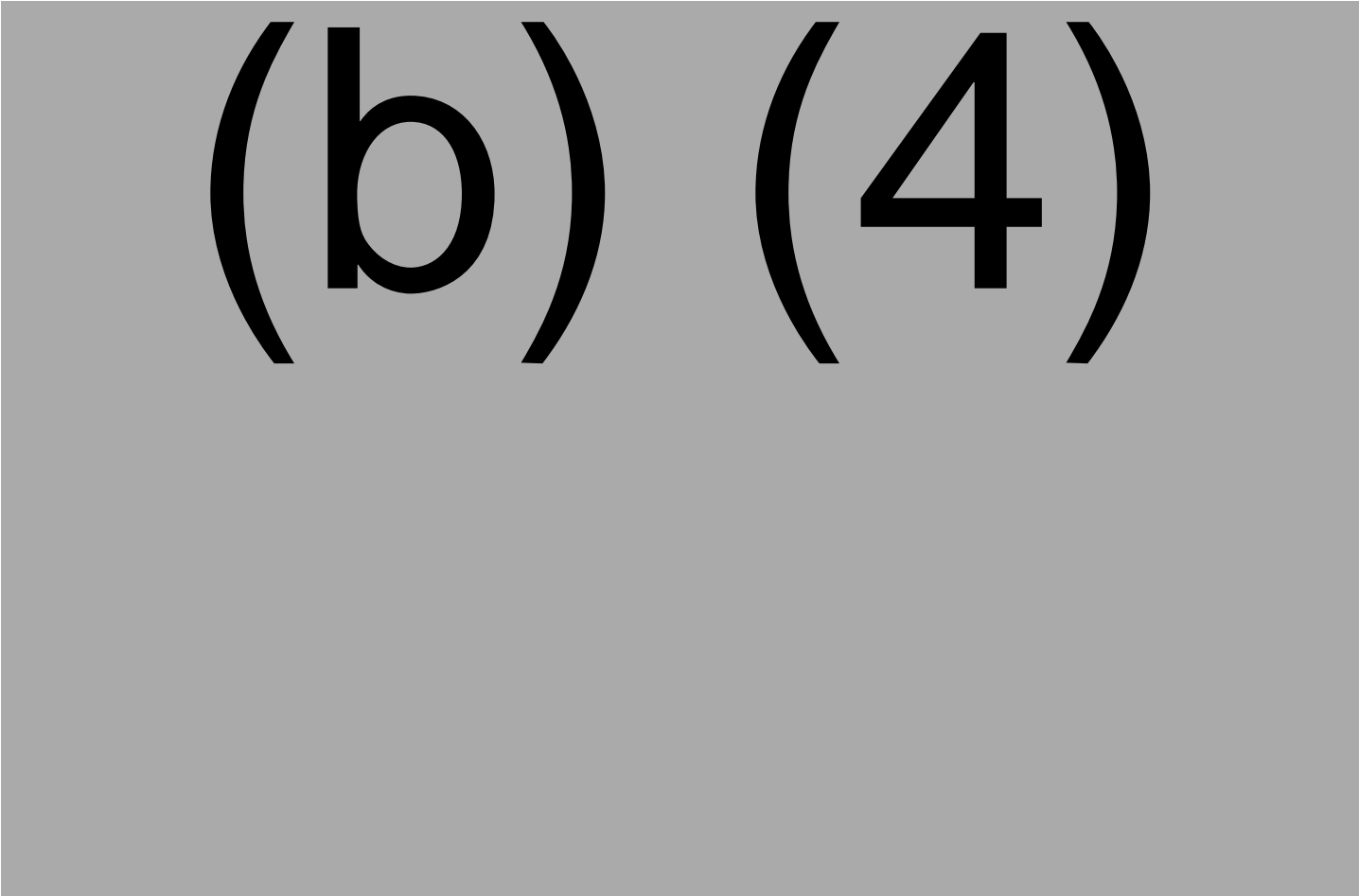
No exclusive non-clinical genotoxicity studies were performed for mRNA-1283.

(b) (4)



(b) (4)





(b) (4)

Based on the totality of data, the risk of genotoxicity is low/negligible for mRNA-1283.

4.5 Carcinogenicity

No carcinogenicity studies were conducted with mRNA-1283.

NONCLINICAL CONCLUSION

Overall, the results from nonclinical pharmacology, pharmacokinetics, and toxicology studies completed using mRNA-1283 or other mRNA vaccines comprised of the SM-102-containing LNPs and developed using holistic mRNA-based platform demonstrated that mRNA-1283 is well tolerated, safe, and elicits a robust and effective immune response against SARS-CoV-2 in animals and support the licensure of this vaccine.

MODULE 5**5.0 BIOANALYTICAL AND ANALYTICAL METHODS FOR HUMAN STUDIES**

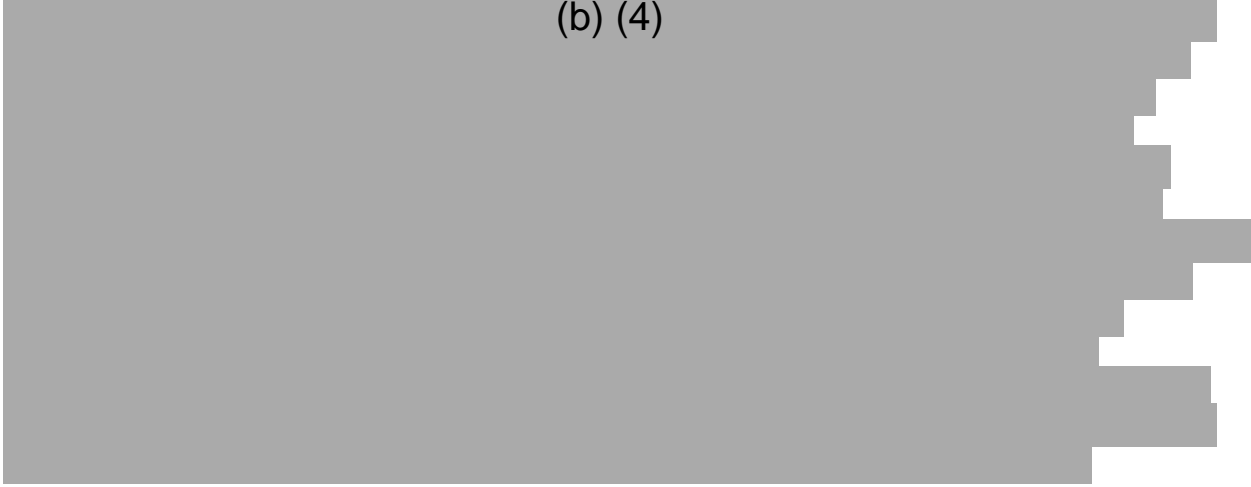
Assays used to support the Clinical Immunogenicity and Efficacy Endpoints are as follows:

A. Immunogenicity Assays**1. Validation of the Pseudotype Virus Neutralization Assay Ancestral Anti-Spike (D614G), and Omicron (BA.1) variant by Duke University; Studies P101 and P201**

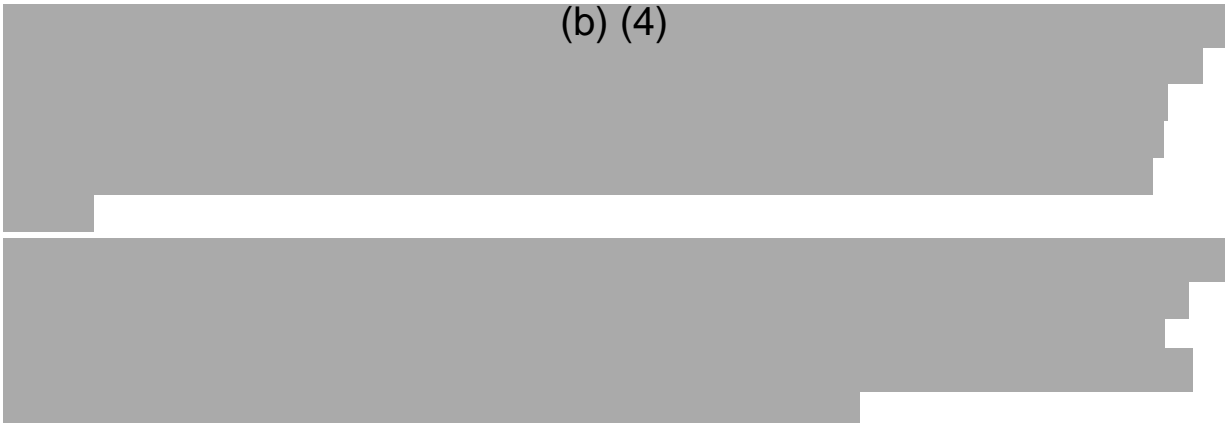
The proprietary serological assay was developed, qualified, and validated by the “Neutralizing Antibody Core” Laboratory at the Duke University Medical Center.

The SARS-CoV-2 Spike (container D614G mutation) Pseudotype Virus Neutralization Assay (PsVNA) in 293/ACE2 cells was used to measure neutralizing antibody titers against SARS-CoV-2 in sera from clinical study participants after vaccination.





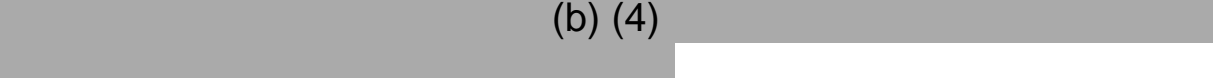
(b) (4)

**Parameters assessed and Results**

(b) (4)





(b) (4)



Reviewers' Conclusion: *Based on the data submitted in the validation report, the assay has been successfully validated for its intended purpose of quantifying the SARS-CoV-2 neutralizing antibodies in vaccine recipients.*

(b) (4)

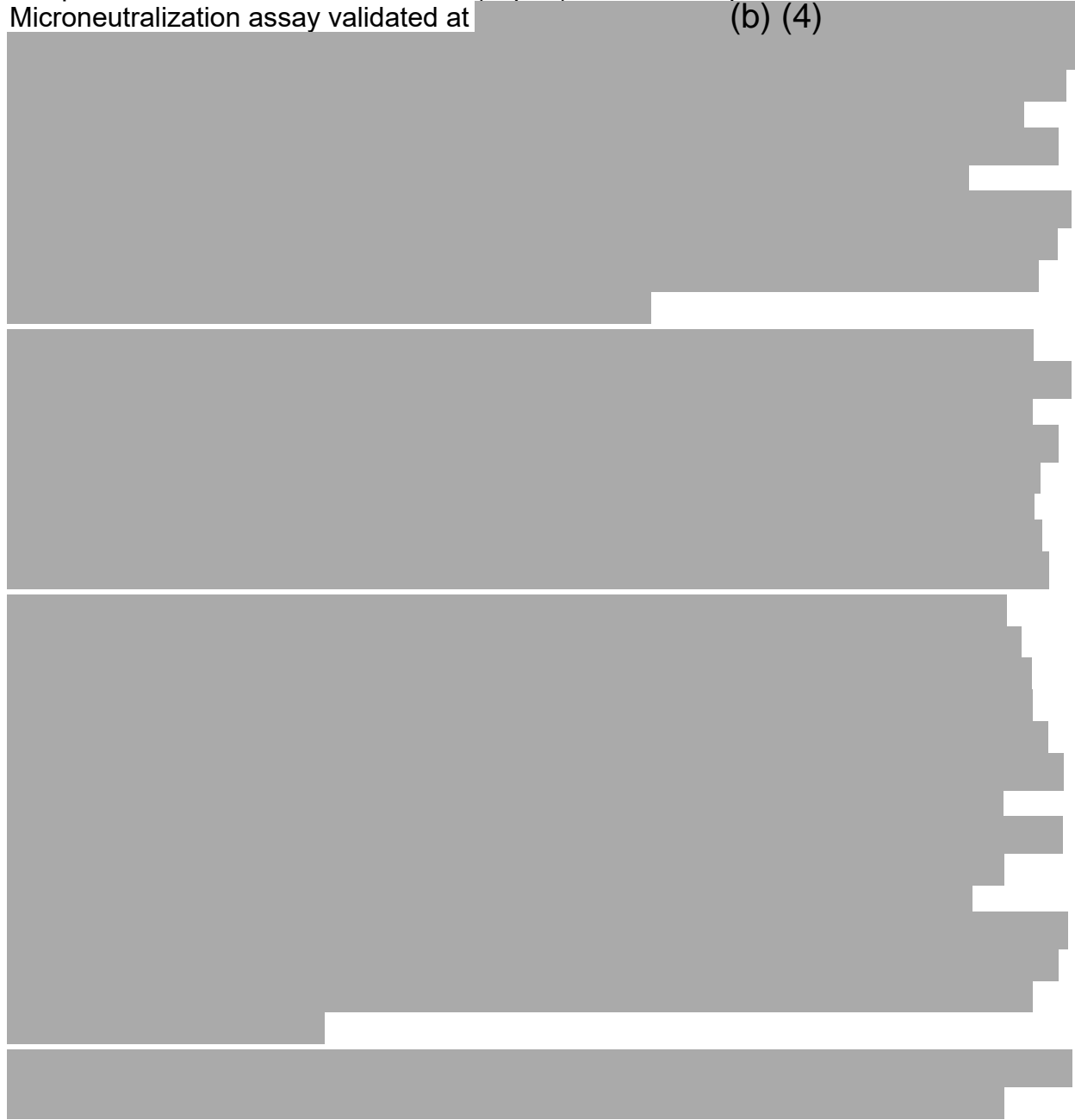


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Reviewer's comments and conclusion: *Based on the data submitted in the Omicron BA.1 modified validation report, the assay has been adequately validated for its intended purpose of quantifying the SARS-CoV-2 Omicron BA.1 variant-specific neutralizing antibodies in vaccine recipients.*

2. Validation of the Pseudotype Virus Neutralization Assay for Anti-Spike Ancestral (D614G) VAC62, Omicron (BA.4/BA.5) VAC137 and XBB.1.5 VAC150 variant by (b) (4); Studies P301 and P301 (Japan)

Another Pseudotype virus neutralization (PsVNA) assay was used to assess the clinical samples from studies P301 and P301 (Japan). This is a Reporter Virus based Microneutralization assay validated at (b) (4)



(b) (4)



3. Validation of the Live Virus Neutralization Assay Ancestral (Wu-1) with an in situ ELISA readout from (b) (4) for quantification of neutralizing antibodies; Studies P101

A live virus microneutralization (MN) assay was developed to quantify the SARS-CoV-2 neutralizing antibodies in serum or plasma samples from individuals who have received

a SARS-CoV-2 vaccine. The assay was developed, qualified, and validated at the (b) (4).

(b) (4)

Parameters assessed and Results

(b) (4)

(b) (4)


Reviewers' comments and Conclusion: *This assay has been successfully validated and is appropriate for its intended purpose of quantifying the SARS-CoV-2 neutralizing antibodies in vaccine recipients.*

B. Diagnostic Assays

1. (b) (4) SARS CoV-2 RT-PCR assay using the (b) (4)
Validated at (b) (4)

(b) (4) SARS CoV-2 specific RT-PCR assay was used at (b) (4) for baseline SARS CoV-2 serostatus determination and at (b) (4) for the COVID-19 case confirmation for primary efficacy endpoint analysis in Study P301. The assay is an FDA Emergency Use Authorized In Vitro Diagnostic test for the qualitative detection of SARS CoV-2 nucleic acid in nasal and nasopharyngeal swabs from infected people. In brief, (b) (4) SARS-CoV-2 RT-PCR assay is a (b) (4)

(b) (4)



Reviewer's comments and conclusion: Based on the review of the (b) (4) RT-PCR assay, it is suitable for its intended purpose of qualitative detection of SARS CoV-2 nucleic acid. In the assay verification reports, (b) (4) verified the assay performance in their labs. Both labs are deemed proficient in handling clinical samples and performing the RT-PCR assay.

2. Elecsys N protein IgG Assay

The assay is a commercially available kit from Roche Diagnostics that has been approved by the FDA under an Emergency Use Authorization. Elecsys® Anti-SARS-CoV-2 immunoassay is intended for the qualitative detection of antibodies to SARS-CoV-2 in human serum and plasma. The assay uses a recombinant nucleocapsid (N) protein antigen for the determination of antibodies against SARS-CoV-2. The test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. The qualitative results are reported as reactive (i.e., positive for anti-SARS-CoV-2 N antibodies; cutoff index [COI] ≥ 1.0) or nonreactive (i.e., negative for anti-SARS-CoV-2 N antibodies; COI < 1.0). The assay was used to screen samples taken at baseline and also samples from asymptomatic individuals who may be infected but did not develop symptoms of the disease post vaccination. The assay performance was verified in (b) (4) before routine testing of clinical samples. Accuracy, Precision and Correlation were verified. Accuracy was assessed by (b) (4)

(b) (4) Similarity, Precision assessment met the predefined acceptance criteria, and no discrepant results were observed.

Reviewer's comments and conclusion: *The same assay was used during the original BLA approval of SPIKEVAX and was also reviewed at the time of licensure. The assay is fit for its intended purpose and (b) (4) has demonstrated adequate assay performance in their lab.*

Information Requests

The following IR#19 was sent to Moderna on February 18, 2025 and the responses were received on March 3, 2025 in amendment 25 (Seq 0026)

1. In BLA 125835/0, for the primary efficacy endpoint for Study P301, COVID-19 cases according to CDC case definition were determined using the (b) (4) SARS-CoV-2 RT-PCR assay. The corresponding assay verification report and addendums were conducted by (b) (4) central lab primarily using nasopharyngeal swabs with one addendum for nasal swabs, where only positive samples were tested for the nasal swabs. However, in Study P301, almost all samples were derived from nasal swabs and evaluated at (b) (4) central lab. Please submit an assay verification report demonstrating adequate RT-PCR assay performance in the (b) (4) central lab using nasal swabs with an adequate number of both positive and negative samples.

Sponsor Response: The verification report for (b) (4) SARS-CoV-2 RT-PCR assay from nasal swabs at (b) (4) central lab is provided in m5.3.1.4/RT-PCR in the BLA.

2. In BLA 125835/0, under Section "2.3.1.2 RT-PCR ((b) (4))" of the document entitled "Clinical Pharmacology Studies Summary - Summary of Biomarker Assays used in mRNA-1283 Program", the assay verification report referenced appears to be incorrect.

An RT-PCR assay is listed, but the assay verification report is for an antibody detection assay. Please reference and/or submit the correct document.

Sponsor Response: The RT-PCR report referenced in Section 2.3.1.2 RT-PCR ((b) (4)) of the document "Clinical Pharmacology Studies Summary – Summary of Biomarker Assays Used in mRNA-1283 Program" has been correctly hyperlinked to the verification report for ((b) (4)) SARSCoV-2 RT-PCR assay from nasal swabs at ((b) (4)) central lab provided in the above response for Item #1.

Reviewer's comments and conclusion: *The sponsor provided the requested information and the assay verification report for the ((b) (4)) RT-PCR assay run in ((b) (4)). The report was reviewed and found to be acceptable. No further action is required.*

APPENDICES

Appendix 1

(b) (4)

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Appendix 3

(b) (4)

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Table 3. Summary of the mRNA-1283 DP Lots Placed on Stability

ID	CMO Lot #	Target RNA content (mg/mL)	Lot Type	Date of Manufacture	Fill Volume (mL)	Manufacturing Site	Container Closure	Conditions		Latest Timepoint used for analysis (months)
								Temperature	Duration (months)	
(b) (4)	(4)	0.05	PPQ	(b) (4)	(4)	(b) (4)	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C	12 (b) (4) (b) (4)	N/A ^a N/A ^a (b) (4)
		0.05	PPQ			(b) (4)	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C	12 (b) (4) (b) (4)	N/A ^a N/A ^a (b) (4)
		0.05	PPQ			(b) (4)	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C	12 (b) (4) (b) (4)	N/A ^a N/A ^a (b) (4)
		(b) (4)	(b) (4)			ModernaTX Norwood	(b) (4)	(b) (4)		
		(b) (4)	Development			(b) (4)	COC PFS	(b) (4) (b) (4) -25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C	(b) (4) (b) (4) (b) (4) (b) (4) (b) (4)	(b) (4) (b) (4) 12 (b) (4) (b) (4)
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		
		0.05	Development			ModernaTX Norwood	COC PFS	(b) (4) (b) (4) -25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C	(b) (4) (b) (4) (b) (4) (b) (4) (b) (4)	(b) (4) (b) (4) 9 (b) (4) (b) (4)
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		
		0.05	Development			ModernaTX Norwood	COC PFS	(b) (4) -25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C	(b) (4) (b) (4) (b) (4) (b) (4)	(b) (4) (b) (4) 9 (b) (4) (b) (4)
		0.05	Development			ModernaTX Norwood	COC PFS	-25°C to -15°C 2°C to 8°C	(b) (4) (b) (4)	6 (b) (4)
		0.05	Development			ModernaTX Norwood	COC PFS	-25°C to -15°C	6	6
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		
		0.05	Development			ModernaTX Norwood	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4)°C	12 (b) (4) (b) (4)	1 2 (b) (4)
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		

(b) (4)

a Not included in statistical analysis as fewer than three timepoints are available.

b Not used for the shelf-life assessment as stored in (b) (4) .

Table 4. Updated Stability Summary for mRNA-1283 DP Lots (Based on data submitted in BLA STN 125835.38)

ID	CMO Lot #	Target RNA content (mg/mL)	Lot Type	Date of Manufacture	Fill Volume (mL)	Manufacturing Site	Container Closure	Conditions		Latest Timepoint used for analysis (months)
								Temperature	Duration (months)	
(b) (4)	(4)	0.05	PPQ	(b) (4)	(4)	(b) (4)	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4) C	12 (b) (4) (b) (4)	6 (b) (4) (b) (4)
		0.05	PPQ			(b) (4)	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4) C	12 (b) (4) (b) (4)	6 (b) (4) (b) (4)
		0.05	PPQ			(b) (4)	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4) C	12 (b) (4) (b) (4)	6 (b) (4) (b) (4)
		0.05	PPQ			(b) (4)	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4) C	12 (b) (4) (b) (4)	3 (b) (4) (b) (4)
		0.05	PPQ			(b) (4)	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4) C	12 (b) (4) (b) (4)	3 (b) (4) (b) (4)
		0.05	PPQ			(b) (4)	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4) C	12 (b) (4) (b) (4)	3 (b) (4) (b) (4)
		0.05	PPQ			(b) (4)	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4) C	12 (b) (4) (b) (4)	3 (b) (4) (b) (4)
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		
		0.05	Development			ModernaTX Norwood	COC PFS	(b) (4) (b) (4)	(b) (4) (b) (4) (b) (4) (b) (4)	(b) (4) (b) (4) (b) (4) (b) (4)
		(b) (4)	(b) (4)			ModernaTX Norwood	(b) (4)	(b) (4)		
		0.05	Development			ModernaTX Norwood	COC PFS	(b) (4) (b) (4)	(b) (4) (b) (4) (b) (4) (b) (4)	(b) (4) (b) (4) (b) (4) (b) (4)
		(b) (4)	(b) (4)			ModernaTX Norwood	(b) (4)	(b) (4)		
		0.05	Development			ModernaTX Norwood	COC PFS	(b) (4) (b) (4)	(b) (4) (b) (4) (b) (4) (b) (4)	(b) (4) (b) (4) (b) (4) (b) (4)
		(b) (4)	(b) (4)			ModernaTX Norwood	(b) (4)	(b) (4)		
		0.05	Development			ModernaTX Norwood	COC PFS	(b) (4) (b) (4)	(b) (4) (b) (4) (b) (4) (b) (4)	(b) (4) (b) (4) (b) (4) (b) (4)
		0.05	Development			ModernaTX Norwood	COC PFS	-25°C to -15°C 2°C to 8°C 23°C to (b) (4) C	(b) (4) (b) (4) (b) (4)	12 (b) (4) (b) (4)
		0.05	Development			ModernaTX Norwood	COC PFS	-25°C to -15°C 2°C to 8°C	(b) (4) (b) (4)	12 (b) (4)
		0.05	Development			ModernaTX Norwood	COC PFS	2°C to 8°C 23°C to (b) (4) C	3 24h	3 24h
		0.05	Development			ModernaTX Norwood	COC PFS	2°C to 8°C 23°C to (b) (4) C	3 24h	3 24h
		(b) (4)	(b) (4)			(b) (4)	(b) (4)	(b) (4)		

(b) (4)	(b) (4)	0.05	Development	(b) (4)	(b) (4)	ModernaTX Norwood	COC PFS	2°C to 8°C	(b) (4)	(b) (4)
								23°C to (b) (4) C	(b) (4)	(b) (4)

(b) (4)

2 pages have been determined to be not releasable: (b)(4)

