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[illegible]

Where: A, C, G and U = AMP, CMP, GMP and N1-Me-ΨMP, respectively;

Me = methyl; p = inorganic phosphate.

mRNA Sequence Molecular Weight (free acid) 1,329,683 Daltons

mRNA Sequence Molecule Length 4,101 nucleotides

mRNA Sequence Elements ORF: 3819 nucleotides

5'UTR: Cap + 57 nucleotides

3'UTR: 119 nucleotides

PolyA tail: 105 nucleotides

2.3.S.1.2 General Properties

CX-024414 is intended for further processing into mRNA-1273 Drug Product, an mRNA/lipid-based product. CX-024414 is not intended for direct injection. The general properties of CX-024414 are summarized in [Table 1](#).

Table 1: General Properties of CX-024414

Description	CCI
Appearance	
pH	
Partition coefficient	
Aqueous solubility	
Non-aqueous solubility	
Extinction coefficient	

2.3.S.2 Manufacture

2.3.S.2.1 Manufacturer(s)

Each manufacturer, including contractors, and each proposed production site or facility involved in the manufacturing are described in [Table 2](#).

Table 2: Manufacturers of CX-024414

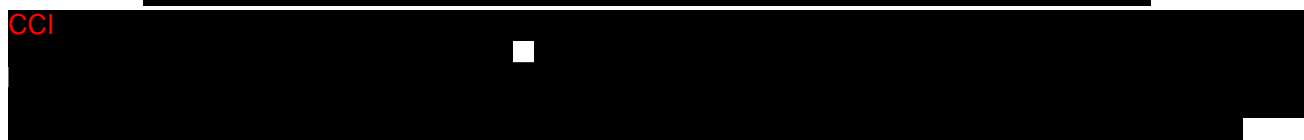
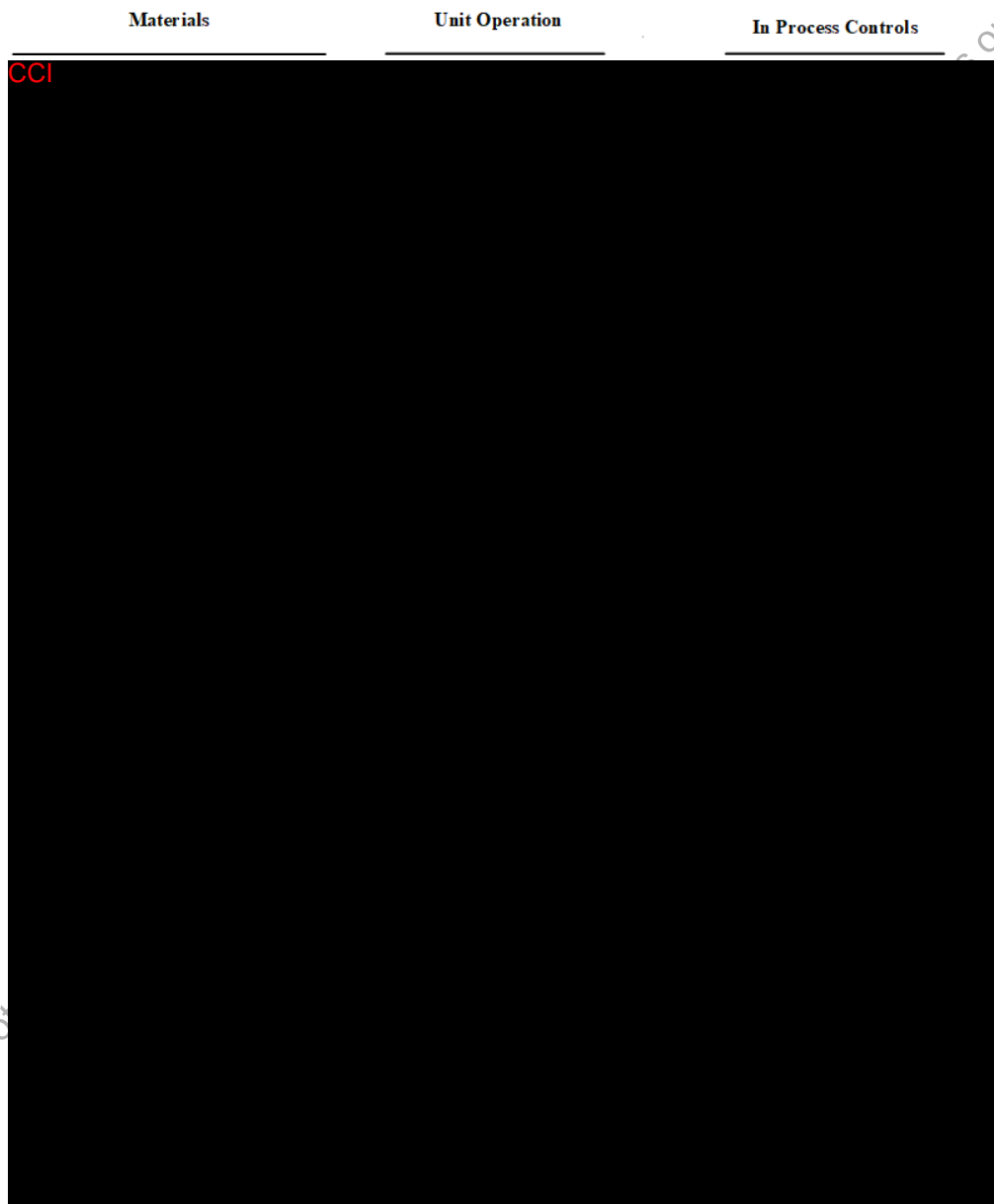
Manufacturers	Location	Responsibility
Lonza AG	Lonzastrasse 3930 Visp Switzerland	<ul style="list-style-type: none"> Manufacture of CX-024414 (20L, 60 L and 75 L IVT Scale) Quality control testing (excluding identity testing)
Microsynth AB	Schützenstrasse 15 9436 Balgach Switzerland	<ul style="list-style-type: none"> Quality Control testing for identity of CX-024414
Moderna Biotech Spain S.L.	C/ Julián Camarillo nº31 28037 Madrid Spain	<ul style="list-style-type: none"> Release and stability testing (all methods excluding Bioburden)
ModernaTX, Inc.	One Moderna Way Norwood, MA, 02062 USA	<ul style="list-style-type: none"> Manufacturer of CX-024414 (10 L, 60 L and 75 L IVT scales)
ModernaTX, Inc.	One Moderna Way Norwood, MA, 02062 USA	<ul style="list-style-type: none"> Release, Stability, In-process testing 10 L, 60 L and 75 L IVT CX-024414 manufactured at ModernaTX Inc. Norwood. Release, stability, in-process testing of 20 L, 60 L and 75 L IVT CX-024414 manufactured at Lonza Biologics Inc. (excluding Bacterial endotoxin and bioburden)
ModernaTX, Inc.	210 Rustcraft Road Dedham, MA, 02026 USA	
Lonza Biologics, Inc.	101 International Drive Portsmouth, NH, 03801 USA	<ul style="list-style-type: none"> Manufacture of CX-024414 (20 L, 60 and 75 L IVT scale) Release testing (bacterial endotoxin and bioburden for 20 L, 60 L and 75L IVT scales CX-024414 manufactured at site) Stability testing for Bacterial endotoxin 20 L, 60 L, and 75 L IVT scales CX- 024414 manufactured at site

2.3.S.2.2 Description of Manufacturing Process and Process Controls

Flow Diagram

A flow diagram of the manufacturing process is provided in [Figure 2](#).

Figure 2: Overview of the Manufacturing Process



Narrative description of the manufacturing process(es):

In Vitro Transcription

The purpose of the IVT step is to synthesize multiple copies of full-length, uncapped RNA from a DNA template (plasmid). CCI [REDACTED]

[REDACTED] Nucleotides, plasmid and IVT Reaction Buffer are thawed prior to use.

The IVT reaction includes the nucleotides, plasmid, pyrophosphatase, T7 RNA polymerase, IVT Reaction Buffer and Process Water to meet a defined reaction volume. CCI [REDACTED]

In Vitro Transcription Tangential Flow Filtration (IVT TFF)

The IVT TFF step is performed for buffer exchange and to concentrate the QIVT intermediate.

CCI [REDACTED]

CCI

The IVT TFF harvest is detached from the TFF system and mixed to ensure solution homogeneity prior to sampling for RNA concentration at the end of IVT TFF to determine the total mass of RNA as an input for oligo dT affinity chromatography.

First Oligo dT Chromatography

The IVT TFF harvest is loaded onto a custom affinity chromatography resin

CCI

The pool is transferred to the dT TFF operation or refrigerated/chilled if storage is necessary prior to dT TFF. The chromatography system and the column are then cleaned per established procedures.

dT Tangential Flow Filtration

The dT TFF step is performed for buffer exchange and to concentrate the dT eluate pool.

CCI

CCI

The dT TFF harvest in the Cap Reaction vessel is detached from the TFF system and mixed to ensure solution homogeneity prior to sampling for RNA concentration. The dT TFF harvest is stored in the Cap Reaction vessel until further processing.

Capping

CCI

Cap Tangential Flow Filtration

The cap TFF step is performed for buffer exchange and to concentrate the QCap product.

CCI

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

The cap TFF harvest is detached from the TFF system and mixed to ensure solution homogeneity prior to sampling for mRNA concentration at the end of cap TFF to determine the total mass of mRNA as an input for the second oligo dT affinity chromatography step. The cap TFF harvest is stored until further processing.

Second Oligo dT Chromatography

The second oligo dT chromatography step utilizes the same resin and capture chemistry described for the first oligo dT chromatography step.

CCI

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

CCI

The dT2 eluate pool is then transferred to the final TFF operation or refrigerated if storage is necessary prior to final TFF.

Final Tangential Flow Filtration

The final TFF step is performed for buffer exchange and to concentrate the dT2 eluate pool.

CCI

The final TFF harvest is detached from the TFF system and mixed to ensure homogeneity prior to sampling for mRNA concentration as an input for the clarification step. The final TFF harvest is stored until further processing.

Clarification, Post-Clarification Hold, and Storage

If the TFF harvest was refrigerated prior to processing, it is mixed while warmed to room temperature before use. Once the target temperature range has been reached, mixing and heating are stopped.

CCI

CCI

The CX-024414 mRNA is mixed to ensure homogeneity prior to sampling for mRNA concentration. Prior to dispensing, the number of CX-024414 mRNA containers and fill volume for each container are calculated to target a fixed amount of mRNA per container at target percentage of the container volume.

The bulk CX-024414 mRNA is aseptically dispensed into containers as described in to the target fill weight.

Storage

CX-024414 mRNA is stored at -90 to -60°C, after an optional interim storage temperature at -15 to -25°C, in containers as defined in

Reprocessing

Reprocessing is not performed for any CX-024414 mRNA process step.

2.3.S.2.3 Control of Materials

Raw Materials

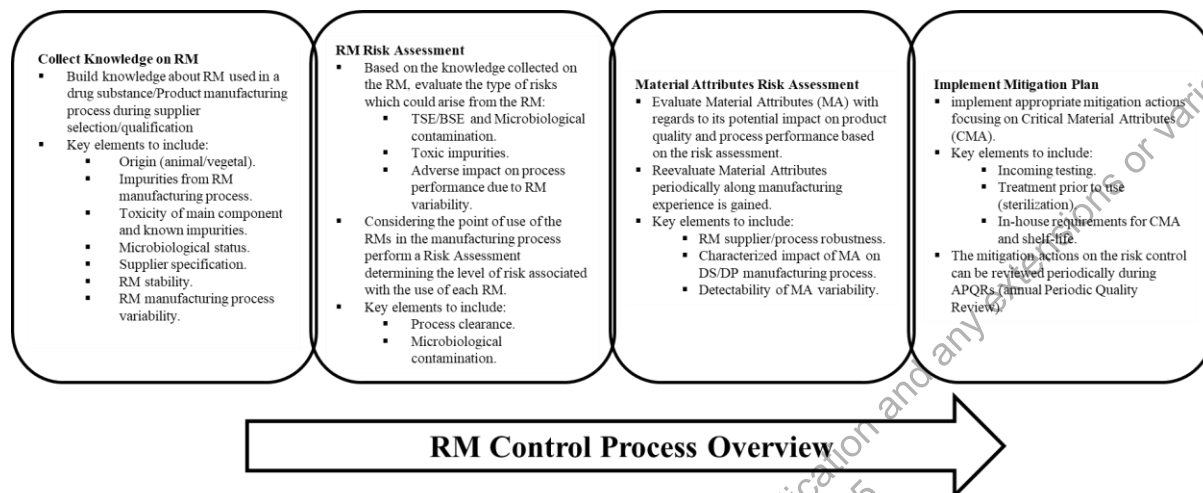
Quality of materials is assured through a rigorous system consisting of supplier evaluation, supplier qualification, audits, quality agreements, incoming goods testing, internal release procedure, package selection, shipping, storage conditions, expiry dates, and/or sterility requirements, based on the risk and criticality of supplier and/or material.

Raw materials are received from qualified suppliers. The supplier qualification process demonstrates the supplier has an effective and acceptable Quality Management System in place and can meet the minimum quality requirements of the process. Qualified suppliers have been assessed and qualified using a supplier risk level review and audit requirements based on the materials to be sourced from the supplier. At ModernaTX, Inc., qualification includes verification of release assay performance. Supplier performance is maintained and monitored through routine surveillance audits, periodic re-verification of release assay performance, quality agreements, and change notification agreements based on supplier risk level.

Incoming raw materials are released based on the Certificate of Acceptance and/or incoming goods testing. The Certificate of Acceptance and/or testing results are compared against the material specification prior to material release. All incoming raw materials are stored in a GMP warehouse. An overview of the Raw Material control process is provided in [Figure 3](#). In instances of material transfer within the global manufacturing network, raw materials initially received at a manufacturing site and released may be transferred to another manufacturing site and received as

released goods for manufacturing based on a risk-based approach that will be managed through the Quality Management System accordingly.

Figure 3: Raw Material Control Process Overview



Adapted from Concept Paper: Management and control of raw materials. Version 1, Nov 29th 2017

Raw materials used in the production of CX 024414 mRNA are categorized as compendial and non compendial. Raw materials are supplied from approved, qualified suppliers and are released prior to use per controlled incoming material specifications and current GMP (cGMP) guidelines. At a minimum, all raw materials are confirmed to conform with Certificates of Analysis (CoAs) or Certificates of Compliance, which includes verification of bovine spongiform encephalopathy/transmissible spongiform encephalopathy (BSE/TSE) certificates, either as part of raw material selection and/or during pre-release prior to use, per site procedures. In addition, all non compendial raw materials are tested for identification or other material attributes.

More detailed information is provided in

Materials of Biological Origin

There are no starting materials of animal or human origin used in the manufacture of CX-024414 mRNA.

Starting Materials

The starting materials in the manufacture of CX-024414 are the linearized plasmid template and the nucleotides ATP, CTP, GTP, N1-Me-ΨTP.

Linearized Plasmid Template

A unique linearized DNA plasmid template specific for CX-024414 is manufactured at ModernaTX, Inc. (Norwood, MA, USA). The plasmid contains the molecular elements of the CX-024414 mRNA including the 5' untranslated region, the coding region [open reading frame (ORF)], the 3' untranslated region and the polyA tail. CCI [REDACTED]

[REDACTED] The current plasmid map has all the appreciable elements annotated.

CCI [REDACTED]

The plasmid map has been purposely designed and minimizes the elements in the plasmid backbone to eliminate unnecessary DNA elements for IVT based mRNA production resulting in the currently utilized backbone.

The full plasmid DNA sequence and the plasmid map are provided in

Generation of Host Cell Line

The lineage of the host cell line is illustrated in [Figure 4](#).

Figure 4: Lineage of Host Cell Line

CCI



Four wild type strains of *E. coli* were purchased from the American Type Culture Collection. The growth and productivity of each strain was assayed in shake flasks. Similar growth (OD600) was observed for all strains; however, MG1655 presented with the greatest plasmid DNA productivity (as measured by plasmid miniprep). Additionally, MG1655 is a very well known, easily transformed, and robust strain. MG1655 was selected as the wild type host based on productivity and its well-understood characteristics.

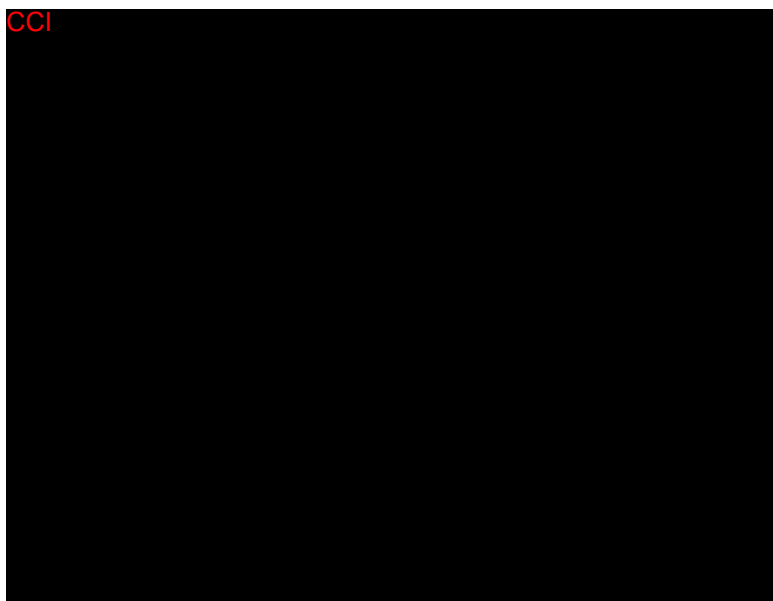
A description of the genetic modifications, transformation and purification of the host cell line are provided in

Plasmid Cell Banking System

The cell banking system is two-tiered, including a master cell bank (MCB) and a working cell bank (WCB).

An overview of the cell banking process is provided in [Figure 5](#).

Figure 5: Overview of Plasmid Cell Banking



Further details of the manufacturing of the cell banks, their release results, stability testing and qualifications are provided in

2.3.S.2.4 Controls of Critical Steps and Intermediates

Critical Process Parameters

Critical process parameters (CPPs) and their proven acceptable range (PARs) for the CX-024414 mRNA manufacturing process are provided in [Table 3](#). Excursions outside of these established ranges are reported, investigated, and assessed by the Quality Unit per established standard operating procedures.

Table 3: Critical Process Parameters

Step	CPP	PAR	Rationale
CCI			
CCI			

Microbial Control

Manufacturing operations utilize single-use, disposable materials for product contact equipment whenever possible. These materials are either received as gamma irradiated/autoclaved by the manufacturer or in-house or are intended to be sanitized in place. The exceptions to this are the chromatography and tangential flow filtration (TFF) skids, which use clean in place cycles for equipment sanitization.

The operations and manufacturing controls specifically intended for microbial control of the CX-024414 mRNA manufacturing process are provided in [REDACTED]. Any deviations incurred in the implementation of these microbial controls are assessed for product impact

In-Process Holds

The maximum duration that process intermediates may be held at [REDACTED]; the maximum intermediate hold duration at [REDACTED]

Resin and Membrane Lifecycles and Column Reuse

Studies using scale-down models were used to evaluate the maximum number of cycles that the dT chromatography resin and the TFF membrane can be used. These studies demonstrated that the resin may be re-used for processing multiple batches to a maximum of [REDACTED]. The TFF membrane can be used for all four TFF operations that occur within a single batch.

Critical In-Process Controls

The critical in-process testing performed for the CX-024414 mRNA manufacturing process is listed in Table 4. The in-process sample matrix was qualified as described in ICH Q2.

Table 4: Critical In-process Controls

Step	CIPC	Acceptance Criteria
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]

2.3.S.2.5 Process Validation and/or Evaluation

Process Performance Qualification Results

A Process Validation Master Plan (PVMP) was developed to ensure that the commercial manufacturing process for CX-024414 mRNA is capable of reliably and consistently delivering quality product.

PPQ acceptance criteria were defined in each PPQ protocol. PPQ was performed at multiple sites to increase and secure vaccine supply. A minimum of three PPQ lots were completed at each site to demonstrate process consistency. For sites that manufacture CX-024414 mRNA on multiple, independently operated process trains, the initial process train at the site included at least 3 PPQ lots. Each subsequent process train at the site completed at least one PPQ lot.

The PPQ batches are eligible for commercial use provided that the entire process validation according to the Validation Master Plan is successful and completed.

The PPQ has been initiated for 20 L IVT scale to demonstrate process consistency for CX-024414 mRNA manufacturing at Lonza AG in Visp, Switzerland. The process parameters and the proven acceptable ranges (PARs) were determined prior to the initiation of PPQ activities.

The PPQ for the 60 L IVT scale began in January 2021 for CX-024414 mRNA manufacturing at Lonza AG (Lonza Visp) on kit 4, kit 5 and kit 6. The three kits are equivalent (equivalent equipment installed) therefore the initial validation of the process was performed on Kit 4. The process was subsequently validated on Kit 5 and Kit 6. Similar to the 20 L IVT scale, process parameters and PARs were determined prior to initiation of PPQ activities.

The PPQ for the 75 L IVT scale for CX-024414 mRNA was performed at Lonza Visp on kit 5. Process parameters and PARs were determined prior to initiation of PPQ activities. To validate the scale up to 75 L IVT, three PPQ batches (54001, 54002, 54003) were produced.

Three additional kits, Kit 11, 12 and 13, were introduced for manufacturing CX-024414 mRNA at Lonza Visp. Again, process parameters and PARs were determined prior to initiation of PPQ. To validate the new kits, three PPQ batches (114001, 124001, 134001) were produced.

All PPQs for the 20 L, 60 L and 75 L IVT scales were completed at Lonza Biologics, Inc. in Portsmouth, NH to demonstrate process consistency for CX-024414 mRNA manufacturing process.

In addition, the results of the critical process parameters, PP, critical and non-critical in-process controls and Drug Substance testing of the CX-024414 mRNA PPQ batches were within the specifications and the prior defined acceptance criteria. Based on the outcome of the PPQ exercise, the CX-024414 manufacturing process has been successfully validated in Lonza, Visp at the 20 L IVT initial scale B as well as 60 L and 75 L IVT final scale B.

More complete information on process validation is provided in

Continued Process Verification (CPV)

Following PPQ, a CPV program was established to ensure robust process control across the lifespan of CX-024414 mRNA manufacturing. The CPV program includes monitoring process controls as well as all CX-024414 mRNA CQAs defined in

A risk assessment is performed for each parameter type (CPP, CQA, etc.) and appropriate response to a control signal will be applied which may include formal deviation in quality management system or assessment within CPV reports.

Resin Reuse Study

The CX-024414 mRNA manufacturing process includes two dT chromatography unit operations. The capture of polyA-containing RNA is achieved by a sequence-specific hydrogen bonding affinity interaction between poly-adenosine and the immobilized dT ligand. Non-bound and weakly bound impurities are washed from the column, and the product is eluted from the resin through modulation of mobile phase ionic strength and temperature.

Data from development studies support use of the resin for the initial target of CCI. Additional concurrent at scale qualification is being performed to verify and if applicable extend total number of cycles. Sanitization, neutralization, and storage procedures, as well as in-process controls, ensure resin functionality, microbial control, mitigation of chemical risks, and prevention of cross-contamination.

More complete information on resin reuse studies is provided in [REDACTED].

Extractables and Leachables

A systematic risk assessment was performed for evaluating extractables and leachables in biopharmaceutical manufacturing systems. An overall leachable risk rating (LRR) was calculated for each material as a weighted sum of the following risk parameters: distance along the production stream, exposure temperature, exposure duration, process fluid interaction and dilution ratio.

For consumables identified as product contact at Lonza Visp, no consumables were assessed as high risk; most consumables were assessed as medium and low risk.

Medium Risk materials were assessed for risk based upon their compliance to applicable guidance/monographs (USP Class VI) for use in pharmaceutical manufacturing.

Based on the risk assessment performed and additional actions taken, it is concluded that the product contact materials used in the mRNA-1273 drug substances and process pose negligible safety risk to humans at the levels currently assessed in the toxicological risk assessment.

2.3.S.2.6 Manufacturing Process Development (CX-024414, Lonza AG)

The initial development of CX-024414 mRNA was conducted using equipment in ModernaTX, Inc.'s small-scale Personalized Vaccine Unit (PVU). In the PVU, the manufacturing operations for mRNA are completed as part of an integrated comprehensive process encompassing both CX-024414 mRNA and mRNA-1273 Lipid Nanoparticle (LNP) processes for each lot of mRNA-1273 Drug Product and does not include a separate release of the mRNA.

Large-scale manufacturing process development was completed in the laboratories at ModernaTX, Inc. to establish unit operations and process parameter ranges for the manufacture of CX-024414 mRNA at a 10 L IVT scale (Scale A) utilizing ModernaTX, Inc.'s 10 L IVT scale mRNA platform process. The mRNA process was developed to achieve targeted strength, safety, and purity product quality attributes and to be comparable to previously manufactured MPI lots.

This process was subsequently transferred to the GMP manufacturing area to enable the manufacture of CX-024414 mRNA (lot 4007220001 through lot 4007220005) at the 10 L IVT scale for the use in the manufacture of mRNA-1273 DP for use in clinical studies. Small-scale studies were conducted, and process changes implemented after the manufacture of CX-024414 mRNA Lot 4007220001 with the aim to increase the expected productivity of the 10 L IVT manufacturing process. Lots 4007220003 through 4007220005 represent the PPQ lots for the Scale A CX-024414 mRNA process.

The process was transferred to Lonza Biologics, Inc. (Portsmouth, NH) for an additional scale-up to a 20 L IVT scale (PN 40075, Initial Scale B). One development lot, one GMP lot (prior to PPQ), and three PPQ lots were performed to establish the 20 L IVT scale process at Lonza. One development lot and one GMP lot were performed prior to PPQ as this was the first time Moderna's CX-024414 mRNA process was transferred to Lonza.

Initial Scale B was also transferred to Lonza Visp (20 L IVT), and several changes were made to accommodate the site and scale changes. Changes were made to material sourcing due to regional availability operations to maintain aseptic processing aligned with facility fit, and operational improvements.

The full commercial scale has been increased and has been firstly implemented at the US manufacturing sites with the 60 L IVT scale (PN 40074) (Scale B Final) before transfer to kits 4, 5 and 6 at the Lonza Visp site. Subsequently, the process has been scaled-up to 75 L IVT (PN40102).

Subsequently, three additional kits, 11, 12 and 13, were introduced for manufacturing CX-024414 mRNA at Lonza Visp. To accommodate a larger eluted volume from the Oligo dT Chromatography steps for the 75 L IVT process, a 1000 L single-use mixer (SUM) is used instead of a 500 L SUM in these kits to mitigate the risk of insufficient capacity in the pooling vessel.

This manufacturing process is described in

A comparability exercise was performed and described in

In conclusion, the one GMP Initial Scale B (20 L IVT) Pre-PPQ batch, as well as the three Initial Scale B (20 L IVT) PPQ batches of CX-024414 mRNA manufactured at Lonza Visp demonstrated that the pre-change and post-change manufacturing processes and quality attributes were comparable to previous 20 L IVT lots manufactured in the US.

The one Scale B (60 L IVT) Pre-PPQ batch together with seven Scale B (60 L IVT) PPQ batches of CX024414 manufactured at Lonza Visp demonstrated that the pre-change and post-change manufacturing processes and quality attributes were comparable to the previous 20 L IVT lots except the following: The purity of CCI for the Pre-PPQ batch meets the CCI release specification but fell lower on the expected comparability range of CCI due to extended hold time. The purity of mRNA-1273 LNP is at CCI with this batch of mRNA, which meets the expected comparability range of CCI of mRNA-1273 LNP. PPQ lots will continue to be assessed.

For the 75 L IVT Scale B process, three PPQ batches of CX024414 manufactured at Lonza Visp demonstrated that the pre-change and post-change manufacturing processes and quality attributes were comparable to the 60 L IVT Scale B lots. Residual DNA results are reported to align with specifications; comparability acceptance criteria review will be assessed using raw data.

All together, the results from the Scale B (75 L IVT scale) PPQ verification lots of CX-024414 manufactured at Lonza (Visp, Switzerland) on kits 11, 12 and 13 demonstrated that the pre-change and post-change manufacturing processes and quality attributes were comparable.

2.3.S.3 Characterisation

2.3.S.3.1 Elucidation of Structure and Other Characteristics

In addition to release and stability assessment of product quality, orthogonal methods have been performed to determine the structural, physicochemical, and biological activity properties of the CX-024414 mRNA (Table 5). The techniques used and the results are summarized in the table below. Unless otherwise indicated, data were generated from process development lot MTDS20002, designated as interim reference material; GMP lots 4007220001 and 8410000103; and PPQ lots 400722003, 400722004, and 400722005. The data generated from these analyses confirm the chemical and physical structure and characterize the physicochemical and functional attributes of CX-024414, as described in more details in }.

Table 5: Elucidation of Structure Summary for CX-024414

Attribute/Characteristic	Method	Results Summary
CCI		
Biological Activity		
CCI		
CCI		

2.3.S.3.2 Impurities

Product-Related Impurities

Product-related impurities ([Table 6](#)) are molecular variants arising during manufacture or storage with properties different from those of the desired product with respect to activity, efficacy, and safety. Characterization of CX-024414 mRNA identified the presence of minor amounts of product variants representative of those commonly found in the profile of mRNA-based therapeutics, such as short mRNA impurities, high molecular impurities and polyA tail variants, double-stranded RNA (dsRNA), and cap variants. The presence, impact, and ability of the process to control such impurities is discussed in

An overview of product-related impurities is presented in [Table 6](#).

Table 6: Overview of CX-024414 mRNA Product-related Impurities

Product-related Impurities	Description	Control Strategy
Short mRNAs resulting from either premature termination of transcription or in-process degradation	Short mRNAs can be generated either by: Premature termination of transcription during IVT Degradation of the mRNA during the manufacturing process These short mRNA impurities will lack either the 5' cap or the 3' polyA tail and therefore cannot be translated.	CCI
High-molecular-weight impurities representing mRNAs longer than the CX024414 generated via transcriptional read-through	mRNAs longer than the CX024414 can be generated from read-through transcription. Read-through transcription can occur either in instances where the polymerase remains engaged with the linearized plasmid template following transcription of the polyA tail, resulting in continued transcription from the complementary strand of the template, or transcription from any closed circular plasmid impurities potentially present in the linearized plasmid template.	
Uncapped mRNA and other cap variants and degradants	Uncapped and other cap variants can be present in the CX024414 if the capping reaction fails to go to completion or mRNA exposed to extreme conditions (heat and acidic).	
Point mutations, insertions/deletions	All polymerases have an intrinsic error rate that can lead to either incorporation of the wrong base (point mutation), or insertions/deletions where a base is erroneously added or skipped. Point mutations can lead to mis-incorporation of a single amino acid in the protein if the point mutation results in a codon that codes for a different amino acid. Insertions/deletions of 1 or 2 nucleotides within a protein coding region would lead to frame shifts that could produce truncated prefusion stabilized Spike protein of 2019-novel Coronavirus (SARS-CoV-2).	
dsRNA	dsRNA can potentially be formed during IVT. dsRNA can be recognized by receptors in the innate immune system, leading to production of immune-stimulatory cytokines.	

Process-Related Impurities

Process-related impurities (Table 7) that may be present in CX-024414 mRNA include residual process material and in vitro transcription (IVT) impurities derived from or generated during the manufacturing process. Please refer to [REDACTED] for a discussion of each of these impurities and provide data demonstrating the ability of the process to clear them down to residual levels in the final filtered bulk mRNA. Lots evaluated include development, GMP clinical supplies, and process performance qualification (PPQ) lots.

CX-024414 mRNA is synthesized by IVT reaction templated against the linearized plasmid template for CX-024414. CCI [REDACTED]

[REDACTED] Tangential flow filtration (TFF) is performed to exchange buffer and concentrate the quenched IVT product. CCI [REDACTED]

[REDACTED] TFF is performed to adjust concentration and prepare the RNA for capping. Enzymatic capping is performed using CCI [REDACTED]

CCI

A third TFF is performed, then capped mRNA is purified by a second dT oligonucleotide chromatography step. A final TFF is performed to concentrate the mRNA into a CCI, followed by CCI filtration.

Table 7: Overview of CX-024414 mRNA Process-related Impurities

Impurity	Test Method	Target Criteria or Acceptance Criteria
Residual DNA template	qPCR (SOP-0491)	CCI
Residual protein	NanoOrange (Characterization for dT2)	CCI
	ELISA (Characterization)	
	LC-MS (Characterization)	
Low molecular weight	N/A	Report results
Residual ethanol and isopropanol	GC	Not tested for release
Residual dT	HPLC-UV	Not tested for release
Residual ethanol	GC	CCI

Abbreviations: ELISA = enzyme-linked immunosorbent assay; GC = gas chromatography; N/A = not applicable; QC = quality control; LC-MS = liquid chromatography with mass spectrometry; qPCR = quantitative polymerase chain reaction; SOP = standard operating procedure

2.3.S.4 Control of the Drug Substance

2.3.S.4.1 Specification

Testing of CX-024414 is performed in accordance with the specification listed in [Table 8](#).

Table 8: Specification for CX-024414

Test	Analytical Method	Acceptance Criteria
Appearance	Visual Ph. Eur 2.2.2, 2.9.20 USP <790>, <631>	Clear, colorless solution, essentially free of visible particulates
Identity	Reverse Transcription/Sanger Sequencing	Sequence matches 100% description of coding region
Total RNA content	UV	CCI
Purity	RP-IP-HPLC	
Product-related impurities	RP-IP-HPLC	
% 5' Capped	RP-UPLC-UV	
% PolyA tailed RNA (% Tailless RNA)	RP-HPLC	
pH	USP <791> Ph. Eur. 2.2.3	
Bacterial endotoxin	USP <85> Ph. Eur. 2.6.14	
Bioburden	USP <61>, Ph. Eur. 2.6.12	

2.3.S.4.2 Analytical Procedures

The proposed drug substance specification has been designed to ensure safety and quality of the product, with the objective of patient safety. Acceptance criteria for tests for appearance, identity, mRNA content, purity, impurities and microbiological quality were established based on compliance with regulations, guidances and compendial methods, and are justified based on characterization studies, the manufacturing process, available batch analyses data, and stability results using validated analytical methods. A full description of each method used is provided in

2.3.S.4.3 Validation of Analytical Procedures

The analytical procedures used for testing CX-024414 have been validated by ModernaTX, Inc.

The analytical procedures used for testing of CX-024414 were confirmed as suitable for their intended use through executed method validation experiments. The % purity method by RP-HPLC and the % polyA tailed RNA method by RP-HPLC were demonstrated to be stability indicating.

A summary of the validation parameters provided in [Table 9](#) is provided

Table 9: Summary of Validation Parameters of the Analytical Methods for CX-024414

Attribute	Method	Method Parameter
Identity	Reverse Transcription/ Sanger Sequencing	Specificity
Total RNA Content	UV	Specificity, linearity, accuracy, precision, intermediate precision, range, robustness
Purity Product-related impurities	RP-IP-HPLC	Specificity, linearity, precision, intermediate precision, reproducibility, accuracy, range, QL, detection limit, prepared solution stability
% 5' Capped	RP-HPLC-UV	Specificity, linearity, accuracy, precision, intermediate precision range, robustness, QL, sample stability
% PolyA Tailed RNA	RP-HPLC	Specificity, linearity, accuracy, precision, intermediate precision, range, robustness, QL, sample stability
Residual DNA	qPCR	Specificity, linearity, accuracy, precision, intermediate precision range, robustness, detection limit, quantitation limit

2.3.S.4.4 Batch Analyses

CX-024414 GMP lots are intended for further manufacturing into mRNA-1273 Drug Product GMP lots. Batch analysis data provided in [Table 10](#) through [Table 21](#) are generated for CX-024414 according to specification approved at time of release.

Table 10: Batch Analyses Data for GMP CX-024414 Scale A (10 L IVT) and Initial Scale B (20 L IVT) Lots – US Sites

CX-024414 GMP Lot Number		4007220001	4007220002	4007220003	4007220004	4007220005	4007520001 Lonza Lot 918988	4007520002 Lonza Lot 922693	4007520003 Lonza Lot 928769	4007520004 Lonza Lot 930143
Date of Manufacture		03Apr2020	04May2020	20May2020	04Jun2020	18Jun2020	28Jul2020	12Aug2020	25Aug2020	31Aug2020
Manufacturing Location		ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)
Purpose		Phase 3 IND	Phase 3 IND	Phase 3 IND 10 L IVT Scale A PPQ	Phase 3 IND 10 L IVT Scale A PPQ	Phase 3 IND 10 L IVT Scale A PPQ	20 L IVT GMP	20 L IVT Initial Scale B PPQ	20 L IVT Initial Scale B PPQ	20 L IVT Initial Scale B PPQ
IVT Scale		10 L	10 L	10 L	10 L	10 L	20 L	20 L	20 L	20 L
Yield (grams)		CCI								
Release Specification		SPC-1995, Version 1	SPC-1995, Version 1	SPC-1995, Version 1	SPC-1995, Version 1	SPC-1995, Version 1	SPC-1118, Version 1	SPC-1118, Version 1	SPC-1118, Version 1	SPC-1118, Version 1
Test	Acceptance Criteria	Results								
Appearance (SOP-0278)	Clear, colorless solution	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
	Essentially free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates
Identity by Reverse Transcription/Sanger Sequencing (SOP-0492)	Sequence matches 100% description of coding region	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Total RNA content by UV (SOP-0249)	CCI									
Purity by RP-HPLC (SOP-0321)	CCI									
Product-related impurities by RP-HPLC (SOP-0321)	Report % Post main peak area	< LOQ ^(a)	< LOQ ^(a)	< LOQ ^(a)	< LOQ ^(a)	< LOQ ^(a)	< LOQ ^(a)	< LOQ ^(a)	< LOQ ^(a)	< LOQ ^(a)
% 5' Capped by RP-UPLC-UV (SOP-0467)	CCI									
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC (SOP-0291)	CCI									
Residual DNA template by qPCR (SOP-0491)	CCI									
pH (SOP-0288)	CCI									
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14 (SOP-0352)	CCI									
Bioburden USP <61>, Ph. Eur. 2.6.12 (SOP-0480)	CCI									

Abbreviations: LOQ = Limit of Quantitation

- a) LOQ is CCI
- b) With the implementation of the parameter change as described in Table 4, Section 3.2.S.2.6 (CX-024414), an expected increase in productivity was achieved and the resultant yield of purified CX-024414 was increased by CCI
- c) Bioburden tested using 100 mL per Deviation QE-002523.

Table 11: Batch Analyses Data for GMP CX-024414 Scale A (10 L IVT) and Initial Scale B (20 L IVT) Lots (continued) – US Sites

CX-024414 GMP Lot Number		4007220001	4007220002	4007220003	4007220004	4007220005	4007520001	4007520002	4007520003	4007520004
Date of Manufacture		03Apr2020	04May2020	20May2020	04Jun2020	18Jun2020	Lonza Lot 918988	Lonza Lot 922693	Lonza Lot 928769	Lonza Lot 930143
Manufacturing Location		ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)
Purpose		Phase 3 IND	Phase 3 IND	Phase 3 IND 10 L IVT Scale A PPQ	Phase 3 IND 10 L IVT Scale A PPQ	Phase 3 IND 10 L IVT Scale A PPQ	20 L IVT GMP	20 L IVT Initial Scale B PPQ	20 L IVT Initial Scale B PPQ	20 L IVT Initial Scale B PPQ
IVT Scale		10 L	10 L	10 L	10 L	10 L	20 L	20 L	20 L	20 L
Yield (grams)		CCI								
Release Specification		SPC-1995, Version 1	SPC-1995, Version 1	SPC-1995, Version 1	SPC-1995, Version 1	SPC-1995, Version 1	SPC-1118, Version 1	SPC-1118, Version 1	SPC-1118, Version 1	SPC-1118, Version 1
Test	Acceptance Criteria	Results								
Appearance (SOP-0278)	Clear, colorless solution	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
	Essentially free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates
Identity by Reverse Transcription/Sanger Sequencing (SOP-0492)	Sequence matches 100% description of coding region	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Total RNA content by UV (SOP-0249)	CCI									
Purity by RP-HPLC (SOP-0321)	CCI									
Product-related impurities by RP-HPLC (SOP-0321)	CCI									
	Report % Post main peak area	< LOQ (a)	< LOQ (a)	< LOQ (a)	< LOQ (a)	< LOQ (a)	< LOQ (a)	< LOQ (a)	< LOQ (a)	< LOQ (a)
% 5' Capped by RP-UPLC-UV (SOP-0467)	CCI									
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC (SOP-0291)	CCI									
Residual DNA template by qPCR (SOP-0491)	CCI									
pH (SOP-0288)	CCI									
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14 (SOP-0352)	CCI									
Bioburden USP <61>, Ph. Eur. 2.6.12 (SOP-0480)	CCI									

Abbreviations: LOQ = Limit of Quantitation

- a) LOQ is CCI
- b) With the implementation of the parameter change as described in [redacted], an expected increase in productivity was achieved and the resultant yield of purified CX-024414 was increased by CCI
- c) Bioburden tested using 100 mL per Deviation QE-002523.

Table 12: Batch Analyses Data for GMP CX-024414 Scale A (10 L IVT) and Initial Scale B (20 L IVT) Lots (continued) — US Sites

CX-024414 GMP Lot Number		4007520007	4007520006	4007520008
Date of Manufacture		Lonza Lot 933032 02Oct2020	Lonza Lot 933031 25Sep2020	Lonza Lot 933033 10Oct2020
Manufacturing Location		Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)
Purpose		20 L IVT GMP Lot	20 L IVT GMP Lot	20 L IVT GMP Lot
IVT Scale		20 L	20 L	20 L
Yield (grams)		CCI		
Release Specification		SPC-1118, Version 1	SPC-1118, Version 1	SPC-1118, Version 1
Test	Acceptance Criteria	Results		
Appearance (SOP-0278)	Clear, colorless solution	Conforms	Conforms	Conforms
	Essentially free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates
Identity by Reverse Transcription/Sanger Sequencing (SOP-0492)	Sequence matches 100% description of coding region	Conforms	Conforms	Conforms
Total RNA content by UV (SOP-0249)	CCI			
Purity by RP-HPLC (SOP-0321)	CCI			
Product-related impurities by RP-HPLC (SOP-0321)	CCI			
% 5' Capped by RP-UPLC-UV (SOP-0467)	Report % Post main peak area	< LOQ	< LOQ	< LOQ
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC (SOP-0291)	CCI			
Residual DNA template by qPCR (SOP-0491)	CCI			
pH (SOP-0288)	CCI			
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14 (SOP-0352)	CCI			
Bioburden USP <61>, Ph. Eur. 2.6.12 (SOP-0480)	CCI			

LOQ = Limit of Quantitation

Table 13: Batch Analyses Data for GMP CX-024414 Final Scale B (60L IVT) Lots – US Sites

CX-024414 GMP Lot Number	4007420001	4007420002	4007420003	4007420010	4007420004	4007420016	4007420501
				Lonza Lot 943122			Lonza Lot 944438
Date of Manufacture	20Sep2020	03Oct2020	19Oct2020	08Nov2020	31Oct2020	29Dec2020	03Dec2020
Manufacturing Location	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	Lonza Biologics, Inc. (Portsmouth, NH)	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	Lonza Biologics, Inc. (Portsmouth, NH)
Purpose	60 L IVT GMP	60 L IVT GMP	60 L IVT PPQ	60 L IVT GMP	60 L IVT PPQ	60 L IVT GMP Train 7	60 L IVT GMP Train 2
IVT Scale	60 L	60 L	60 L	60 L	60 L	60 L	60 L
Yield (grams)	CCI						
Release Specification	SPC-1149 Version 1	SPC-1149 Version 1	SPC-1149 Version 1	SPC-1149 Version 2	SPC-1149 Version 1	SPC-1149 Version 2	SPC-1149 Version 2
Test	Acceptance Criteria	Results					
Appearance (SOP-0278)	Clear, colorless solution	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
	Essentially free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates
Identity by Reverse Transcription/Sanger Sequencing (SOP-0492)	Sequence matches 100% description of coding region	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Total RNA content by UV (SOP-0249)	CCI						
Purity by RP-HPLC (SOP-0321)	CCI						
Product-related impurities by RP-HPLC (SOP-0321)	Report % Post main peak area	< LOQ	< LOQ	< LOQ	CCI	< LOQ	CCI
% 5' Capped by RP-UPLC-UV (SOP-0467)	CCI						
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC (SOP-0291)	CCI						
Residual DNA template by qPCR (SOP-0491)	CCI						
pH (SOP-0288)	CCI						
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14 (SOP-0352)	CCI						
Bioburden USP <61>, Ph. Eur. 2.6.12 (SOP-0480)	CCI						

LOQ = Limit of Quantitation

a) LOQ is 5.0%

Table 14: Batch Analyses Data for GMP CX-024414 Final Scale B (60L IVT) Lots (continued) – US Sites

CX-024414 GMP Lot Number	4007420006	4007420007	4007420021	4007421013 Lonza Lot 960389	4007421017 Lonza Lot 963102	4007421022 Lonza Lot 963103	4007420008	4007420009
Date of Manufacture	10Nov2020	12Nov2020	06Jan2021	12Jan2021	21Jan2021	28Jan2021	21Nov2020	23Nov2020
Manufacturing Location	ModernaTX , Inc. Norwood, MA	ModernaTX , Inc. Norwood, MA	ModernaTX , Inc. Norwood, MA	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)	ModernaTX , Inc. Norwood, MA	ModernaTX , Inc. Norwood, MA
Purpose	60 L IVT PPQ	60 L IVT PPQ	60 L IVT PPQ	60 L IVT PPQ	60 L IVT PPQ	60 L IVT PPQ	60 L IVT GMP	60 L IVT GMP
IVT Scale	60 L	60 L	60 L	60 L	60 L	60 L	60 L	60 L
Yield (grams)	CCI							
Release Specification	SPC-1149 Version 2	SPC-1149 Version 2	SPC-1149 Version 2	SPC-1149 Version 2	SPC-1149 Version 2	SPC-1149 Version 2	SPC-1149, Version 2	SPC-1149 Version 2
Test	Acceptance Criteria	Results						
Appearance ^c (SOP-0278)	Clear, colorless solution	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
	Essentially free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates
Identity by Reverse Transcription/Sanger Sequencing (SOP-1019)	Sequence matches 100% description of coding region	Conforms (a)	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Total RNA content by UV (SOP-0995)	CCI							
Purity by RP-HPLC (SOP-0996)	CCI							
Product-related impurities by RP-HPLC (SOP-0996)	Report % Post main peak area	CCI				< LOQ	CCI	
% 5' Capped by RP-UPLC-UV (SOP-0997)	CCI							
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC (SOP-0994)	CCI							

2.3.S Drug Substance – Quality Overall Summary [CX-024414]

CX-024414 GMP Lot Number		4007420006	4007420007	4007420021	4007421013	4007421017	4007421022	4007420008	4007420009
					Lonza Lot 960389	Lonza Lot 963102	Lonza Lot 963103		
Date of Manufacture		10Nov2020	12Nov2020	06Jan2021	12Jan2021	21Jan2021	28Jan2021	21Nov2020	23Nov2020
Manufacturing Location		ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA
Purpose		60 L IVT PPQ	60 L IVT PPQ	60 L IVT PPQ	60 L IVT PPQ	60 L IVT PPQ	60 L IVT PPQ	60 L IVT GMP	60 L IVT GMP
IVT Scale		60 L	60 L	60 L	60 L	60 L	60 L	60 L	60 L
Yield (grams)		CCI							
Release Specification		SPC-1149 Version 2	SPC-1149 Version 2	SPC-1149 Version 2	SPC-1149 Version 2	SPC-1149 Version 2	SPC-1149 Version 2	SPC-1149, Version 2	SPC-1149 Version 2
Test	Acceptance Criteria	Results							
Residual DNA template by qPCR (SOP-1020)	CCI								
pH (SOP-0288)									
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14 (SOP-0352)									
Bioburden USP <61>, Ph. Eur. 2.6.12 (SOP-0480)									

- a) Method number for Identity at time of testing SOP-0492
b) Method number for Residual DNA Template at time of testing SOP-0491
c) SOP-1019 for Lot Number 4007420009

Table 15: Batch Analyses Data for GMP CX-024414 Final Scale B (75 L IVT) Lots – US Sites

CX-024414 GMP Lot Number	4007921001	4007921003	4007921005	4007921027 Lonza Lot 996648	4007921028	4007921029
	06Mar2021	20Mar2021	07Apr2021	06May2021	26Jul2021	02Aug2021
Date of Manufacture						
Manufacturing Location	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)
Purpose	75 L IVT PPQ	75 L IVT PPQ	75 L IVT PPQ	75 L IVT PPQ	75 L IVT PPQ	75 L IVT PPQ
IVT Scale	75 L	75 L	75 L	75 L	75 L	75 L
Yield (grams)	CCI					
Release Specification	SPC-1149 Version 3	SPC-1149 Version 3	SPC-1149 Version 3	SPC-1149 Version 3	SPC-1149 Version 3	SPC-1149 Version 3
Test	Acceptance Criteria					
Appearance (SOP-0278)	Clear, colorless solution	Conforms	Conforms	Conforms	Conforms	Conforms
	Essentially free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates	Free of visible particulates
Identity by Reverse Transcription/Sanger Sequencing (SOP-1019)	Sequence matches 100% description of coding region	Conforms	Conforms	Conforms	Conforms	Conforms
Total RNA content by UV (SOP-0995)	CCI					
Purity by RP-HPLC (SOP-0996)						
Product-related impurities by RP-HPLC (SOP-0996)	Report % Post main peak area	< QL	< QL	CCI	< QL	CCI
% 5' Capped by RP-UPLC-UV (SOP-0997)	CCI					
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC (SOP-0994)						
Residual DNA template by qPCR (SOP-1020)						
pH (SOP-0288)						

2.3.S Drug Substance – Quality Overall Summary [CX-024414]

CX-024414 GMP Lot Number		4007921001	4007921003	4007921005	4007921027	4007921028	4007921029
					Lonza Lot 996648		
Date of Manufacture		06Mar2021	20Mar2021	07Apr2021	06May2021	26Jul2021	02Aug2021
Manufacturing Location		ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	ModernaTX, Inc. Norwood, MA	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)	Lonza Biologics, Inc. (Portsmouth, NH)
Purpose		75 L IVT PPQ	75 L IVT PPQ	75 L IVT PPQ	75 L IVT PPQ	75 L IVT PPQ	75 L IVT PPQ
IVT Scale		75 L	75 L	75 L	75 L	75 L	75 L
Yield (grams)		CCI					
Release Specification		SPC-1149 Version 3	SPC-1149 Version 3	SPC-1149 Version 3	SPC-1149 Version 3	SPC-1149 Version 3	SPC-1149 Version 3
Test	Acceptance Criteria	CCI					
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14 (SOP-0352)							
Bioburden USP <61>, Ph. Eur. 2.6.12 (SOP-0480)							

*QL= Quantitation Limit

Table 16: Batch Analyses Data for GMP CX-024414 - Initial Scale B – Lonza Visp (20 L IVT)

CX-024414 GMP Lot Number		Lot 4007520801 Lonza Visp Lot 1000	Lot 4007520802 Lonza Visp Lot 1001	Lot 4007520803 Lonza Visp Lot 1002	Lot 4007520804 Lonza Visp Lot 1003
Date of Manufacture		11Nov20	30 Nov 2020	16 Dec 2020	23 Dec 2020
Manufacturing Location		Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)
Purpose		Comparability Lot	Comparability Lot	Comparability Lot	Comparability Lot
IVT Scale		20 L IVT	20 L IVT	20 L IVT	20 L IVT
Yield (grams)		CCI			
Release Specification		SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)			
Test	Acceptance Criteria	Result	Result	Result	Result
Appearance	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution
	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles
Identity by RT/Sanger Sequencing	Sequence matches 100% description of coding region	Conforms	Conforms	Conforms	Conforms
Total RNA Content by UV	CCI				
Purity by RP-HPLC	CCI				
Product-related impurities by RP-HPLC	CCI				
	Report % Post main peak area	CCI			
% 5' Capped by UPLC-UV	CCI				
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC	CCI				
Residual DNA template by qPCR	CCI				
pH	CCI				
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14	CCI				
Bioburden USP <61>, Ph. Eur. 2.6.12	CCI				

Table 17: Batch Analyses Data for GMP CX-024414 batches –Final Scale B – Lonza Visp (60 L IVT)

CX-024414 Pre-PPQ Lot Number		Lot 4007421005 Lonza Visp Lot 41001	4007421006 (Lonza lot 41002)	4007421008 (Lonza lot 41003)	4007421009 (Lonza lot 41004)
Date of Manufacture		23 Jan 2021	12 Feb 2021	24 Feb 2021	11 Mar 2.21
Manufacturing Location		Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)
Purpose		Comparability Lot	PPQ Lot	PPQ Lot	PPQ Lot
IVT Scale		60 L IVT	60 L IVT	60 L IVT	60 L IVT
Yield (grams)		CCI			
Release Specification		SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)
Test	Acceptance Criteria	Result	Result	Result	Result
Appearance	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution
	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles
Identity by RT/Sanger Sequencing	Sequence matches 100% description of coding region	Conforms	Conforms	Conforms	Conforms
Total RNA Content by UV	CCI				
Purity by RP-HPLC					
Product-related impurities by RP-HPLC	Report % Post main peak area	CCI			
% 5' Capped by UPLC-UV	CCI				
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC					
Residual DNA template by qPCR					
pH					
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14					
Bioburden USP <61>, Ph. Eur. 2.6.12					

*No valid bioburden TAMC available due to analytical error

Table 18: Batch Analyses Data for GMP CX-024414 batches –Final Scale B – Lonza Visp (60 L IVT)

CX-024414 Pre-PPQ Lot Number		Lot 4007421005 Lonza Visp Lot 41001	4007421006 (Lonza lot 41002)	4007421008 (Lonza lot 41003)	4007421009 (Lonza lot 41004)
Date of Manufacture		23 Jan 2021	12 Feb 2021	24 Feb 2021	11 Mar 2021
Manufacturing Location		Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)
Purpose		Comparability Lot	PPQ Lot	PPQ Lot	PPQ Lot
IVT Scale		60 L IVT	60 L IVT	60 L IVT	60 L IVT
Yield (grams)		CCI			
Release Specification		SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)
Test	Acceptance Criteria	Result	Result	Result	Result
Appearance	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution
	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles
Identity by RT/Sanger Sequencing	Sequence matches 100% description of coding region	Conforms	Conforms	Conforms	Conforms
Total RNA Content by UV	CCI				
Purity by RP-HPLC	CCI				
Product-related impurities by RP-HPLC	Report % Post main peak area	CCI	CCI		
% 5' Capped by UPLC-UV	CCI				
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC	CCI				
Residual DNA template by qPCR	CCI				
pH	CCI				
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14	CCI				
Bioburden USP <61>, Ph. Eur. 2.6.12	CCI				

*No valid bioburden TAMC available due to analytical error

Table 19: Batch Analyses Data for GMP CX-024414 batches –Final Scale B – Lonza Visp (60 L IVT)

CX-024414 Pre-PPQ Lot Number		Lot 40074210067 Lonza Visp Lot 51001	4007421092 Lonza Visp Lot 61001	4007421093 Lonza Visp Lot 61002	4007421094 Lonza Visp Lot 61003
Date of Manufacture		13-Mar-2021	11 May 2021	22 May 2021	1 Jun 2021
Manufacturing Location		Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)
Purpose		PPQ Lot	PPQ Lot	PPQ Lot	PPQ Lot
IVT Scale		60 L IVT	60 L IVT	60 L IVT	60 L IVT
Yield (grams)		CCI			
Release Specification		SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)
Test	Acceptance Criteria	Result	Result	Result	Result
Appearance	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution
	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles
Identity by RT/Sanger Sequencing	Sequence matches 100% description of coding region	Conforms	Conforms	Conforms	Conforms
Total RNA Content by UV	CCI				
Purity by RP-HPLC	CCI				
Product-related impurities by RP-HPLC	Report % Post main peak area	CCI			
% 5' Capped by UPLC-UV	CCI				
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC	CCI				
Residual DNA template by qPCR	CCI				
pH	CCI				
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14	CCI				
Bioburden USP <61>, Ph. Eur. 2.6.12	CCI				

Table 20: Batch Analyses Data for GMP CX-024414 batches –Final Scale B – Lonza Visp (75 L IVT)

CX-024414 Pre-PPQ Lot Number		Lot 4010221001 Lonza Visp Lot 54001	Lot 4010221003 Lonza Visp Lot 54002	Lot 4010221004 Lonza Visp Lot 54003	Lot 4010222010 Lonza Visp Lot 44001	Lot 4010222040 Lonza Visp Lot 64001
Date of Manufacture		07-Oct-2021	23Oct2021	19Nov2021	04-Feb-2022	06-Apr-2022
Manufacturing Location		Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)
Purpose		PPQ Lot	PPQ Lot	PPQ Lot	PPQ Lot	PPQ Lot
IVT Scale		75 L IVT	75 L IVT	75 L IVT	75 L IVT	75 L IVT
Yield (grams)		CCI				
Release Specification		SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 1 (ModernaTX, Inc.) CHIV-376350 Version 2 (Lonza Visp)	SPC-1233 Version 3 (ModernaTX, Inc.) CHVI-423034 (Lonza Visp)	SPC-1233 Version 3 (ModernaTX, Inc.) CHVI-423034 (Lonza Visp)
Test	Acceptance Criteria	Result	Result	Result	Result	Results
Appearance	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution	Clear, colourless solution	Clear, Colourless	Clear, Colourless
	Essentially free of visible particles ^(a)	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particles	Essentially free of visible particulates	Essentially free of visible particulates
Identity by RT/Sanger Sequencing	Sequence matches 100% description of coding region	Conforms	Conforms	Conforms	Conforms	Conforms
Total RNA Content by UV	CCI					
Purity by RP-HPLC						
Product-related impurities by RP-HPLC	Report % Post main peak area	CCI				
% 5' Capped by UPLC-UV	CCI					
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC						
Residual DNA template by qPCR						
pH						
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14						
Bioburden USP <61>, Ph. Eur. 2.6.12						

a) The specification has been updated from “particles” to “particulates” after the manufacture of the first kit 5 batches

b) For the first kit 5 batches, specification was CCI and then changed to CC

c) For batch 64001, specification changed to CCI

d) For the first kit 5 batches, specification was CCI and then changed to C

e) Sample preparation for endotoxin test was not documented, no valid result available, covered under DR-879235

Table 21: Batch Analyses Data for GMP CX-024414 batches – Final Scale B (75 L IVT) (Cont.)

CX-024414 PPQ Lot Number		Lot 401022049 Lonza Visp Lot 114001	Lot 4010222060 Lonza Visp Lot 124001	Lot 4010222068 Lonza Visp Lot 134001
Date of Manufacture		03-April-2022	03-April-2022	28-May-2022
Manufacturing Location		Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)	Lonza AG (Lonza Visp)
Purpose		PPQ verification	PPQ verification	PPQ verification
IVT Scale		75 L IVT	75 L IVT	75 L IVT
Yield (grams)		CCI		
Release Specification		CHVI-432870 (Lonza Visp)	CHVI-432870(Lonza Visp)	CHVI-432870(Lonza Visp)
Test	Acceptance Criteria	Result	Result	Result
Appearance	Clear, colourless solution	Clear, Colourless	Clear, Colourless	Clear, Colourless
	Essentially free of visible particulates	Essentially free of visible particulates	Essentially free of visible particulates	Essentially free of visible particulates
Identity by RT/Sanger Sequencing	Sequence matches 100% description of coding region	Conforms	Conforms	Conforms
Total RNA Content by UV	CCI			
Purity by RP-HPLC	CCI			
Product-related impurities by RP-HPLC	Report % Post main peak area	CCI		
% 5' Capped by UPLC-UV	CCI			
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC	CCI			
Residual DNA template by qPCR	CCI			
pH	CCI			
Bacterial endotoxin USP <85>, Ph. Eur. 2.6.14	CCI			
Bioburden USP <61>, Ph. Eur. 2.6.12	CCI			

2.3.S.4.5 Justification of Specification

Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities. Specifications are chosen to confirm the quality of the CX-024414 mRNA rather than to establish full characterization and should focus on those characteristics found to be useful in ensuring the safety and efficacy of the CX-024414 mRNA (ICH Q6B and Q11).

A Critical Quality Attribute is defined as a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality per ICH Q8(R2)—*Pharmaceutical Development*.

Table 22 summarizes the CQAs and the corresponding justifications for the CX-024414 mRNA based on risk assessment performed and process and product characterization knowledge gained for CX-024414 mRNA and other mRNAs.

Table 22: Critical Quality Attributes Assessment for CX-024414 mRNA

Quality Attribute	Classification	Justification
Appearance	CQA	CCI
pH	CQA	
Identity	CQA	
Bacterial Endotoxins	CQA	
Bioburden	CQA	
RNA Content	Process Control	
RNA Purity	CQA	
RNA Impurities (Product-Related)	CQA	
% 5' Capped	CQA	
% PolyA Tailed RNA	CQA	
% Tailless RNA	CQA	

The specification for release of CX-024414 mRNA is presented in . The specification includes the attributes, test methods, and acceptance criteria that together confirm the quality of CX-024414 mRNA lots and ensure each lot is acceptable for use in DP manufacturing.

Acceptance criteria were established according to ICH Q6B guidelines and reflect historical product quality data from preclinical and clinical lots, release results for manufactured CX-024414 mRNA, stability study results, relevant development data, and analytical method performance data.

The rationale for the establishment and justification of the release specification is provided in

2.3.S.5 Reference Standards or Materials

Reference Materials for Commercial Use

The reference material program has been established in accordance with ICH Q6B, *Specification: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*. It consists of an in-house primary reference material (PRM) and a working reference material (WRM), prepared and qualified from a CX-024414 lot for testing of CX-024414 mRNA, mRNA-1273 LNP and mRNA-1273 Drug Product. An overview of the commercial reference material is provided in Table 23.

The reference material was prepared and qualified for release and stability testing in the RNA content by anion exchange high-performance liquid chromatography (refer to , and Section 3.2.P.5.2 for respective analytical procedures):

Table 23: Commercial Reference Material

Lot Number	Manufacturing		Released for Use	Use	
	Process	DOM		Sample Type	Test Methods
DH-03180.1 (PRM and WRM)	CX-024414 PPQ 2)	17 June 2020	Qualification completed Release for use	CX-024414 mRNA mRNA-1273 LNP, mRNA-1273 DP	mRNA Purity Poly A Tail RNA content by AEX-HPLC (Reference material)

Abbreviations: AEX-HPLC = anion exchange high-performance liquid chromatography; DOM = date of manufacture; DP = drug product; LNP = lipid nanoparticle; PPQ = process performance qualification; PRM = primary reference material; WRM = working reference material

PRM/WRM lot DH-03180.1 is intended for release and stability testing of commercial lots. It was generated from CX-024414 mRNA process performance qualification (PPQ) Lot 4007220004, which was manufactured using the Scale A (10 L IVT) process (changes between Scale A and Final Scale B manufacturing processes are presented in). To generate

DH-03180.1, post-filtration material from PPQ Lot 4007220004 was diluted CCI and then dispensed into CCI tubes with CCI fill volume. During dispensing, the diluted bulk CX-024414 material was routinely inverted to minimize concentration gradients. PRM Lot DH-03180.1 is stored at CCI

The PRM/WRM (DH-03180.1) was characterized before use and will be re-qualified annually. The stability study supporting ongoing use of the commercial reference material is summarized will be evaluated for CCI and for CCI.

A summary of the qualification results from lot release and characterization testing of DH-03180.1 is provided in Table 24 and Table 25 below. The qualification report MQR-0017 is provided. The stability monitoring program is summarized in Table 26 below.

Table 24: CX-024414 Primary Reference Material, Lot DH-03180.1 Release Testing

Attribute	Target Criteria	Results
Appearance ^(a)	Clear, colorless solution, essentially free of visible particulates	Clear, colorless, free of visible particulates
Identity by RT Sanger sequencing	Sequence matches 100% description of coding region	Conforms
Total RNA content by UV	CCI	
Purity by RP-HPLC		
Report % post-main peak area		
< LOO		
% 5' Capped by RP-UPLC-UV		
% PolyA tailed RNA (%PolyA tailed RNA / tailless RNA) by RP-HPLC		
Residual DNA template by qPCR		
pH		
Bacterial endotoxin ^(a)	CCI	
Bioburden ^(a)		

Abbreviations: EU = endotoxin unit(s); qPCR = quantitative polymerase chain reaction; RP-HPLC = reverse-phase high-performance liquid chromatography; RP-UPLC-UV = reverse-phase high-performance liquid chromatography with ultraviolet detection; RT = reverse transcription
a) Testing performed at release of lot 4007220004

Table 25: CX-024414 Primary Reference Material, Lot DH-03180.1 Characterization Results

Attribute	Description	Results
CCI		

Attribute	Description	Results
CCI		
CCI		

Table 26: CX-024414 Reference Material, Lot DH-03180.1 Stability Monitoring

Attribute	Target Criteria
Appearance	Clear, colorless solution, essentially free of visible particulates
Identity by RT Sanger sequencing	Sequence matches 100% description of coding region
Total RNA content by UV	CCI
Purity by RP-HPLC	
% 5' Capped by RP-UPLC-UV	
% PolyA tailed RNA (% Tailless RNA) by RP-HPLC	
Residual DNA template by qPCR	
pH	
Bacterial endotoxin	

Abbreviations: EU = endotoxin unit(s); RP-HPLC = reverse-phase high-performance liquid chromatography; RP-UPLC-UV = reverse-phase ultra-performance liquid chromatography and ultraviolet detection; RT = reverse transcription

The new Reference Material lot RM-4007421130 is qualified against Reference Material lot DH-03180.1. The qualification report MQR-0351 and the Certificate of Analysis of new reference material lot RM-4007421130 are provided in Section 3.2.S.5.

Future Reference Materials

Future mRNA Reference Materials will be prepared from commercial CX-024414 lots. Reference Materials will be qualified against an established Primary Reference Material Qualification protocol **MQP-0241**. A certificate of analysis must be generated and approved prior to the Reference Material being used for GMP testing. Characterization of a Primary Reference Material includes meeting the release criteria outlined in the approved specification. At a minimum, the stability of the Reference Material is re-qualified annually.

2.3.S.6 Container Closure System

CX-024414 mRNA is stored frozen in gamma irradiated, single-use storage bags as a low bioburden material. The CX-024414 mRNA is dispensed into bags utilizing aseptic connections. The filled bags are directly processed or stored at -90°C to -60°C prior to mRNA-1273 LNP

manufacture. Two storage bag options are available as described below.

The Mobius storage bag assemblies are manufactured from PureFlex film, which is composed of ultra-low density polyethylene (ULDPE) product contact and ethylene vinyl acetate (EVA) support structure. The bags are equipped with three ports with C-Flex and silicone tubing. The single-use systems are individually packaged prior to being gamma-irradiated at a minimum exposure of 25 kGy, capable of ensuring a sterility assurance level of 10⁻⁶. Liquid particle count levels are certified in compliance with USP <788>.

The CCI storage bag assemblies are manufactured from CCI film, which is composed of ultra-low density polyethylene (ULDPE) product contact and ethylene vinyl acetate (EVA) and polyamide (PA) support structure. The bags are equipped with three ports with silicone tubing.

The single-use systems are individually packaged prior to being gamma-irradiated at a minimum exposure of 25 kGy, capable of ensuring a sterility assurance level of 10⁻⁶. Liquid particle count levels are certified in compliance with USP <788>. The results of USP <87> (in vitro Biological Reactivity) and USP <88> (in vivo Biological Reactivity) and ISO 10993-4 (Hemolysis) testing conducted by the manufacturer indicates that the primary container materials of construction are non-cytotoxic. The materials also meet the requirements for physicochemical testing as described in USP <661> (Plastic Packaging Systems and their Materials of Construction). The storage bags meet the USP Class VI requirements and are determined to be nonpyrogenic at a level of < 0.25 EU/mL per USP <85>.

Each incoming lot is received with a Certificate of Quality, which documents completion of the gamma irradiation cycle. The storage system is rated at an operating range of -80°C to 60°C CCI max operating temperature of 40°C), which accommodates the CX-024414 manufacturing process parameters. The manufacturer has assigned a post gamma irradiation sterilization shelf life of two years (3 years for CCI).

There are no functional secondary packaging components.

2.3.S.7 Stability

2.3.S.7.1 Stability Summary and Conclusions

The CX-024414 mRNA registration stability program was executed according to ICH Q1A (R2), *Stability Testing of new Drug Substances and Products*, and ICH Q5C, *Stability Testing of Biotechnological/Biological Products*. The approved shelf life for CX-024414 utilized in the manufacture of prototype mRNA-1273 DP is currently set at 9 months when stored at -15°C to -25°C in the commercial container closure system defined in 4}. An initial shelf-life of 36 months is proposed for CX-024414 stored in the commercial container closure system, defined in Section 3.2.S.6 {CX-024414}, when stored at the recommended long-term storage condition of -60°C to -90°C. Moving forward, Moderna will utilize the long term

storage temperature range of -60°C to -90°C for CX-024414 to ensure higher starting levels of purity in the mRNA-1273 drug product.

The shelf-life is justified from the purity statistical model. The lots used in the purity modeling analysis were manufactured using a development process, PVU scale process, the initial Scale B process and the Scale B process. Modeling included data from development lots from additional variant RNAs to incorporate sequence differences in the statistical model.

Stability and characterization studies were designed to evaluate product stability under various stressed and long-term storage conditions. All lots were manufactured using the manufacturing process described in [REDACTED]. Stability samples were stored in containers made of the same materials (high-density polyethylene bottle with a polyethylene terephthalate glycol cap) as the commercial closure system. For stability studies performed at Lonza AG (Visp, Switzerland) stability samples are stored in containers that are made of a high-purity fluoreopolymer film.

Size-based RNA purity and polyA tailed RNA, as determined by reverse-phase high-performance liquid chromatography (RP-HPLC), were demonstrated to be stability indicating (refer to [REDACTED] for analytical procedures).

An overview of the CX-024414 mRNA stability program is outlined in Table 27. The data from these studies are presented in [REDACTED].

Table 27: Summary of CX-024414 Batch Information

Lot Number	Usage	Manufacturing Site	Date of Manufacture
MTDS20002	Toxicology, Development	ModernaTX, Inc. Cambridge, MA	March 26, 2020
4007220001	Phase 3 Clinical Supplies (10 L IVT)	ModernaTX, Inc. Norwood, MA	April 03, 2020
4007220002	Phase 3 Clinical Supplies (10 L IVT)	ModernaTX, Inc. Norwood, MA	May 04, 2020
4007220003	Phase 3 Clinical Supplies (10 L IVT PPQ)	ModernaTX, Inc. Norwood, MA	May 20, 2020
4007220004	Phase 3 Clinical Supplies (10 L IVT PPQ)	ModernaTX, Inc. Norwood, MA	June 04, 2020
4007220005	Phase 3 Clinical Supplies (10 L IVT PPQ)	ModernaTX, Inc. Norwood, MA	June 18, 2020
4007520001	GMP (20 L IVT)	Lonza Biologics, Inc. Portsmouth, NH	July 28, 2020
4007520004	GMP (20 L IVT PPQ)	Lonza Biologics, Inc. Portsmouth, NH	August 31, 2020
4007420003	GMP (60 L IVT PPQ)	ModernaTX, Inc. Norwood, MA	October 19, 2020
4007420004	GMP (60 L IVT PPQ)	ModernaTX, Inc. Norwood, MA	October 31, 2020
4007420006	GMP (60 L IVT PPQ)	ModernaTX, Inc. Norwood, MA	November 10, 2020
4007420007	GMP (60 L IVT PPQ)	ModernaTX, Inc. Norwood, MA	November 12, 2020
4007421017	GMP (60 L IVT PPQ)	Lonza Biologics, Inc. Portsmouth, NH	January 21, 2021
4007420021	GMP (60 L IVT PPQ)	ModernaTX, Inc. Norwood, MA	January 06, 2021
4007421013	GMP (60 L IVT PPQ)	Lonza Biologics, Inc. Portsmouth, NH	January 21, 2021
Lonza Lot 1002 (4007520803)	GMP (20 L IVT PPQ)	Lonza AG (Visp, Switzerland)	16 Dec 2020
Lonza Lot 1003 (4007520804)	GMP (20 L IVT PPQ)	Lonza AG (Visp, Switzerland)	23 Dec 2020
(Lonza lot 1014)	GMP (20 L IVT PPQ)	Lonza AG (Visp, Switzerland)	21 Mar 2021
4007421005 (Lonza lot 41001)	GMP (60 L IVT Pre-PPQ)	Lonza AG (Visp, Switzerland)	23 Jan 2021
4007421006	GMP	Lonza AG	12 Feb 2021

Lot Number	Usage	Manufacturing Site	Date of Manufacture
(Lonza lot 41002)	(60 L IVT PPQ)	(Visp, Switzerland)	
4007421008 (Lonza lot 41003)	GMP (60 L IVT PPQ)	Lonza AG (Visp, Switzerland)	24 Feb 2021
4007421009 (Lonza lot 41004)	GMP (60 L IVT PPQ)	Lonza AG (Visp, Switzerland)	11 Mar 2021
4007421067 (Lonza lot 51001)	GMP (60 L IVT PPQ)	Lonza AG (Visp, Switzerland)	13 Mar 2021
4007421092 (Lonza lot 61001)	GMP (60 L IVT PPQ)	Lonza AG (Visp, Switzerland)	11 May 2021
4010221001 (Lonza lot 54001)	GMP (75 L IVT PPQ)	Lonza Visp, Switzerland	06 Oct 2021
4010222049 (Lonza lot 114001)	GMP (75 L IVT PPQ)	Lonza Visp, Switzerland	03 Apr 2022

The stability protocols are described in [Table 28](#) to [Table 35](#).

Table 28: Stability Protocol for Development CX-024414

Condition ^(a)	Time Interval (Months)					
	0	1	3	6	9	12
-20°C ± 5°C/Ambient RH	XY	X	X	X	X	X
5°C ± 3°C/Ambient RH		X	N/A	N/A	N/A	N/A

a = Container closure: sterile 30 mL PETG bottle with HDPE cap

X = Appearance, % Purity by RP-HPLC, % Poly A Tail by RP-HPLC, % 5' Capped by RP-HPLC, Total RNA Content by NaOH digest

Y = pH, Bacterial Endotoxins, Bioburden, Residual DNA, Identity

N/A = Not tested per the stability protocol

Table 29: Stability Protocol for GMP CX-024414 Manufactured at ModernaTX, Inc. and Lonza Biologics, Inc. (Portsmouth, NH) 10 L

Condition ^(a)	Time Interval (Months)								
	0	3	6	9	12	18	24	36	48
-20°C ± 5°C/Ambient RH	X,Y,Z,C	X	XY	X	XYZ	XY	XYZ	XYZ	XYZ
5°C ± 3°C/Ambient RH		X	XY	N/A	N/A	N/A	N/A	N/A	N/A

a = Container closure: sterile 5 mL PETG bottle with HDPE cap

X = Appearance, pH % Purity by RP-HPLC, Product-related impurities by RP-HPLC, % Poly A Tail by RP-HPLC, Total RNA Content by NaOH digest

Y = % 5' Capped

Z = Bacterial Endotoxin

C = Bioburden, Residual DNA, Identity (Refer to)

N/A = Not tested per the stability protocol

Table 30: Stability Protocol for GMP CX-024414 20 L IVT Scale

Condition ^(a)	Time Interval (Months)									
	0	1 ^(b)	3	6	9	12	18	24	36	48
-20°C ± 5°C/Ambient RH	X,Y,Z,C	N/A	X	XY	X	XYZ	XY	XYZ	XYZ	XYZ
5°C ± 3°C/Ambient RH		X	X	XY	N/A	N/A	N/A	N/A	N/A	N/A

a = Container closure: sterile 5 mL PETG bottle with HDPE cap b = Timepoint testing applicable to lots initiated on stability after 26Aug20
X = Appearance, pH % Purity by RP-HPLC, Product-related impurities by RP-HPLC, % Poly A Tail by RP-HPLC, Total RNA Content by NaOH digest
Y = % 5' Capped
Z = Bacterial Endotoxin
C = Bioburden, Residual DNA, Identity (Refer to
N/A = Not tested per the stability protocol

Table 31: Stability Protocol for GMP CX-024414 60 L IVT Scale

Condition ^(a)	Time Interval (Months)										
	0	1	2	3	6	9	12	18	24	36	48
-20°C ± 5°C/ Ambient RH	X,Y,Z,C	N/A	N/A	X	XY	X	XYZ	XY	XYZ	XYZ	XYZ
5°C ± 3°C/ Ambient RH		X	X	XY	N/A	N/A	N/A	N/A	N/A	N/A	N/A

a = Container closure: sterile 5 mL PETG bottle with HDPE cap
X = Appearance, pH % Purity by RP-HPLC, Product-related impurities by RP-HPLC, % Poly A Tail by RP-HPLC, Total RNA Content by NaOH digest
Y = % 5' Capped
Z = Bacterial Endotoxin
C = Bioburden, Residual DNA, Identity (Refer to
N/A = Not tested per the stability protocol

Table 32: Stability Protocol for GMP PPQ CX-024414 75 L IVT Scale (ModernaTX, Inc.)

Condition ^(a)	Time Interval (Months)							
	0	1	3	6	12	24	36	48
-20°C ± 5°C/Ambient RH	X,Y,Z,C	N/A	X	XY	XYC	XYC	XYC	XYZ
5°C ± 3°C/Ambient RH		X	XY	N/A	N/A	N/A	N/A	N/A

a = Container closure: sterile 5 mL PETG bottle with HDPE cap
X = Appearance, pH % Purity by RP-HPLC, Product-related impurities by RP-HPLC, % Poly A Tail by RP-HPLC, Total RNA Content by NaOH digest
Y = % 5' Capped
Z = Bacterial Endotoxin
C = Bioburden, Residual DNA, Identity (Refer to
N/A = Not tested per the stability protocol

Table 33: Stability Protocol for GMP PPQ CX-024414 75L IVT Scale (Lonza Biologics)

Condition ^(a)	Time Interval (Months)							
	0	1	3	6	12	24	36	48
-20°C ± 5°C	X,Y,Z,C	N/A	X	XY	XYZ	XYZ	XYZ	XYZ
5°C ± 3°C		X	XY	N/A	N/A	N/A	N/A	N/A

a = Container closure: sterile 5 mL PETG bottle with HDPE cap
X = Appearance, pH % Purity by RP-HPLC, Product-related impurities by RP-HPLC, % Poly A Tail by RP-HPLC, Total RNA Content by NaOH digest
Y = % 5' Capped
Z = Bacterial Endotoxin
C = Bioburden, Residual DNA, Identity (Refer to
N/A = Not tested per the stability protocol

Table 34: Stability Protocol for GMP CX-024414 75L IVT Manufactured at ModernaTX, Inc. and Lonza AG (Visp, Switzerland) – CCI Bags

Condition ^(a)	Time Interval (Months)								
	0	1	2	3	6	9	12	18	24
-20°C ± 5°C/Ambient RH	X,Y,Z,C,D	N/A	N/A	X	XY	X	XYZ	XY	XYZD
5°C ± 3°C/Ambient RH		X	X	XY	XY	N/A	N/A	N/A	N/A

a = Container closure: 50 mL Aramus bag (high-purity fluoropolymer film)
X = Appearance, pH % Purity by RP-HPLC, Product-related impurities by RP-HPLC, % Poly A Tail by RP-HPLC, Total RNA Content
Y = % 5' Capped
Z = Bacterial Endotoxin
C = Residual DNA, Identity
D = Bioburden
N/A = Not tested per the stability protocol

Table 35: Stability Protocol for GMP CX-024414 75 L IVT Scale

Condition ^(a)	Time Interval (Months)									
	0	1	3	6	9	12	18	24	36	48
-60°C to -90°C	X,Y,Z	X	X	XY	XYZ	XYZ	XY	XYZ	XYZ	XYZ

a = Container closure: sterile 5 mL PETG bottle with HDPE cap
X = Appearance, % Purity, Product-related impurities, % Poly A Tail, % Tailless RNA
Y = pH, Total RNA Content, % 5' Cap
Z = Bacterial Endotoxin

Stability conclusion and stability data are provided in } and
3.2.S.7.3 {CX-024414}, respectively.

Freeze-Thaw Cycling Stability Study for CX-024414

A freeze-thaw stability study was performed using Development CX-024414 Lot MTDS20002. CX-024414 samples were subjected to a series of CCI freezing and thawing cycles at room temperature and assessed for purity by RP-HPLC. No notable changes in the CX-024414 purity were observed after CCI freeze-thaw cycles. The data are presented in

Stability Conclusions

Based on all the available stability data, the use period for GMP CX-024414 stored at -60°C to -90°C is 36 months.

2.3.S.7.2 Post-Approval Stability Protocol and Stability Commitment

For information regarding the post-approval stability, please refer to

2.3.S.7.3 Stability Data

Stability data are provided in }.