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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)
BLA	Biologics License Application
CMV	cytomegalovirus
COVID-19	coronavirus disease 2019
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
ERD	enhanced respiratory disease
EUA	Emergency Use Authorization
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
IDR	identity and ratio
IM	intramuscular(ly)
IV	intravenous(ly)
LNP	lipid nanoparticle
MERS-CoV	Middle East respiratory syndrome coronavirus
nAb	neutralizing antibody
NHP	nonhuman primate
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PIV3	parainfluenza virus type 3
PK	pharmacokinetic(s)
PSVNA	pseudotyped virus neutralization assay
RBD	receptor-binding domain
S	spike
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
UTR	untranslated region
VOC	variant of concern

Abbreviation	Definition
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)
XBB.1.16	subvariant of Omicron

## 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)'s scalable mRNA/LNP technology platform allowed for a rapid response to the 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 18 years of age and older.

Starting in the Summer of 2021, an increase in COVID-19 cases was observed in many populations around the world, including those who had been vaccinated, due to the emergence of the Delta variant (B.1.617.2) of SARS-CoV-2. This variant of SARS-CoV-2 was shown to have increased pathogenicity as compared to prior variants ([Barouch 2022](#); [Siddle et al 2022](#); [Twohig et al 2022](#)). Subsequently, in the Fall of 2021, multiple regulatory bodies worldwide authorized a booster vaccine for use in adults following the observed waning of effectiveness after primary series immunization. Administration of a first booster dose increased the antibody responses against variants and improved vaccine effectiveness against the Delta variant.

In November 2021, the Omicron variant (B.1.1.529; BA.1) emerged as the most antigenically divergent variant to date with > 30 mutations in the spike protein ([Hastie et al 2021](#)). Soon after its emergence, the Omicron variant rapidly became dominant worldwide, driving a wave of infections and COVID-19 disease. The 2-dose mRNA-1273 had been shown to be effective against COVID-19 and hospitalization due to COVID-19 caused by SARS-CoV-2 variants, including Alpha, Beta, Delta, and Gamma. However, the effectiveness of the original vaccine formulations appeared to be reduced with respect to infections with the Omicron variant ([Bruxvoort et al 2021](#); [Tseng et al 2022](#)).

The morbidity and mortality associated with COVID-19 caused by antigenically divergent variants such as Omicron and the decreased effectiveness of mRNA-1273 against Omicron infection created the need to develop a booster with enhanced immunogenicity to improve protection against COVID-19 and help decrease the burden on hospitals and healthcare systems ([Gilbert et al 2022](#); [Khoury et al 2021](#)). On 31 Aug 2022, the US FDA amended the mRNA-1273 EUA to authorize an Omicron-containing (BA.4/BA.5) bivalent formulation of mRNA-1273 (ie, mRNA-1273.222) for use as a single booster dose at least 2 months following primary or booster vaccination. Other agencies worldwide authorized the Omicron BA.1-containing bivalent formulation of mRNA-1273 (ie, mRNA-1273.214) and subsequently the Omicron BA.4/BA.5-containing bivalent formulation throughout the second half of 2022. Authorizations for both bivalent boosters were based on preclinical and clinical data demonstrating superior

immune responses to the variant in the vaccine compared with the original mRNA-1273 booster, a noninferior antibody response to SARS-CoV-2 (D614G), and a similar safety profile as mRNA-1273 (Chalkias et al 2022a; Chalkias et al 2022b).

The SARS-CoV-2 virus is continually evolving by accumulating mutations, some which offer a significant growth advantage, leading to establishment of VOC strains that become dominant in circulation. The emergence of the Omicron variant was a significant evolutionary shift where an unprecedented number of mutations were observed that enabled significant immune escape against immunity provided by prototype vaccines and/or immunity provided by infection with SARS-CoV-2 strains prior to Omicron. Although the development and deployment of Omicron-containing vaccine boosters, specifically mRNA-1273.214 (BA.1-containing bivalent) and mRNA-1273.222 (BA.4/BA.5-containing bivalent), substantially enhanced protection against the early Omicron strains, the Omicron virus family has continued to rapidly evolve. New subvariants have emerged in early 2023 (eg, XBB.1.5, XBB.1.9.1, and XBB.1.16) with additional growth advantages, increased transmissibility, and the ability to escape authorized BA.1- or BA.4/BA.5-containing bivalent booster vaccine- or infection-derived immunity. Given the evident immune escape that these new variants exhibit to current vaccines, updating vaccine strain compositions to match such strains more closely is critical to maintaining protection against COVID-19.

The complex nature of SARS-CoV-2's continuing evolution 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. A framework to identify VOCs and 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.

The Sponsor's ongoing monitoring and variant response effort has enabled the development and preclinical evaluation of more than 19 monovalent and bivalent vaccine compositions. For example, based on real-time monitoring efforts, the Sponsor initiated the development and evaluation process for variants including XBB.1, BQ.1.1, and BN.1 in early Fall of 2022, and in early 2023, CH.1.1, XBB.1.5/1.9.1, and XBB.1.16 (among others). Additionally, from these analyses, antigenically similar variants are categorized into subvariant or "subfamily" groups, where the majority of these antigenic regions are the same except for a small number of additional mutations that are not predicted to significantly impact antibody neutralization. For example, based on these assessments, the Sponsor has categorized XBB, BA.2.75, and BA.5 as distinct subfamilies. These subfamilies are predicted to respond similarly to functional antibodies that neutralize the virus. This has been corroborated by assessments of viral neutralization by sera from BA.4/BA.5 bivalent-boosted individuals from Study mRNA-1273-P205 Part H (Study P205H), in which study participants that had received 3 prior doses of mRNA-1273 were boosted with mRNA-1273.222. A subset of sera from Study P205H were tested using the Sponsor's internal, nonvalidated PSVNA, assessing neutralization against XBB.1, XBB.1.5, and XBB.1.16. All 3 XBB subvariants were neutralized similarly, likely due to antigenic similarity in their RBD. This subfamily matching approach also indicates that it is possible to reliably predict

the immunogenicity of a variant antigen from neutralization studies using sera from animals immunized with another variant antigen from the same subfamily. For instance, the neutralization titers of sera from XBB.1.5-immunized mice against XBB.1.16 would be expected to reliably predict similar titers from XBB.1.16-immunized mice.

The Sponsor has recently evaluated multiple XBB-containing vaccines concurrently, including the following: 1) the monovalent mRNA-1273.815 vaccine that contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the XBB.1.5/XBB.1.9.1 (note: the spike protein of XBB.1.9.1 is identical to that of XBB.1.5) subvariant of Omicron; (2) the monovalent mRNA-1273.116 vaccine that contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the XBB.1.16 subvariant of Omicron; (3) the bivalent mRNA-1273.231 vaccine, which is a coformulation of the mRNA-1273.045 vaccine (a monovalent vaccine containing a single mRNA encoding the SARS-CoV-2 S-2P antigen of the BA.4/BA.5 subvariants of Omicron) and the mRNA-1273.815 vaccine, and (4) the bivalent mRNA-1273.234 vaccine, which is a coformulation of the mRNA-1273.045 vaccine and the mRNA-1273.116 vaccine. All mRNAs are formulated into a mixture of 4 lipids: SM-102, cholesterol, DSPC, and PEG2000-DMG.

Nonclinical studies conducted with XBB.1.5- and XBB.1.16-containing vaccines are herein summarized to support registration of an XBB-containing vaccine for the 2023-2024 season.

#### 2.4.1.2 Nonclinical Test Materials

Preclinical mRNA-1273, mRNA-1273.045, 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, mRNA-1273.045, mRNA-1273.815, and mRNA-1273.222 clinical Drug Products. Preclinical material for mRNA-1273.116 was prepared with the representative manufacturing process conditions for mRNA-1273.116, with the exception of the IDR sequence present in the 3' UTR. The Sponsor routinely includes IDR sequences of up to 25 nucleotides in the 3' UTR to facilitate analytical detection. Because UTR regions are noncoding and therefore will not be translated into proteins, these modifications have no impact on the quality attributes, stability profile, functional activity, or safety of the mRNA product. The Sponsor has performed extensive in vitro and in vivo equivalence studies to confirm this.

mRNA-1273 encodes the S-2P antigen of the Wuhan-Hu-1 isolate of SARS-CoV-2, mRNA-1273.045 encodes the S-2P antigen of the SARS-CoV-2 Omicron BA.4/BA.5 subvariants (note: the spike protein of BA.5 is identical to that of BA.4), mRNA-1273.815 encodes the S-2P antigen of the SARS-CoV-2 Omicron XBB.1.5/XBB.1.9.1 subvariants (note: the spike protein of XBB.1.9.1 is identical to that of XBB.1.5), and mRNA-1273.116 encodes the S-2P of the SARS-CoV-2 Omicron XBB.1.16 subvariant. All vaccines include 2 proline mutations introduced to stabilize the spike protein into the prefusion conformation. The mRNA-1273.231 and mRNA-1273.234 vaccines included mRNA-1273.045 combined with mRNA-1273.815 or mRNA-1273.116, respectively, in a 1:1 ratio of separately formulated mRNA vaccines to generate a bench side mix. mRNA-1273.222 is a coformulation of the mRNA-1273 and mRNA-1273.045 vaccines. mRNA-1273.045 and mRNA-1273.222 were used as active controls in the preclinical studies and mRNA-1273 was given as a primary series. All vaccines were formulated into a mixture of 4 lipids: SM-102, cholesterol, DSPC, and PEG2000-DMG.

The preclinical monovalent mRNA-1273.045 vaccine was based on sequence -GISAID: EPI\_ISL\_12548717 - and encoded the following substitutions from the original mRNA-1273 vaccine: T19I, L24-, P25-, P26-, A27S, H69-, V70-, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K.

The preclinical monovalent mRNA-1273.815 vaccine was based on sequence -GISAID: EPI\_ISL\_16134259 - and encoded the following substitutions from the original mRNA vaccine: T19I, L24-, P25-, P26-, A27S, V83A, G142D, Y144-, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486P, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K.

The preclinical monovalent mRNA-1273.116 vaccine was based on sequence - GenBank: OQ931660, GISAID: EPI\_ISL\_17619088 – and encoded the following substitutions from the original mRNA-1273 vaccine: T19I, L24-, P25-, P26-, A27S, V83A, G142D, Y144-, H146Q, E180V, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478R, E484A, F486P, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K.

All vaccines were encapsulated in an LNP through a modified ethanol-drop nanoprecipitation process as previously described ([Hassett et al 2019](#)). Briefly, ionizable, structural, helper, and polyethylene glycol lipids were mixed with mRNA-1273 in acetate buffer, pH 5.0, at a ratio of 2.5:1 (lipids: mRNA). The mixture was neutralized with tris(hydroxymethyl)aminomethane hydrochloride, pH 7.5, sucrose was added as a cryoprotectant, and the final solution was sterile-filtered. Vials were filled with formulated LNP and stored frozen at -20°C until further use. The preclinical vaccine product underwent analytical characterization, which included the determination of particle size and polydispersity, encapsulation, mRNA purity, double-stranded RNA content, osmolality, pH, endotoxin, and bioburden, and the material was deemed acceptable for the in vivo study ([Corbett et al 2020a](#)).

Details of the nonclinical test materials used in the pharmacokinetic and toxicology studies are described in Module 2.6.4 and Module 2.6.7, respectively.

### 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).

In support of the development of XBB-containing mRNA vaccines for the 2023-2024 season, nonclinical in vivo pharmacology studies were conducted in BALB/c mice. These studies evaluated immunogenicity of XBB-containing mRNA vaccines given as a primary series, or as a booster dose following primary series vaccination with mRNA-1273.

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

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 [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.



## 2.4.2 PHARMACOLOGY

Nonclinical primary pharmacology evaluations supporting 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 2020b), 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 BLA mRNA-1273 Module 2.4 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 XBB-containing vaccines. These pharmacology results are fully summarized in Module 2.6.2.

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

Study Type/Description	Test Article Dose	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
<b>Primary Pharmacology</b>					
Evaluation of Immunogenicity of a Primary Series of Monovalent and Bivalent SARS-CoV-2 XBB.1.5-containing Vaccines in Mice	PBS control 0 mRNA-1273.045 <sup>a</sup> mRNA-1273.815 <sup>b</sup> mRNA-1273.222 <sup>c</sup> mRNA-1273.231 <sup>d</sup> 1 µg (primary series)	Mouse, BALB/c	IM; Day 1, 22 (primary series)	No	<a href="#">MOD-6037</a>
Evaluation of Immunogenicity of Monovalent and Bivalent SARS-CoV-2 XBB.1.5-containing Vaccine Boosters in Mice	PBS control 0 mRNA-1273 <sup>e</sup> 0.5 µg (primary series)  PBS control 0 mRNA-1273.045 <sup>a</sup> mRNA-1273.815 <sup>b</sup> mRNA-1273.222 <sup>c</sup> mRNA-1273.231 <sup>d</sup> 1.0 µg (booster)	Mouse, BALB/c	IM; Day 1, 22 (primary series [Dose 1 and Dose 2])  IM; Day 92 (booster [Dose 3])	No	<a href="#">MOD-5827</a>

Study Type/Description	Test Article Dose	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Evaluation of Immunogenicity of Monovalent and Bivalent SARS-CoV-2 XBB.1.16-containing Vaccine Boosters in Mice	PBS control 0 mRNA-1273 <sup>e</sup> 0.5 µg (primary series)  mRNA-1273.116 <sup>f</sup> mRNA-1273.234 <sup>g</sup> 1.0 µg (booster)	Mouse, BALB/c	IM; Day 1, 22 (primary series [Dose 1 and Dose 2])  IM; Day 71 (booster [Dose 3])	No	<a href="#">MOD-5972</a>

Abbreviations: GLP=good laboratory practice; IM=intramuscular; 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-1273.045 is a monovalent vaccine that contains a single mRNA that encodes the S-2P antigen of the BA.4/BA.5 subvariants of Omicron. The spike protein of BA.5 is identical to that of BA.4.
- <sup>b</sup> mRNA-1273.815 is a monovalent vaccine that contains a single mRNA that encodes the S-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>c</sup> mRNA-1273.222 is a coformulation of the mRNA-1273 and mRNA-1273.045 vaccines.
- <sup>d</sup> mRNA-1273.231 is a 1:1 bench side mix of separately formulated mRNA-1273.045 and mRNA-1273.815 vaccines.
- <sup>e</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>f</sup> mRNA-1273.116 is a monovalent vaccine that contains a single mRNA that encodes the S-2P antigen of the XBB.1.16 subvariant of Omicron.
- <sup>g</sup> mRNA-1273.234 is a 1:1 bench side mix of separately formulated mRNA-1273.045 and mRNA-1273.116 vaccines.

### 2.4.2.1 Primary Pharmacology

In support of the development of XBB-containing mRNA vaccines for the 2023-2024 season, nonclinical in vivo pharmacology studies were conducted in BALB/c mice. These studies evaluated immunogenicity of XBB-containing mRNA vaccines given as a primary series or as a booster dose following primary series vaccination with mRNA-1273. The results are summarized as follows:

- In a non-GLP immunogenicity study using BALB/c mice, after a 2-dose primary series, monovalent mRNA-1273.815 and bivalent mRNA-1273.231 elicited robust S-2P bAb titers and potently neutralized the XBB subfamily strains XBB.1.5 and XBB.1.16, with neutralization titers that were >45-fold higher than those elicited by monovalent mRNA-1273.045 or bivalent mRNA-1273.222. mRNA-1273.231 had numerically higher titers against the ancestral and BA.4/BA.5 strains compared with mRNA-1273.815, consistent with the inclusion of BA.4/BA.5 in the bivalent vaccine. All vaccines elicited nAb titers against XBB.1.5 that were similar to nAb titers against XBB.1.16.

- In a non-GLP immunogenicity study using BALB/c mice, administering monovalent mRNA-1273.815 or bivalent mRNA-1273.231 as a booster following a primary series of mRNA-1273 elicited robust S-2P bAb titers and high nAb responses against the XBB subfamily strains XBB.1.5 and XBB.1.16, with mRNA-1273.815 driving higher nAb responses than mRNA-1273.231. mRNA-1273.231 had numerically higher titers against ancestral and BA.4/BA.5 strains compared with mRNA-1273.815, consistent with the inclusion of BA.4/BA.5 in the bivalent vaccine. nAb titers against XBB.1.5 and XBB.1.16 were also comparable in this booster study.
- In a non-GLP immunogenicity study using BALB/c mice, administering monovalent mRNA-1273.116 or bivalent mRNA-1273.234 as a booster following a primary series of mRNA-1273 elicited robust S-2P bAb titers as well as neutralizing titers against XBB subfamily strains XBB.1.16 and XBB.1.5. nAb titers against XBB.1.16 were comparable to those against XBB.1.5.

Overall, animals vaccinated with XBB-containing vaccines (mRNA-1273.815, mRNA-1273.116, mRNA-1273.231, and mRNA-1273.234), either as a primary series or as a booster dose, elicited robust S-2P bAb titers and potentially neutralized both XBB.1.5 and XBB.1.16 similarly. The results suggest that such XBB-containing vaccines are likely to substantially boost protection against XBB subfamily strains including XBB.1.5, XBB.1.9.1, or XBB.1.16. The results also collectively support the subfamily approach proposed by the Sponsor, wherein preclinical immunogenicity studies performed with an XBB strain support registration of a vaccine with strains within the subfamily due to their antigenic similarity. This approach may enable more rapid deployment of future variant matched vaccines based on preclinical data from closely matched subfamily variants generated during routine monitoring.

### 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.

**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; PEG2000-DMG=1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000.

<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.

**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; CoV=coronavirus; 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; 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; 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

Nonclinical pharmacology studies of variant-containing mRNA vaccines are clinically tolerated and support the clinical efficacy and safety of these vaccines to be administered in response to emerging VOCs.

Overall, animals vaccinated with XBB-containing vaccines (mRNA-1273.815, mRNA-1273.116, mRNA-1273.231, and mRNA-1273.234), either as a primary series or as a booster dose, elicited robust S-2P bAb titers and potently neutralized both XBB.1.5 and XBB.1.16 similarly. The results suggest that such XBB-containing vaccines are likely to substantially boost protection against XBB subfamily strains including XBB.1.5, XBB.1.9.1, or XBB.1.16. The results also collectively support the subfamily approach proposed by the Sponsor, wherein preclinical immunogenicity studies performed with an XBB strain support registration of a vaccine with strains within the subfamily due to their antigenic similarity. This approach may enable more rapid deployment of future variant matched vaccines based on preclinical data from closely matched subfamily variants generated during routine monitoring.

Given that there were no new safety concerns observed with XBB-containing vaccines in nonclinical pharmacology studies, 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 XBB-containing vaccines.

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