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## LIST OF ABBREVIATIONS

Abbreviation	Definition
BA.4/BA.5	subvariants of Omicron (the spike protein of BA.5 is identical to that of BA.4)
bAb	binding antibody
BLA	Biologics License Application
CMV	cytomegalovirus
COVID-19	coronavirus disease 2019
DSPC	1,2-distearoyl sn glycerol-3-phosphocholine
ERD	enhanced respiratory disease
EMA	European Medicines Agency
ETF	Emergency Task Force
FDA	Food and Drug Administration
GISAID	Global Initiative on Sharing All Influenza Data
GLP	Good Laboratory Practice
hMPV	human metapneumovirus
ICH	International Council for Harmonisation
IgG	immunoglobulin G
IM	intramuscular(ly)
IV	intravenous(ly)
JN.1	BA.2.86.1.1 subvariant of Omicron
KP.2	BA.2.86.1.1.11.1.2 subvariant of Omicron
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
NPI	nascent peptide imaging
OECD	Organisation for Economic Co-operation and Development
PBS	phosphate-buffered saline
PEG2000-DMG	1,2-dimyristoyl-rac-glycerol-3-methoxypolyethylene glycol-2000
PIV3	parainfluenza virus type 3
PK	pharmacokinetics
pp65	phosphoprotein 65
prME	premembrane and envelope
PSVN	pseudotyped virus neutralization assay

Abbreviation	Definition
RBD	receptor-binding domain
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
SoA	summary of analysis
TAG-CO-VAC	Technical Advisory Group on COVID-19 Vaccine Composition
VOC	variant of concern
VOI	variant of interest
VSV	vesicular stomatitis virus
WHO	World Health Organization
XBB.1.5	subvariant of Omicron
XBB.1.5/XBB.1.9.1	subvariants of Omicron (the spike protein of XBB.1.9.1 is identical to that of XBB.1.5)

## 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, and China in December 2019, and the disease quickly spread globally.

ModernaTX, Inc. (the Sponsor)'s scalable mRNA/LNP technology platform 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 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™).

Starting in 2021, the emergence of SARS-CoV-2 variants resulted in breakthrough cases, and subsequently a public health need for immunization against these antigenically divergent strains. Given the evident immune escape that VOCs exhibit to current vaccines, updating vaccine strain compositions to match such strains more closely is critical to maintaining protection. In response, variant-specific booster vaccines were recommended by WHO TAG-CO-VAC, EMA, and FDA, starting in 2022 with recommendations for bivalent vaccines. In 2022, bivalent variant-specific mRNA-1273 booster vaccines were authorized, with both ancestral and Omicron BA.1 (mRNA-1273.214) and ancestral and Omicron BA.4/BA.5 (mRNA-1273.222) being developed and authorized. In 2023, monovalent Omicron subvariant XBB.1.5 vaccine was recommended, and monovalent variant-specific mRNA-1273 vaccine (mRNA-1273.815) was authorized to address rises in infection from the XBB family Omicron subvariants.

In August 2023, the WHO designated a new strain BA.2.86 as a variant under monitoring based on a significant accumulation of mutations (>30) compared to an early Omicron (BA.2) parental lineage. This strain quickly gave rise to sublineages, and based on updated information, BA.2.86 and its sublineages (including JN.1 which has one additional mutation relative to BA.2.86) were classified as VOIs due to the rapid increase in prevalence across WHO countries (WHO 2023). The JN.1 strain overtook the XBB lineage as the predominant strain by January 2024 and exhibited potential for immune escape in individuals who received the most recent vaccine boosters. The JN.1 variant continues to be the most commonly sequenced strain globally, with additional subvariants of JN.1 having more recently emerged. These subvariants of JN.1, such as KP.2 (alias for BA.2.86.1.1.11.1.2) which has 3 additional mutations in the spike protein versus JN.1 including 2 in the RBD (R346T and F456L), are predicted to be antigenically similar to JN.1. As stated by the WHO TAG-CO-VAC and EMA Emergency Task Force (ETF) in April 2024, as virus evolution is expected to continue from JN.1, future formulations of COVID-19 vaccines should aim to induce enhanced neutralizing antibody responses to JN.1 and its descendent lineages (EMA 2024; WHO 2024). As one approach, the WHO TAG-CO-VAC therefore recommends use of a monovalent JN.1 lineage antigen in vaccines.

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. As also recommended by health agencies for COVID-19 strain updates, a framework to identify VOCs and to test updated vaccine candidates is therefore critical to preserve neutralization responses and protection against the infection/severe disease caused by SARS-CoV-2. The Sponsor has established such a process for continuous monitoring of emerging variants, classification of variants based on incorporation of immune-evading mutations, and subsequent testing of vaccine candidates matched to these variants in preparation for deployment should health agencies request it.

Therefore, and in line with the WHO TAG-CO-VAC and EMA ETF recommendations, nonclinical studies conducted with a monovalent JN.1-containing vaccine (mRNA-1273.167) are herein summarized to support registration of an JN.1-containing vaccine for the 2024-2025 season.

#### **2.4.1.2 Nonclinical Test Materials**

Preclinical mRNA-1273, mRNA-1273.167, mRNA-1273.815, and mRNA-1273.222 vaccines used in these studies were prepared with the same method as the Good Manufacturing Practice mRNA-1273 clinical Drug Products.

mRNA-1273 vaccine (monovalent) contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the Wuhan-Hu-1 (ancestral) strain. mRNA-1273.167 vaccine (monovalent) contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the JN.1 subvariant of Omicron. mRNA-1273.815 vaccine (monovalent) contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of Omicron XBB.1.5 and was authorized in September 2023. mRNA-1273.222 vaccine (bivalent) is a coformulation of mRNA-1273 (ancestral) and mRNA-1273.045 (contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the BA.4/BA.5 subvariants of Omicron) and was authorized in September 2022. 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).

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 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 [REDACTED], 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 JN.1-containing mRNA vaccine (mRNA-1273.167) for the 2024-2025 season given the dominance of JN.1 and its antigenically related subvariants, which are predicted to be similarly neutralized by JN.1 vaccine elicited antibodies. To support this, nonclinical in vivo pharmacology studies were conducted in BALB/c mice. These studies evaluated immunogenicity of mRNA-1273.167 given as a primary series, or as a booster dose following primary series vaccination with mRNA-1273. Additionally, the immunogenicity of mRNA-1273.167 as 5<sup>th</sup> booster was evaluated in BALB/c mice previously immunized with mRNA-1273 vaccines to allow assessment of updated boosters where prior immunity is more diverse based on exposure to multiple strain antigens. Such studies are in line with regulatory expectations to generate preclinical immunogenicity data that supports 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 hamsters, and rhesus macaques (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), 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 the 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 JN.1-containing vaccine. These pharmacology results are fully summarized in .

**Table 1: Summary of Pharmacology Program for JN.1-containing Vaccines**

Study Type/Description	Test Article Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
<b>Primary Pharmacology</b>					
Evaluation of immunogenicity of a primary series of a monovalent SARS-CoV-2 JN.1 containing mRNA-1273 vaccine in mice	PBS: 0 mRNA-1273.815 <sup>a</sup> and mRNA-1273.167 <sup>b</sup> : 1	Mouse, BALB/c	IM; Day 1, 22 (primary series)	No	
Evaluation of immunogenicity of monovalent SARS-CoV-2 JN.1 containing vaccine boosters in mice	MOD-6560: PBS control: 0 mRNA-1273 <sup>c</sup> : 0.5 (primary series) mRNA-1273.815 <sup>a</sup> and mRNA-1273.167 <sup>b</sup> : 1.0 (booster)	Mouse, BALB/c	IM; Day 1, 22 (primary series) Day 54 (booster)	No	
	MOD-6094: PBS control: 0 mRNA-1273 <sup>c</sup> : 0.5 (primary series [Dose 1 and Dose 2]) mRNA-1273.222 <sup>d</sup> : 0.5 (booster [Dose 3]) mRNA-1273.815 <sup>a</sup> : 0.5 (booster [Dose 4]) mRNA-1273.815 <sup>a</sup> and mRNA-1273.167 <sup>b</sup> : 1.0 (booster [Dose 5])		IM; Day 1, 22 (primary series [Dose 1 and Dose 2]) Day 106 (booster [Dose 3]) Day 187 (booster [Dose 4]) Day 210 (booster [Dose 5])		

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.

<sup>a</sup> mRNA-1273.815 is a monovalent vaccine that contains a single mRNA that encodes the PS-2P antigen of the XBB.1.5/XBB.1.9.1 subvariants of Omicron. The spike protein of XBB.1.9.1 is identical to that of XBB.1.5.

<sup>b</sup> mRNA-1273.167 vaccine (monovalent) contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the JN.1 subvariant of Omicron.

<sup>c</sup> mRNA-1273 is a monovalent vaccine that contains a single mRNA that encodes the S-2P antigen of the Wuhan-Hu-1 isolate of SARS-CoV-2.

<sup>d</sup> mRNA-1273.222 is a coformulation of the mRNA-1273 and mRNA-1273.045 vaccines.



The results from the primary pharmacology studies with the JN.1-containing mRNA vaccine (mRNA-1273.167) in mice are summarized as follows:

- After a 2-dose primary series, mRNA-1273.167 elicited high bAb titers (S-2P IgG), indicating a strong immunological response. When assessed using the Sponsor's VSV-based PSVN assay, mice that received mRNA-1273.167 neutralized JN.1 and KP.2 variants, with high titers measured against both variants. As expected, given the significant antigenic differences between JN.1 and XBB.1.5, lower neutralization titers measured against the XBB.1.5. By contrast, mRNA-1273.815 drives high levels of neutralization against XBB.1.5, with low neutralization against both JN.1 and KP.2.
- After a 2-dose primary series with mRNA-1273, boosting (Dose 3) with mRNA-1273.167 elicited S-2P IgG bAb responses comparable to boosting with mRNA-1273.815. When assessed using the Sponsor's VSV-based PSVNA, mice boosted (Dose 3) with mRNA-1273.167 effectively neutralized JN.1 and cross-neutralized KP.2, an antigenically related strain, with lower neutralization titers measured against the antigenically distant strain XBB.1.5. In contrast, mice boosted with mRNA-1273.815 elicited the highest titers versus XBB.1.5, with lower neutralization measured against JN.1 and KP.2.
- In mice that had previously received a primary series with mRNA-1273 (Dose 1 and 2) and boosters with mRNA-1273.222 (Dose 3) and mRNA-1273.815 (Dose 4), a 5<sup>th</sup> booster (Dose 5) with mRNA-1273.167 further increased nAb titers against JN.1 (2.5-fold) and KP.2 (1.7-fold), but not against XBB.1.5. In contrast, boosting with mRNA-1273.815 as a 5<sup>th</sup> dose did not further increase titers against JN.1, KP.2, or XBB.1.5, as the high pre-Dose 5 XBB.1.5 titers likely prevented additional boosting.

Overall, the test articles were well tolerated, and animal health monitoring did not reveal any adverse findings. The results of these studies not only demonstrate the immunogenicity of the JN.1 new variant vaccine (mRNA-1273.167), but also indicates the relative antigenic distance between the XBB and JN.1 variants, as mRNA-1273.167 neutralized matched (JN.1) or similar strain (KP.2) robustly with lower neutralization titers measured against XBB.1.5. Similarly, mRNA-1273.815 neutralized matched strain (XBB.1.5) robustly, with lower neutralization measured against JN.1 and KP.2. These data support the potential of a JN.1 monovalent formulation in driving increased immunogenicity against JN.1 as well as closely related subvariants such as KP.2.

### 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 (ie, 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  $\beta$ -oxidative metabolites cleared via both the renal and hepatic routes of elimination. These ester hydrolysis and  $\beta$ -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
<b>Biodistribution</b>					
Single-dose tissue distribution study	mRNA-1647 <sup>a</sup>	Rat, Sprague Dawley	IM injection dose of 100 µg on Day 1	No	5002121 Amendment 2
<b>Metabolite Identification</b>					
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.

<sup>a</sup> 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. 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
<b>Repeat-Dose Toxicity</b>					
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706 <sup>a</sup>	Rat, Sprague Dawley	IM; 0, 13, 65, 129 µg/dose <sup>b</sup> (Days 1, 15, 29)	Yes	5002045
1-month (3 doses) repeat-dose study with 2-week recovery	mRNA-1706 <sup>a</sup>	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 <sup>c</sup>	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 <sup>d</sup>	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 <sup>e</sup>	Rat, Sprague Dawley	IM; 0, 8.9, 27, 89 µg/dose <sup>f</sup> (Days 1, 15, 29, 43)	Yes	5002034
6-week (4 doses) repeat-dose study with 2-week recovery	mRNA-1443 <sup>g</sup>	Rat, Sprague Dawley	IM; 0, 9.6, 29, 96 µg/dose <sup>h</sup> (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
<b>In Vitro Genotoxicity</b>					
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 <sup>®</sup> GM-020) <sup>i</sup>	<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) <sup>i</sup>	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
<b>In Vivo Genotoxicity</b>					
In vivo mammalian erythrocyte micronucleus test	mRNA-1706 <sup>a</sup>	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 <sup>j, k</sup>	Yes	9800399
In vivo mammalian erythrocyte micronucleus test	NPI luciferase mRNA <sup>l</sup>	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.125012 NGLPICH.BTL
<b>Reproductive and Developmental Toxicity</b>					
Combined developmental and perinatal/postnatal reproductive toxicity study	mRNA-1273 <sup>m</sup>	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
<b>Other Toxicology</b>					
5-week (2 doses) repeat-dose immunogenicity and toxicity study	mRNA-1273 <sup>m</sup>	Rat, Sprague Dawley	IM; 0, 30, 60, 100 µg/dose (Days 1 and 22)	No	2308-123

Abbreviations: CMV=cytomegalovirus; F=female; DSPC=1,2-distearoyl-sn-glycero-3-phosphocholine; 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; PIV3=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=2019 novel coronavirus; SM-102=a custom-manufactured ionizable lipid; SoA=summary of analysis.

<sup>a</sup> 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.

<sup>b</sup> 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%.

- <sup>c</sup> 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.
- <sup>d</sup> 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.
- <sup>e</sup> 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.
- <sup>f</sup> 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%.
- <sup>g</sup> 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.
- <sup>h</sup> 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%.
- <sup>i</sup> 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.
- <sup>j</sup> 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.
- <sup>k</sup> 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.
- <sup>l</sup> 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.
- <sup>m</sup> 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 JN.1-containing mRNA vaccine (mRNA-1273.167) for the 2024-2025 season given the dominance of JN.1 and its antigenically related subvariants, which are predicted to respond similarly to functional antibodies that neutralize the virus. To support this, nonclinical in vivo pharmacology studies evaluated immunogenicity of mRNA-1273.167 given as a primary series, or as a booster dose following primary series vaccination with mRNA-1273. Additionally, the immunogenicity of mRNA-1273.167 as 5<sup>th</sup> booster was evaluated in BALB/c mice previously immunized with mRNA-1273 vaccines to allow assessment of updated boosters where prior immunity is more diverse based on exposure to multiple strain antigens.

In Study MOD-6764, the JN.1 new variant vaccine, administered as a primary series, elicited high titers against JN.1 and cross-neutralized the antigenically related JN.1 subvariant, KP.2. Notably, lack of cross-neutralization between XBB and JN.1 indicated that all immunity was solely provided by the 2-dose primary vaccination series and was specific to the strain delivered in the vaccine formulation. A small reduction in KP.2 neutralization in animals vaccinated with mRNA-1273.167 reflects the impact of the 3 mutations in KP.2 relative to JN.1. This was also noted in similar assessments of new variant vaccines in past studies where neutralization reduction from a small number of stepwise mutations found in subvariants of the vaccine strain could be captured in this primary immunization model but was not subsequently seen in humans with diverse immune experience from prior immunization and infections across a range of SARS-CoV-2 variants.

In Studies MOD-6560 and MOD-6094, the JN.1 new variant vaccine administered as a 3<sup>rd</sup> or 5<sup>th</sup> dose further increased nAb titers against JN.1 and KP.2, but not against XBB.1.5. The XBB.1.5 active control in these studies, the currently approved vaccine composition, by contrast drove highest titers against XBB.1.5 but elicited low responses against JN.1 and KP.2.

Across all studies, there was low cross-neutralization between JN.1 and XBB, demonstrating the antigenic differences between these strains. Notably, any small differences in titers against JN.1 and KP.2 neutralization seen following the 2-dose primary series with mRNA-1273.167 was further diminished in the booster studies, likely due to the impact of more diverse immune background elicited by prior vaccinations. The robust immunity in humans derived from multiple vaccinations and/or infection is similarly likely to drive much greater resistance to escape from such stepwise viral evolution.

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 a JN.1-containing vaccine.

Overall, these data showing strong cross-neutralization between JN.1 and closely related sublineage KP.2 by a JN.1-containing vaccine, corroborate WHO TAG-CO-VAC's and EMA's ETF April 2024 recommendations ([EMA 2024](#); [WHO 2024](#)) of the use of a monovalent JN.1 lineage antigen in vaccines to enhance vaccine-induced immune responses to circulating SARS-CoV-2 variants specifically those evolving from JN.1. In prior seasons, similar nonclinical



results have supported the strain recommendation and approvals of BA.4/BA.5 bivalent vaccine and XBB.1.5 monovalent vaccine updates, and upon approval and launch of the new variant vaccines real world evidence as well as clinical immunogenicity results have aligned closely with new variant animal studies results.

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Data up to date as of 12 June 2025

## 2.4.6 REFERENCES

European Medicines Agency. EMA recommendation to update the antigenic composition of authorized COVID-19 vaccines for 2024-2025. [Internet] Amsterdam: European Medicines Agency; 2024 Apr 30 [cited 2024 Apr 30]. Available from: [https://www.ema.europa.eu/en/documents/other/ema-recommendation-update-antigenic-composition-authorized-covid-19-vaccines-2024-2025\\_en.pdf](https://www.ema.europa.eu/en/documents/other/ema-recommendation-update-antigenic-composition-authorized-covid-19-vaccines-2024-2025_en.pdf)

International Conference on Harmonisation (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). 09 November 2011 [cited 2022 May 26]. Available from: <https://database.ich.org/sites/default/files/S2%28R1%29%20Guideline.pdf>.

World Health Organization. Coronavirus disease (COVID-19) pandemic: update on COVID-19 variants. [Internet] Geneva: World Health Organization; 2023 Dec 18 [cited 2023 Dec 20]. Available from: [https://www.who.int/docs/default-source/coronaviruse/18122023\\_jn.1\\_ire\\_clean.pdf?sfvrsn=6103754a\\_3](https://www.who.int/docs/default-source/coronaviruse/18122023_jn.1_ire_clean.pdf?sfvrsn=6103754a_3)

World Health Organization. Statement on the antigen composition of COVID-19 vaccines. [Internet] Geneva: World Health Organization; 2024 Apr 16 [cited 2024 Apr 28]. Available from: <https://www.who.int/news/item/26-04-2024-statement-on-the-antigen-composition-of-covid-19-vaccines>