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LIST OF ABBREVIATIONS

Abbreviation	Definition
ACE2	angiotensin-converting enzyme 2
BA.1	subvariant of Omicron
bAb	binding antibody
CMV	cytomegalovirus
COVID-19	coronavirus disease 2019
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
ERD	enhanced respiratory disease
FDA	Food and Drug Administration
GLP	Good Laboratory Practice
GMT	geometric mean titer
hMPV	human metapneumovirus
ICH	International Council for Harmonization
IgG	immunoglobulin G
IM	intramuscular(ly)
IV	intravenous
LNP	lipid nanoparticle
MERS-CoV	Middle East respiratory syndrome coronavirus
mRNA	messenger RNA
nAb	neutralizing antibody
NHP	nonhuman primate
NOAEL	no observed adverse effect level
OECD	Organization for Economic Co-operation and Development
PBS	phosphate-buffered saline
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PIV3	parainfluenza virus type 3
PK	pharmacokinetic(s)
S-2P	spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
SARS-CoV	severe acute respiratory syndrome coronavirus
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SM-102	a custom-manufactured ionizable lipid
TIC	total intact concentration
US	United States
WHO	World Health Organization
XEC	a recombinant lineage of KS.1.1 (JN.1.13.1.1.1) and KP.3.3 (JN.1.11.1.3.3), which are subvariants of JN.1

DESCRIPTION OF SARS-COV-2 VARIANTS AND MRNA-1273 DRUG PRODUCTS

Strain	Test Material	mRNA Encodes SARS-CoV-2:	Strain Definition
Wuhan-Hu-1	mRNA-1273	S-2P	Original strain
BA.4/BA.5	mRNA-1273.045	S-2P	BA.4/BA.5 are subvariants of Omicron (the spike protein of BA.5 is identical to that of BA.4)
XBB.1.5	mRNA-1273.815	S-2P	XBB.1.5 is a subvariant of Omicron (note: spike protein of XBB.1.9.1 is identical to XBB.1.5)
JN.1	mRNA-1273.167	S-2P	BA.2.86.1.1 is a subvariant of Omicron
KP.2	mRNA-1273.712	S-2P	JN.1.11.1.2 is a subvariant of JN.1
LP.8.1	mRNA-1273.251	S-2P	JN.1.11.1.1.1.3.8.1 is a subvariant of JN.1

Abbreviations: mRNA = messenger RNA; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

2.4.1 OVERVIEW OF NONCLINICAL TESTING STRATEGY

2.4.1.1 Background

Coronaviruses are a large family of viruses including MERS-CoV and SARS-CoV that cause illness ranging from the common cold to more severe diseases. An outbreak of COVID-19 caused by SARS-CoV-2 began in Wuhan, Hubei Province, China in December 2019, and the disease quickly spread globally.

ModernaTX, Inc. (the Sponsor) used a scalable mRNA/LNP technology platform that allowed for a rapid response to the COVID-19 pandemic and was used to develop mRNA-1273, an LNP-encapsulated mRNA-based vaccine against SARS-CoV-2. mRNA-1273 contains a single mRNA that encodes the SARS-CoV-2 spike protein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P). mRNA-1273 was proven highly effective against COVID-19 following SARS-CoV-2 infection and has been licensed or conditionally approved across multiple regions for the prevention of COVID-19 in individuals 6 months of age and older (SPIKEVAX™).

The complex nature of the continuing evolution of SARS-CoV-2 makes it impossible to accurately predict which virus strains will gain dominance in any particular region of the world and how long a strain will remain dominant. Health authorities recommend a framework to identify variants of concern and test updated COVID-19 vaccines, which is critical to preserve neutralization responses and protection against the infection/severe disease caused by SARS-CoV-2. The Sponsor has established a process to monitor emerging variants, classify them by immune-evading mutations, test matching vaccine candidates, and deploy them if requested by health authorities. This approach was effective in enabling preparation of variant-specific mRNA-1273 booster vaccines that were authorized in 2022 (a bivalent vaccine containing the original and Omicron BA.4/BA.5 or Omicron BA.1 vaccines), in 2023 (monovalent Omicron subvariant XBB.1.5-containing vaccine), and in 2024 (monovalent JN.1- or KP.2-containing vaccines).

The current variant landscape (early 2025) is dominated by multiple JN.1 descendants, such as XEC and LP.8.1, with LP.8.1 rapidly increasing and overtaking other variants. LP.8.1, which has acquired 9 spike protein mutations compared to JN.1, was classified as a variant under monitoring by the WHO on 24 Jan 2025 ([WHO 2025a](#)). Studies indicate that LP.8.1 has immune evasion capabilities versus currently approved JN.1 and KP.2 vaccines and high ACE2 binding, potentially supporting a growth advantage ([Liu et al 2025](#)). As of week 5 of 2025, LP.8.1 represented 13.9% of globally available sequences, a significant rise from 1.9% just 6 weeks earlier in epidemiological week 51 of 2024 ([WHO 2025b](#)). In the US, COVID-19 variant surveillance estimated that LP.8.1 represented 48% to 62% of cases as of 29 Mar 2025, with a 95% prediction interval ([CDC 2025](#)).

The Sponsor's risk assessment indicates that a vaccine update to LP.8.1 will be most effective at neutralizing currently circulating strains, with cross-neutralization likely against older JN.1 strains that no longer circulate, as well as JN.1 strains yet to emerge. Preliminary investigations suggest that, given its rapid rise and immune evasion capabilities, LP.8.1-containing vaccines should be investigated as a candidate vaccine for the 2025-2026 season. Therefore, nonclinical

studies conducted with an LP.8.1-containing vaccine (mRNA-1273.251) are herein summarized to support registration of an LP.8.1 new variant vaccine for the 2025-2026 season.

2.4.1.2 Nonclinical Test Materials

Preclinical mRNA-1273, mRNA-1273.045, mRNA-1273.815, mRNA-1273.167, mRNA-1273.712, and mRNA-1273.251 vaccines used in these studies were prepared with the same process as the mRNA-1273 clinical drug products. Refer to [Description of SARS-CoV-2 Variants and mRNA-1273 Drug Products](#) for variant definitions and the corresponding drug products.

All vaccines were formulated into a mixture of 4 lipids: SM-102, cholesterol, DSPC, and PEG2000-DMG.

Details of the nonclinical test materials used in previously conducted PK and toxicology studies are described in Module 2.6.4 and Module 2.6.7, respectively, that have been previously submitted to respective health authorities to support the original mRNA-1273. No new PK and toxicology studies have been conducted for variant-specific mRNA-1273 vaccines.

2.4.1.3 Nonclinical Testing Program

The nonclinical testing program supporting licensure and/or conditional approval of mRNA-1273 or variant-containing formulations of mRNA-1273 across multiple regions was designed to adhere to international regulatory guidelines, the intended clinical development program, and traditional pharmacology and toxicology principles and was consistent with ICH guidelines for biological drug development, including ICH S6(R1) (Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals) and appropriate GLP regulations that were applicable when studies were conducted. The pivotal nonclinical safety studies were conducted according to the OECD Principles of Good Laboratory Practice (ENV/MC/CHEM[98]17) or GLP regulations in other countries that are signatories to the OECD Mutual Acceptance of Data agreement (eg, US FDA Code of Federal Regulations Title 21, Part 58: Good Laboratory Practice for Nonclinical Laboratory Studies).

As in previous years, a “platform concept” strategy has been employed by the Sponsor to support mRNA-1273 and variant-containing mRNA-1273 vaccines, where the safety and tolerability of mRNA vaccines that encode various antigens developed with the Sponsor’s mRNA-based platform using SM-102-containing LNPs, including but not limited to mRNA-1273, have been evaluated in multiple GLP-compliant repeat-dose toxicity studies in Sprague Dawley rats. This strategy has been accepted by regulatory authorities and is considered relevant and sufficient to support clinical development of mRNA-1273 and variant-containing mRNA-1273 vaccines, because there is consistency in the toxicological data across GLP toxicity studies regardless of the antigen expressed, demonstrating that the toxicity associated with mRNA vaccines formulated in LNPs is driven primarily by the LNP composition and to a lesser extent, the biologic activity of the antigen(s) encoded by the mRNA. Moreover, given that there were no new safety concerns observed with variant-containing mRNA-1273 vaccines in the nonclinical pharmacology studies described in Module 2.6.2, toxicological data generated with the mRNA-1273 vaccine, as well as other mRNA vaccines formulated in the same LNPs

(Module 2.6.6), adequately characterize target organs of toxicity and inform the nonclinical risk assessment for variant-containing mRNA-1273 vaccines.

The Sponsor has initiated development of a monovalent LP.8.1-containing mRNA vaccine (mRNA-1273.251) for the 2025-2026 season, given the rapid rise and immune evasion capabilities of LP.8.1. To support this, nonclinical in vivo pharmacology studies were conducted in BALB/c mice. These studies evaluated immunogenicity of mRNA-1273.251 given as a primary series or as a booster dose. Additionally, the Sponsor has expanded the research to include investigating immunogenicity of a booster dose given after various primary series regimens, aiming to more closely reflect the diverse human immune experience. These studies align with regulatory expectations to generate preclinical immunogenicity data that support the effectiveness of an updated vaccine formulation.

The route of administration of the mRNA vaccines used in the nonclinical in vivo pharmacology studies was IM, consistent with the clinical route.

2.4.2 PHARMACOLOGY

Nonclinical primary pharmacology evaluations supporting initial licensure and/or conditional approval of mRNA-1273 across multiple geographic regions were conducted in young and aged mice (BALB/c, BALB/cJ, C57/BL6J, and B6C3F1/J strains), golden Syrian hamster, and rhesus macaque (NHPs) animal models to characterize the immunogenicity of mRNA-1273, as well as its effects on viral replication and disease progression after SARS-CoV-2 challenge, and to evaluate its safety profile and its potential for vaccine-associated ERD after viral challenge (Corbett et al 2020), which has previously been observed with vaccines against respiratory syncytial virus (Kim et al 1969) and measles (Polack 2007) and in animal models of SARS-CoV vaccination (Czub et al 2005, Deming et al 2006, Bolles et al 2011). Additionally, the immunogenicity of mRNA-1273 was assessed in GLP and non-GLP repeat-dose toxicity studies in Sprague Dawley rats (Module 2.6.6). Refer to Module 2.4, supporting the original mRNA-1273, for additional details including the rationale for animal models used as well as the results of these studies.

Table 1 summarizes the additional nonclinical pharmacology studies performed in support of the LP.8.1-containing vaccine (mRNA-1273.251). These pharmacology results are fully summarized in Module 2.6.2.

Table 1: Summary of Pharmacology Program for LP.8.1-containing Vaccines

Study Type/Description	Test Article: Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Evaluation of immunogenicity of a primary series of a SARS-CoV-2 LP.8.1-containing mRNA-1273 vaccine in mice	PBS control: 0 mRNA-1273.712 ^a , mRNA-1273.167 ^b , and mRNA-1273.251 ^c : 1	Mouse, BALB/c	IM; Day 1, 22 (primary series)	No	MOD-7407

Study Type/Description	Test Article: Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Evaluation of immunogenicity of a monovalent SARS-CoV-2 LP.8.1-containing mRNA-1273 vaccine booster in mice	<p>PBS control: 0</p> <p><u>Regimen 1 Primary Series</u> mRNA-1273^d: 0.5 (Dose 1 and Dose 2)</p> <p><u>Regimen 2 Primary Series</u> mRNA-1273^d+ mRNA-1273.045^e: 0.5 (Dose 1)</p> <p>mRNA-1273.815^f+ mRNA-1273.712^a: 0.5 (Dose 2)</p> <p><u>Booster (Dose 3)</u> mRNA-1273.712^a, mRNA-1273.167^b, and mRNA-1273.251^c: 1</p>	Mouse, BALB/c	<p>IM; Day 1, 22 (primary series regimen)</p> <p>Day 65 (booster)</p>	No	MOD-7345.1273

Abbreviations: GLP = good laboratory practice; IM = intramuscular; mRNA = messenger RNA;

PBS = phosphate-buffered saline; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

- ^a mRNA-1273.712 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the KP.2 subvariant of JN.1. mRNA-1273.712 was provided as a monovalent vaccine and a bivalent vaccine (co-administered with mRNA-1273.815 in a 1:1 benchside mix).
- ^b mRNA-1273.167 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the JN.1 subvariant of Omicron.
- ^c mRNA-1273.251 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the LP.8.1 subvariant of JN.1.
- ^d mRNA-1273 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the Wuhan-Hu-1 (original) strain.
- ^e mRNA-1273.045 is a single mRNA encoding the SARS-CoV-2 S-2P antigen of the BA.4/BA.5 subvariants of Omicron. In this study, mice receive bivalent vaccine containing 1:1 benchside mix of separately formulated mRNA-1273.045 and mRNA-1273 (original) (also referred to as bivalent mRNA-1273.222 vaccine).
- ^f mRNA-1273.815 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the XBB.1.5 subvariant of Omicron.

The results from the primary pharmacology studies with the LP.8.1-containing mRNA vaccine (mRNA-1273.251) in mice are summarized as follows:

MOD-7407 (Primary Series)

- The primary series administration of mRNA-1273.251 (LP.8.1), demonstrated high immunogenicity, supported by a robust increase in S-2P bAb (IgG) titers after Dose 2. The GMTs were comparable after Dose 2 for mRNA-1273.251, mRNA-1273.712, and mRNA-1273.167 (<2-fold change in GMT values between groups). The mRNA-1273.251 primary series induced potent nAb titers against LP.8.1 (matched), higher than mRNA-1273.712 and mRNA-1273.167. Additionally, mRNA-1273.251 effectively cross-neutralized JN.1 lineage strains (JN.1, KP.2, and XEC), with nAb titers comparable or higher than those elicited by mRNA-1273.712 or mRNA-1273.167.

MOD-7345.1273 (Booster)

- After the primary series (Regimen 1 or Regimen 2), boosting with mRNA-1273.251 increased S-2P bAb titers (IgG) to levels comparable to mRNA-1273.712 and mRNA-1273.167.
- Mice boosted with mRNA-1273.251 showed increased neutralizing titers to LP.8.1 that cross-neutralized related JN.1 lineage strains (JN.1, KP.2, and XEC). The overall nAb response after Dose 3 in groups receiving Regimen 2 was higher than that in the groups receiving Regimen 1, consistent with these animals having greater immune experience with JN.1 lineage strains, having received mRNA-1273.712 (KP.2) as part of their primary series vaccination.

Overall, the test articles were well tolerated, and animal health monitoring did not reveal any adverse findings. The results of these studies demonstrate the robust immunogenicity of the LP.8.1 new variant vaccine (mRNA-1273.251). The cross-neutralization of other JN.1 lineage strains support the potential of this formulation to boost protection to other antigenically similar strains within the same subfamily (eg, JN.1, KP.2, and XEC). The higher nAb responses in groups receiving Regimen 2, which included exposure to more variant-specific vaccines, support the diverse human immune experience and suggest that this formulation will elicit protective responses in humans.

In conclusion, these data support the potential for LP.8.1 formulation in driving increased immunogenicity and boosting protection against both LP.8.1 and closely related JN.1 lineage strains.

2.4.3 PHARMACOKINETICS

Studies have shown that unformulated mRNA is degraded within minutes in biological fluids and is unlikely to persist in tissues; therefore, the biodistribution of mRNA-based vaccines formulated in LNPs is predicted to be driven by the LNP characteristics and mRNAs that are within LNPs of the same composition (eg, SM-102-containing LNPs) are expected to distribute similarly to the LNPs. Thus, the distribution of mRNA-1647, an mRNA-based CMV vaccine that contains 6 mRNA sequences combined in SM-102-containing LNPs, assessed in a non-GLP, single IM dose biodistribution study evaluations supported licensure and/or conditional approval of mRNA-1273 across multiple geographic regions. This study demonstrated that mRNA was not detectable after 1 to 3 days; the only exceptions were at the site of injection (muscle) and within the draining lymph nodes and spleen where the mRNA had a calculated half-life ranging from 14.9 to 63.0 hours.

No absorption, distribution, metabolism, and excretion studies have been performed with mRNA-1273 or mRNA-1273 variant vaccines; however, the metabolism and elimination of the amino-lipid component in mRNA-1273, SM-102, have been examined in vivo. Overall, the primary circulating species after IV dosing Sprague Dawley rats with SM-102/DMG-containing LNPs was intact SM-102 (>95% of TIC) with principally ester hydrolysis and β -oxidative metabolites cleared via both the renal and hepatic routes of elimination. These ester hydrolysis and β -oxidation products account for the majority of all the detected SM-102 metabolites in both

the urine (>99% of TIC) and bile (approximately 70% of TIC) with the balance comprising intact SM-102. Low molecular weight, hydrophilic metabolites were detected in relatively higher amounts (approximately 10-fold) in urine compared to larger, more hydrophobic metabolites in plasma and bile. The extensive metabolism of SM-102, the oxidative nature of the metabolites, and the multiple, ubiquitous, high capacity systems by which they are formed, combined with the rapid overall clearance of SM-102 (within 24 hours) and the elimination of SM-102 and its metabolites via kidney (metabolites only) and liver (intact SM-102 and metabolites) to <3.0% of the maximum level, indicate that the SM-102 is unlikely to accumulate upon repeat IM dosing or present an issue for elimination in patients with hepatic or renal insufficiency.

Table 2 summarizes the nonclinical PK program for mRNA-1273. These PK results are fully summarized in Module 2.6.4 that was previously submitted to respective health authorities to support the original mRNA-1273. No new PK studies were conducted for variant-specific mRNA-1273 vaccines.

Table 2: Summary of Nonclinical PK Program for mRNA-1273

Study Type	Test Article	Species, Strain	Method of Administration, Dose	GLP	Report Number
Biodistribution					
Single-dose tissue distribution study	mRNA-1647 ^a	Rat, Sprague Dawley	IM injection dose of 100 µg on Day 1	No	5002121 Amendment 2
Metabolite Identification					
In vitro metabolite profiling and identification	SM-102	Rat, monkey, and human hepatocytes	In vitro, 10 µM	No	NCS-BA-2022-010
In vivo metabolite profiling and identification	SM-102	Rat, Sprague Dawley	IV, 0.7 mg/kg	No	QV-0236-DA-RE

Abbreviations: CMV = cytomegalovirus; DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; IM = intramuscular; IV = intravenous; mRNA=messenger RNA; PEG2000-DMG = 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000; PK=pharmacokinetics; SM-102=a custom-manufactured ionizable lipid.

^a mRNA-1647 contains 6 mRNAs that encode the full-length CMV gB and the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex. The 6 mRNAs are combined at a target mass ratio of 1:1:1:1:1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.

2.4.4 TOXICOLOGY

Toxicological data generated from GLP studies by the Sponsor with 6 mRNA-based vaccines, including but not limited to mRNA-1273, demonstrate that the toxicities associated with vaccines formulated in SM-102-containing LNPs are driven primarily by the LNP composition and to a lesser extent, by the biologic activity of the antigen(s) encoded by the mRNA. To support licensure and/or conditional approval of mRNA-1273 across multiple geographic regions, aggregated safety and tolerability platform data from 6 GLP-compliant repeat-dose

toxicity studies in Sprague Dawley rats with 5 mRNA-based vaccines encoding various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs (2 Zika virus vaccines: mRNA-1706 and mRNA-1893; 1 hMPV and PIV3 vaccine: mRNA-1653; and 2 CMV vaccines: mRNA-1647 and mRNA-1443) were used. Additionally, the Sponsor completed GLP and non-GLP repeat-dose studies in Sprague Dawley rats to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses. In these studies, IM doses ranging from 8.9 to 150 µg/dose were administered once every 2 to 4 weeks for up to 8 weeks, and the data were similar and consistent despite the fact that the different mRNA constructs encode different antigens. Toxicities and target organs were consistent with local inflammation at the injection sites and a transient generalized systemic inflammatory/immune system response expected with IM-administered vaccines. The NOAEL across studies was always the highest dose tested (ranging from 40 to 150 µg/dose).

SM-102, the custom lipid used in mRNA-1273, was evaluated in genotoxicity studies as an individual agent using a standard ICH S2 (R1) approach (ICH 2011), including a GLP-compliant bacterial reverse mutation (Ames) test in *Salmonella typhimurium* and *Escherichia coli* and a GLP-compliant in vitro micronucleus test in human peripheral blood lymphocytes. In addition, SM-102 was evaluated for in vivo genotoxicity risk in a GLP-compliant in vivo rat micronucleus test using an mRNA-based vaccine formulated in SM-102 LNPs (mRNA-1706) and a non-GLP-compliant in vivo rat micronucleus test using a reporter mRNA (nascent peptide imaging luciferase mRNA) CCI [REDACTED]. Overall, the genotoxic risk to humans is considered to be low due to minimal systemic exposure following IM administration, limited duration of exposure, and negative in vitro results.

A GLP-compliant combined developmental and perinatal/postnatal reproductive toxicity study was also conducted to assess the potential effects of mRNA-1273 on fertility and pre- and postnatal development in pregnant and lactating female Sprague Dawley rats. Results from this study showed that administration of a 100-µg dose of mRNA-1273 to Sprague Dawley rats did not result in any adverse effects on dams, fetuses, and pups and demonstrated a strong transfer of SARS-CoV-2 S-2P antibodies from dam to fetus and from dam to pup.

Table 3 summarizes the nonclinical toxicity program for mRNA-1273. These toxicology results are fully summarized in Module 2.6.6 that was previously submitted to respective health authorities to support the original mRNA-1273. No new toxicology studies were conducted for variant-specific mRNA-1273 vaccines.

Table 3: Summary of Nonclinical Toxicology Program for mRNA-1273

Study Type	Test Article	Species, Strain	Method of Administration; Dose	GLP	Report Number
Repeat-Dose Toxicity					
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706 ^a	Rat, Sprague Dawley	IM; 0, 13, 65, 129 µg/dose ^b (Days 1, 15, 29)	Yes	5002045
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706 ^a	Rat, Sprague Dawley	IM; 0, 10, 50, 100 µg/dose (Days 1, 15, 29)	Yes	5002231
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1653 ^c	Rat, Sprague Dawley	IM; 0, 10, 50, 150 µg/dose (Days 1, 15, 29)	Yes	5002033
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1893 ^d	Rat, Sprague Dawley	IM; 0, 10, 30, 96 µg/dose (Days 1, 15, 29)	Yes	5002400
6-week (4 doses) repeat-dose study with 2-week recovery	mRNA-1647 ^e	Rat, Sprague Dawley	IM; 0, 8.9, 27, 89 µg/dose ^f (Days 1, 15, 29, 43)	Yes	5002034
6-week (4 doses) repeat-dose study with 2-week recovery	mRNA-1443 ^g	Rat, Sprague Dawley	IM; 0, 9.6, 29, 96 µg/dose ^h (Days 1, 15, 29, 43)	Yes	5002158
8-week (3 doses) repeat-dose study with 2-week recovery	mRNA-1273	Rat, Sprague Dawley	IM; 40 µg/dose (Days 1, 29, and 57)	Yes	2308-245
In Vitro Genotoxicity					
Bacterial reverse mutation test	SM-102	<i>Salmonella typhimurium</i> , <i>Escherichia coli</i>	Incubation for 67 h 29 min with 0, 1.58, 5.0, 15.8, 50, 158, 500, 1581 µg/plate SM-102 with or without supplemented rat liver fraction	Yes	9601567
	PEG2000-DMG (Sunbright® GM-020) ⁱ	<i>Salmonella typhimurium</i> , <i>Escherichia coli</i>	Incubation for 67 h 57 min with 0, 1.58, 5.0, 15.8, 50, 158, 500, 1581 µg/plate PEG2000-DMG with or without supplemented rat liver fraction	Yes	9601035

Study Type	Test Article	Species, Strain	Method of Administration; Dose	GLP	Report Number
Mammalian cell micronucleus test	SM-102	Human peripheral blood lymphocytes	Incubation for 4 and 24 h with 0, 163, 286, 500 µg/mL SM-102 with or without supplemented rat liver fraction	Yes	9601568
	PEG2000-DMG (Sunbright® GM-020) ⁱ	Human peripheral blood lymphocytes	Incubation for 4 and/or 24 h with 0.53.393.3163286 µg/mL PEG2000-DMG with or without supplemented rat liver fraction	Yes	9601036
In Vivo Genotoxicity					
In vivo mammalian erythrocyte micronucleus test	mRNA-1706 ^a	Rat, Sprague Dawley	Single IV; 0, 0.6/6.9 (F), 1.3/15.1, 2.6/30.1, 5.2/60.3 (M) mg/kg RNA/SM-102 ^{j, k}	Yes	9800399
In vivo mammalian erythrocyte micronucleus test	NPI luciferase mRNA ^l	Rat, Sprague Dawley	Single IV; 0, 0.32/6.0, 1.07/20, 3.21/60 mg/kg NPI luciferase RNA/SM-102	No	AF87FU.125 012 NGLPICH.B TL
Reproductive and Developmental Toxicity					
Combined developmental and perinatal/postnatal reproductive toxicity study	mRNA-1273 ^m	Rat, Sprague Dawley	IM; 100 µg/dose (Study Days 1 and 15 [28 and 14 days prior to mating, respectively] and Gestation Days 1 and 13)	Yes	20248897
Other Toxicology					
5-week (2 doses) repeat-dose immunogenicity and toxicity study	mRNA-1273 ^m	Rat, Sprague Dawley	IM; 0, 30, 60, 100 µg/dose (Days 1 and 22)	No	2308-123

Abbreviations: CMV = cytomegalovirus; DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine; F=female; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; h = hour; hMPV = human metapneumovirus; IM = intramuscular; IV = intravenous; M = male; min = minute; mRNA = messenger RNA; NPI = nascent peptide imaging; PEG2000-DMG = 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000; PIV = parainfluenza virus type 3; pp65 = phosphoprotein 65; prME = premembrane and envelope; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SM-102 = a custom-manufactured ionizable lipid; SoA = summary of analysis.

- ^a mRNA-1706 contains a single mRNA sequence that encodes the prME structural proteins of Zika virus combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 20 mM Tris, 8% sucrose, pH 7.4.
- ^b The original dose levels selected were 0, 10, 50, and 100 µg/dose, respectively (SoA issued on 11 October 2016). The calculated dose levels were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064 (SoA issued on 03 May 2017). The change in the reported mRNA content for mRNA-1706 was 29%.
- ^c mRNA-1653 contains 2 distinct mRNA sequences that encode the full-length membrane-bound fusion proteins of hMPV and PIV3. The 2 mRNAs are combined at a target mass ratio of 1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 7% PG, 1 mM DTPA, pH 7.4.

- ^d mRNA-1893 contains a single mRNA sequence that encodes the prME structural proteins of Zika virus in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 100 mM Tris, 7% PG, 1 mM DTPA, pH 7.5.
- ^e mRNA-1647 contains 6 mRNAs that encode the full-length CMV gB and the pentameric gH/gL/UL128/UL130/UL131A glycoprotein complex. The 6 mRNAs are combined at a target mass ratio of 1:1:1:1:1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.
- ^f The original dose levels selected were 0, 10, 30, and 100 µg/dose, respectively (SoA issued on 16 March 2017). The calculated dose levels were revised based on the updated concentration reported for mRNA-1647 Lot No. MTDP17015 (SoA issued on 31 May 2017). The change in the reported mRNA content for mRNA-1647 was -11%.
- ^g mRNA-1443 contains a single mRNA sequence that encodes a phosphorylation mutant of the CMV pp65 protein (ie, deletion of amino acids 435-438) combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 60 mM NaCl, and 7% PG.
- ^h The original dose levels selected were 0, 10, 30, and 100 µg/dose, respectively (SoA issued on 16 March 2017). The calculated dose levels were revised based on the updated concentration reported for mRNA-1443 Lot No. MTDP17017 (SoA issued on 30 May 2017). The change in the reported mRNA content for mRNA-1443 was 4%.
- ⁱ Multiple test articles (Sunbright GM-020 and MC3) were assessed in this study. Only data relevant to the development of mRNA-1273 are discussed in this dossier.
- ^j A dose-range-finding test was performed prior to the main phase of the study, wherein male and female rats (3 animals/sex) were given a single intravenous injection (doses 2.6/30.1, 3.9/45.2, and 5.2/60.3 mg/kg RNA/SM-102 for females, and 2.6/30.1, 5.2/60.3, and 10.3/119.5 mg/kg RNA/SM-102 for males). Doses ≥3.9 mg/kg RNA in the female rat resulted in body weight loss; therefore, the female MTD was determined to be 2.6 mg/kg RNA. In males, 10.3 mg/kg RNA resulted in mortality (2 out of 3 animals) and no clinical signs at 5.2 mg/kg RNA; therefore, the male MTD was determined to be 5.2 mg/kg RNA.
- ^k The original dose levels selected were 0, 1.0, 2.0, 4.0, 0.5, 1.0, and 2.0 mg/kg mRNA-1706, respectively (SoA issued on 11 October 2016). The calculated dose levels were revised based on the updated concentration reported for mRNA-1706 Lot No. MTDP16064 (SoA issued on 03 May 2017). The change in the reported mRNA content for mRNA-1706 was 29%. Doses of SM-102 (mg/kg) were calculated by multiplying the RNA dose (mg/kg) by the ratio of SM-102 concentration (25.5 mg/mL) to RNA concentration (2.2 mg/mL) reported in the revised SoA (issued on 03 May 2017) in Study 9800399.
- ^l The NPI luciferase mRNA is combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 25 mM Tris, 123 g/L sucrose, 1 mM DTPA, pH 7.5.
- ^m mRNA-1273 contains a single mRNA sequence that encodes the full-length SARS-CoV-2 S-2P combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 20 mM Tris, 87 mg/mL sucrose, 17.5 mM sodium acetate, pH 7.5.

2.4.5 INTEGRATED OVERVIEW AND CONCLUSIONS

The Sponsor initiated development of a monovalent LP.8.1-containing mRNA vaccine (mRNA-1273.251) for the 2025-2026 season given the rapid rise and immune evasion capabilities of LP.8.1. To support this, nonclinical in vivo pharmacology studies evaluated immunogenicity of mRNA-1273.251 given as a primary series or as a booster dose, following two different primary series regimens.

In Study MOD-7407, mice administered the LP.8.1 new variant vaccine, mRNA-1273.251, as a primary series induced high S-2P bAb (IgG) titers and neutralizing titers against LP.8.1. The mRNA-1273.251 primary series also induced nAb that cross-neutralized antigenically related JN.1 lineage strains (JN.1, KP.2, and XEC).

In Study MOD-7345.1273, administration of the LP.8.1 new variant vaccine as a booster (Dose 3) was given after 2 different primary series regimens (Regimen 1 and Regimen 2). Regimen 1 enabled assessments using a model that has been previously used for testing updated vaccine candidates, while Regimen 2 was introduced to simulate a diverse immune experience that is more consistent with current human experience. Boosting with mRNA-1273.251 resulted in high S-2P bAb (IgG) titers, indicating a strong immunological response, irrespective of the primary series regimen. The postboost bAb titers for groups receiving mRNA-1273.251 were comparable to those elicited by mRNA-1273.712 and mRNA-1273.167, regardless of the initial primary series regimen and variability in preboost titers.

Boosting with mRNA-1273.251 increased nAb titers against the LP.8.1 strain and cross-neutralized JN.1 lineage strains (JN.1, KP.2, and XEC), irrespective of the primary series regimen. Groups receiving Regimen 2 had a higher overall nAb response, consistent with greater immune experience with a JN.1 lineage strain due to prior vaccination with mRNA-1273.712 (KP.2).

Overall, the results indicate that mRNA-1273.251, given as a primary series or a booster, was most effective in boosting nAb titers to the LP.8.1 strain but also effectively cross-neutralized JN.1 related strains, as well as JN.1 strains yet to emerge.

Toxicological data generated with the mRNA-1273 vaccine, as well as other mRNA vaccines formulated in the same LNPs, effectively characterize the nonclinical safety profile of an LP.8.1-containing vaccine.

2.4.6 REFERENCES

Bolles M, Deming D, Long K, Agnihothram S, Whitmore A, Ferris M, et al. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. *J Virol*. 2011;85(23):12201-15.

Center for Disease Control and Prevention. COVID Data Tracker. Summary of variant surveillance [database on the Internet]. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2025 Mar 28 [cited 2025 Apr 1]. Available from: <https://covid.cdc.gov/covid-data-tracker/#variant-summary>.

Corbett KS, Edwards DK, Leist SR, Abiona OM, Boyoglu-Barnum S, Gillespie RA, et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature*. 2020;586(7830):567-71.

Czub M, Weingartl H, Czub S, He R, Cao J. Evaluation of modified vaccinia virus Ankara based recombinant SARS vaccine in ferrets. *Vaccine*. 2005;23(17-18):2273-9.

Deming D, Sheahan T, Heise M, Yount B, Davis N, Sims A, et al. Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. *PLoS Med*. 2006;3(12):e525. Erratum in: *PLoS Med*. 2007;4(2):e80.

International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use: S2(R1). 9 Nov 2011 [cited 2022 May 26]. Available from: <https://database.ich.org/sites/default/files/S2%28R1%29%20Guideline.pdf>.

Kim HW, Canchola JG, Brandt CD, Pyles G, Chanock RM, Jensen K, et al. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am J Epidemiol*. 1969;89(4):422-34.

Liu J, Yu Y, Yang S, Jian F, Song W, Yu L, et al. Virological and antigenic characteristics of SARS-CoV-2 variants LF.7.2.1, NP.1, and LP.8.1. *Lancet Infect Dis*. 2025;25(3): e128-e130.

Polack FP. Atypical measles and enhanced respiratory syncytial virus disease (ERD) made simple. *Pediatr Res*. 2007;62(1):111-5.

World Health Organization. WHO TAG-VE Risk Evaluation for SARS-CoV-2 Variant Under Monitoring: LP.8.1 [Internet] Geneva: World Health Organization; 2025a Feb 3 [cited 2025 Feb 26]. Available from: https://cdn.who.int/media/docs/default-source/documents/epp/tracking-sars-cov-2/03022025_lp.8.1_ire.pdf?sfvrsn=b89f0899_4&download=true.

World Health Organization. Coronavirus disease (COVID-19) Epidemiological Updates and Monthly Operational Updates [Internet] Geneva: World Health Organization; 2025b Mar 12 [cited 2025 Mar 28]. Available from: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports>.

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