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List of Abbreviations

Abbreviation	Definition
ACE-2	angiotensin converting enzyme 2
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
hMPV	human metapneumovirus
ICH	International Council for Harmonisation
ID ₅₀	infectious dose 50
IgG	immunoglobulin G
IM	intramuscular(ly)
IV	intravenous(ly)
LN	lymph node(s)
LNP	lipid nanoparticle
MERS-CoV	Middle-East respiratory syndrome coronavirus
mRNA	messenger RNA
NHP	nonhuman primate
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PBS	phosphate-buffered saline
PEG	polyethylene glycol
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PIV3	parainfluenza virus type 3
PK	pharmacokinetic(s)
S	spike
S-2P	spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
S-2P.529	Omicron-specific S-2P
SARS-CoV	severe acute respiratory syndrome coronavirus
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2

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Abbreviation	Definition
SM-102	a custom-manufactured ionizable lipid
Th	T helper
TIC	total ion current
WT	wild type
VE	vaccine efficacy
VOC	variant of concern

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2.4.1 OVERVIEW OF NONCLINICAL TESTING STRATEGY

2.4.1.1 Background

Coronaviruses are a large family of viruses that cause illness ranging from the common cold to more severe diseases, such as MERS-CoV and SARS-CoV. 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 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.

Evidence suggests that by 6 months following vaccination with the primary series, waning immunity against ancestral SARS-CoV-2 is evident and neutralization titers against VOCs are low or undetectable (Choi et al 2021). Real-world studies have shown a decline in vaccine efficacy over time, particularly against VOCs (Bruxvoort et al 2021; Puranik et al 2021; Tseng et al 2021). An analysis of data from the Phase 3 Study P301 (NCT04470427) during the Delta variant surge (July to August 2021) showed lower incidence rates of COVID-19 in participants more recently vaccinated than those more remotely vaccinated (Baden et al 2021). Participants with a median follow-up of 7.9 months had lower rates of breakthrough infection (49.0/1000 person years) compared to participants with a median follow-up time of 13 months (77.1/1000 person-years). Accordingly, the Sponsor assessed the immunogenicity of a 50-µg booster of mRNA-1273 in participants previously primed with 2 doses of mRNA-1273. Results showed a robust immune response, and mRNA-1273 was subsequently authorized as a 50-µg single booster dose in individuals 18 years of age and older. Health agencies have also authorized mRNA-1273 to be given as a second booster in certain high-risk populations across multiple regions.

In November 2021, the World Health Organization reported a new VOC, Omicron (BA.1, also known as B.1.1.529), that was detected in South Africa and quickly spread in the US, becoming the dominant circulating variant (WHO 2021). Available evidence suggests that the BA.1 variant has transmission advantage over prior variants, with significant antigenic change and a potential growth advantage. In addition, it contains antibody escape site mutations (such as K417N, T478K, E484A, N501Y). The BA.1 variant contains more than 30 amino acid substitutions. Additional sub-lineages of Omicron have also emerged with one, BA.2, demonstrating increased transmissibility versus BA.1 which subsequently became the predominant circulating variant in most geographical regions. As of May 2022, Omicron sub-lineages BA.4 and BA.5 have been identified and are increasing in circulation in certain geographical regions.

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A test-negative case-control analysis conducted at Kaiser Permanente Southern California using 6,657 SARS-CoV-2 positive specimens collected between 06 Dec 2021 and 23 Dec 2021 showed the 2-dose VE of mRNA-1273 against Omicron infection was 44.0% at 14 to 90 days after the second dose and declined quickly thereafter (Tseng et al 2022). The analysis also assessed the 3-dose VE of mRNA-1273 and found that VE was high (93.7% and 86.0%) against the Delta variant but lower (71.6% and 47.4%) against Omicron infection at 14 to 60 days and >60 days respectively. Additionally, an evaluation of BA.1 neutralization (ID₅₀) in serum samples of participants who received the mRNA-1273 primary series detected Omicron neutralization in 85% of participants 1 month after the primary series, but titers observed were 35-fold lower than titers against the ancestral SARS-CoV-2 with the D614G mutations (hereafter referred to as WA1 D614G) (Pajon et al 2022). Seven months after the primary series and before a booster dose, Omicron neutralization was detected in 55% of participants and the ID₅₀ titers were 8.4-fold lower than titers against WA1 D614G. A booster dose of mRNA-1273, however, was associated with neutralization titers against Omicron that were 20-fold higher than titers against Omicron at 1 month after the second dose of the primary series.

Even with the availability of a 50-μg mRNA-1273 booster, the evolving antigenic variation of SARS-CoV-2 underscores the need for vaccination strategies that induce broader protection, specifically against VOC with increased risk of viral escape. Variant-matched booster vaccines have been suggested as a strategy to focus the antibody response against VOCs compared to the authorized, standard-of-care booster vaccines against COVID-19.

2.4.1.2 Nonclinical Test Materials

The preclinical mRNA-1273 and mRNA-1273.529 Drug Products used in the pharmacology studies were mRNA formulations prepared with the same method as the Good Manufacturing Practice mRNA-1273 and mRNA-1273.529 clinical Drug Products. mRNA-1273 is a monovalent vaccine that contains a single mRNA (CX-024414) that encodes WT SARS-CoV-2 S-2P. mRNA-1273.529 is a monovalent vaccine that contains a single mRNA (CX-031302) that encodes SARS-CoV-2 S-2P for BA.1 (S-2P.529). Both mRNA-1273 and mRNA-1273.529 include 2 proline mutations introduced to stabilize the S protein into the prefusion conformation. mRNA-1273.214 is a bivalent vaccine that contains 2 mRNAs: mRNA-1273 (CX-024414) and mRNA-1273.529 (CX-031302) in a 1:1 ratio. All vaccines were formulated into a mixture of 4 lipids: SM-102, cholesterol, DSPC, and PEG2000-DMG.

The preclinical mRNA-1273.529 vaccine encoded the following substitutions: A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F.

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 PEG lipids were mixed with mRNA-1273 in acetate buffer, pH 5.0, at a ratio of 2.5:1

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(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 2020b](#)).

A noncoding mRNA (UNFIX-01) or PBS was used as a negative control. The UNFIX-01 was synthesized and similarly formulated into LNPs as previously described ([Corbett et al 2020a](#); [Corbett et al 2020b](#)). The UNFIX-01 contains a short mRNA sequence formulated into the same LNP dispersion as mRNA-1273. After delivery into cells, this mRNA is not translated into protein.

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 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 mRNA-1273.214, nonclinical pharmacology evaluations were conducted in mice (BALB/c, K18-hACE2, and 129S2 strains) and NHPs (rhesus macaques) to evaluate the immunogenicity, antigen-specific B cell responses, and protection from Omicron challenge after administration of mRNA-1273 or Omicron-matched vaccines as primary series (mRNA-1273, mRNA-1273.529, or mRNA-1273.214) with or without boosting with mRNA-1273, mRNA-1273.529, or mRNA-1273.214. Additionally, the potential for vaccine-associated ERD after viral challenge was further evaluated in NHPs.

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

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Mice are a pharmacologically relevant model to assess vaccine immunogenicity, and protection results are important predictors of vaccine performance in NHP and human studies. In the challenge evaluations, K18-hACE2 mice were challenged with WA1 D614G (described previously in [Plante et al 2021](#)) or BA.1 isolate (hCoV-19/USA/WI WSLH 221686/2021). The 129S2 mice were challenged with the WA1 2020/N501Y/D614G variant as the substitution of N501Y enables engagement of murine ACE2 and productive infection of conventional strains of laboratory mice ([Gu et al 2020](#); [Liu et al 2021](#); [Rathnasinghe et al 2021](#); [Ying et al 2022](#)).

Nonhuman primates are the species most closely related to humans and have previously recapitulated several important aspects of SARS-CoV infection ([Lu et al 2020](#)). Although SARS-CoV-2 infection in NHPs result only in mild clinical symptoms, infection does cause illness with evidence of pneumonia ([Johansen et al 2020](#)). To assess protection, NHPs were challenged with SARS-CoV 2-Omicron ([Gagne et al 2022](#)).

A ‘platform concept’ strategy has been employed by the Sponsor to support mRNA-1273.214, 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-1273.214, 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 mRNA-1273.214 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 mRNA-1273.214.

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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, 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; Corbett et al 2020a). 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 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 development of mRNA-1273.214. These pharmacology results are fully summarized in Module 2.6.2.

Table 1: Summary of Pharmacology Program for mRNA-1273.214

Study Type/Description	Test Article Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Evaluation of immunogenicity of Omicron-matched mRNA vaccines as primary series in mice	PBS control, mRNA-1273, mRNA-1273.529, mRNA-1273.214 1	Mouse, BALB/c	IM; Day 1, 22	No	MOD-5156
Evaluation of immunogenicity and antigen-reactive B cell response of Omicron-matched mRNA vaccine boosters in mice	mRNA-1273, mRNA-1273.529, mRNA-1273.214 0.25	Mouse, BALB/c	IM; Day 1, 22 (primary series) Day 50 (booster 1) Day 78 (booster 2)	No	MOD-5019

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Study Type/Description	Test Article Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Primary series and booster studies in mice of mRNA-1273 and mRNA-1273.529 immunogenicity and protection from Omicron challenge	mRNA control, mRNA-1273 0.1, 5	Mouse, K18-hACE2	IM; Day 0, 21	No	WASHU-01-MOD-5020
	mRNA control, mRNA-1273 0.25, 5 (primary series) 1 (booster)	Mouse, K18-hACE2	IM; Day 0, 21 (primary series) Day 134/156 (booster)		
	PBS control, mRNA-1273, mRNA-1273.529 0.1, 1	Mouse, BALB/c	IM; Day 1, 22		
	mRNA control, mRNA-1273 5, 0.25 (primary series) mRNA control, mRNA-1273, mRNA-1273.529 1 (booster)	Mouse, 129S2	IM; Day 0, 21 (primary series) Day 98/99 (booster)		
mRNA-1273 primary series and mRNA-1273 versus mRNA-1273.529 booster regimen in a rhesus macaque SARS-CoV-2 Omicron challenge model	mRNA-1273 100 (primary series) mRNA-1273, mRNA-1273.529 50 (booster)	NHP, Rhesus macaque	IM; Week 0, 4 (primary series) Week 41 (booster)	No	VRC-20-857

Abbreviations: GLP = good laboratory practice; IM = intramuscular; mRNA = messenger RNA; NHP = nonhuman primate; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

2.4.2.1 Primary Pharmacology

In support of the development of mRNA-1273.214, the Sponsor has conducted nonclinical studies with mice and NHPs to test the immunogenicity, antigen-reactive B cell responses, and protection offered by a combination of mRNA-1273 or Omicron-matched vaccines as primary series (mRNA-1273, mRNA-1273.529, or mRNA-1273.214) with or without boosting with mRNA-1273, mRNA-1273.529, or mRNA-1273.214. The results are summarized as follows:

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Primary Series – Mice

- After a 2-dose primary series in mice, mRNA-1273.214 showed robust neutralization against WA1 D614G, BA.1, and BA.2, and overall, mRNA-1273.214 provided the broadest neutralization coverage across the variants evaluated.
- After primary series with mRNA-1273, robust binding antibody (IgG) titers against S-2P and S-2P.529 were seen, along with robust neutralizing antibody responses against WA1 D614G. However, most mice had minimal to no neutralizing antibody responses against BA.1 and BA.2 across all groups.
- A low-dose mRNA-1273 primary series (0.1 or 0.25 µg) induced levels of neutralizing antibodies in mice similar to those measured in human sera after completion of a 2-dose primary series with mRNA-1273. However, there was less inhibitory activity against BA.1, which was reflected in breakthrough infections in the upper and lower respiratory tracts after viral challenge. Cytokine and histology analyses confirmed the low-to-minimal protection against BA.1.
- A high-dose mRNA-1273 primary series (5 µg) induced antibodies that neutralized both WA1 D614G and BA.1, although reduced neutralization was observed against BA.1. Mice showed robust protection against both WA1 D614G and BA.1 viral challenge.
- Using mRNA-1273.529 as a primary series induced robust neutralizing antibodies against BA.1; however, neutralizing antibody titers against WA1 D614G, B.1.351 (Beta), and B.1.617.2 (Delta) were lower.

Primary Series Plus Booster(s) – Mice and NHP

- Mice boosted with mRNA-1273.214 had higher neutralizing antibody titers against all 3 variants (WA1 D614G, BA.1, and BA.2) compared with the mice boosted with mRNA-1273 and mRNA-1273.529.
- Boosting mice with mRNA-1273.529 or mRNA-1273.214 induced antigen-reactive B cells to S-2P.529 in the draining LN, while boosting with mRNA-1273 did not. In the spleen, minimal antigen-specific B cell responses were observed across all groups, indicating that antigen-reactive B cells were not in sufficient quantity to be measured in the systemic circulation.
- The serum neutralizing titers in mice 1 month after boosting with mRNA-1273 were increased, although the response to BA.1 was lower than the response to WA1 D614G. Boosting mice with mRNA-1273.529 following a primary series of mRNA-1273 enhanced neutralizing responses against BA.1 and BA.2.

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- Boosting mice with either mRNA-1273 or mRNA-1273.529 resulted in enhanced neutralizing antibody responses against WA1 D614G and was associated with protection in the lower and upper respiratory tract against mouse-adapted WA1 D614G or BA.1 viral challenge along with lower levels of pro-inflammatory cytokines.
- Boosting NHPs with mRNA-1273 or mRNA-1273.529 led to comparable and significant increases in neutralizing antibody responses against all VOCs, including Omicron, and was important for enhancing mucosal antibody binding and neutralization responses.
- Cross-reactive B cells in NHPs were expanded following a boost with mRNA-1273 or mRNA-1273.529, while only mRNA-1273 was capable of boosting memory B cells specific for WA1 alone.
- Significant and equivalent control of virus replication in lower airways was observed in NHPs following either boost. There was no evidence of viral antigen in the lung samples of any vaccinated NHP, and boosted animals displayed histopathologic alterations that were classified as minimal to mild or moderate.
- Two booster doses of mRNA-1273.529 resulted in markedly increased BA.1 and BA.2 neutralization antibody titers in mice, likely indicating that the Omicron-specific memory B cells measured after the third dose of mRNA-1273.529 responded to the fourth dose of mRNA-1273.529.

Additionally, in NHPs, a primary series with mRNA-1273 and a boost with either mRNA-1273 or mRNA-1273.529 drives a predominant Th1-directed immune response, which is not predicted to drive vaccine associated ERD. Prior studies with similar designs to the booster studies described here also showed no vaccine associated ERD in mice, hamsters, and NHPs, as was demonstrated by balanced Th1/Th2 directed immune responses to immunization ([mRNA-1273 Module 2.4](#)). mRNA-1273.214 was clinically tolerated in nonclinical pharmacology studies as indicated by no mortality after dosing with mRNA-1273, mRNA-1273.529, and/or mRNA-1273.214.

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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 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.

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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; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L;

GLP = Good Laboratory Practice; IM = intramuscular; IV = intravenous; mRNA = messenger RNA.

^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.

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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 novel 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) [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.

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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, 5000 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, 5000 µg/plate PEG2000-DMG with or without supplemented rat liver fraction	Yes	9601035
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

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Study Type	Test Article	Species, Strain	Method of Administration; Dose	GLP	Report Number
	PEG2000-DMG (Sunbright® GM-020) ⁱ	Human peripheral blood lymphocytes	Incubation for 4 and/or 24 h with 0, 53.3, 93.3, 163, 286, 500 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.125012 NGLPICH.BTL
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 ⁿ	Rat, Sprague Dawley	IM; 0, 30, 60, 100 µg/dose (Days 1 and 22)	No	2308-123

Abbreviations: CMV = cytomegalovirus; CoV = coronavirus; 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; PIV3 = parainfluenza virus type 3; pp65 = phosphoprotein 65; prME = pre-membrane 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.

^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.

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- ^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.
- ⁿ mRNA-1273 contains a single mRNA sequence that encodes for 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, 10.7 mM sodium acetate, pH 7.5.

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2.4.5 INTEGRATED OVERVIEW AND CONCLUSIONS

Nonclinical pharmacology studies demonstrate that mRNA-1273.214 is clinically tolerated and support the clinical efficacy and safety of the bivalent vaccines, such as mRNA-1273.214, to be administered as a 50-µg booster in response to emerging VOCs.

Overall, mRNA-1273 affords lower neutralization and protection against key VOCs, while mRNA-1273.529 more potently neutralizes BA.1. However, mRNA-1273.529 has lower neutralization against other non-BA.1 variants the ancestral SARS-CoV-2 strain. Bivalent vaccines, such as mRNA-1273.214, demonstrated greater cross-variant neutralization in nonclinical studies. Both mRNA-1273.529 and mRNA-1273.214 showed equivalent or better BA.1 and BA.2 neutralization, protection, and antigen-reactive B cells versus mRNA-1273. In mice, mRNA-1273.214 demonstrated increased potency when compared with monovalent vaccines. Additionally, in NHPs, a primary series with mRNA-1273 and a boost with either mRNA-1273 or mRNA-1273.529 was not associated with ERD. Refer to mRNA-1273.214 Module 2.5 for a summary of results from clinical studies with mRNA-1273 bivalent vaccines.

Given that there were no new safety concerns observed with mRNA-1273.214 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 mRNA-1273.214.

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2.4.6 REFERENCES

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