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Abbreviation	Definition
ACE-2	angiotensin converting enzyme 2
cDC	conventional dendritic cell
CDC	Centers for Disease Control and Prevention
CMV	cytomegalovirus
CoV	coronavirus
COVID-19	coronavirus disease 2019
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
DTPA	diethylenetriamine pentaacetic acid
ERD	enhanced respiratory disease
gB	glycoprotein B
GD	Gestation Day
gH	glycoprotein H
gL	glycoprotein L
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
hMPV	human metapneumovirus
ICH	International Council for Harmonisation
Ig	immunoglobulin
IM	Intramuscular(-ly)
LD	Lactation Day
LLOQ	lower limit of quantitation
LNP	lipid nanoparticle
mAb	monoclonal antibody
mRNA	messenger RNA
NHP	nonhuman primate
NPI	nascent peptide imaging
NTD	N-terminal domain
pDC	plasmacytoid dendritic cell
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PIV3	parainfluenza virus type 3
RBD	receptor binding domain
S	spike

Abbreviation	Definition
S-2P	spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
SARS	severe acute respiratory syndrome
SARS-CoV-1 DIV	double-inactivated severe acute respiratory syndrome coronavirus-1
SARS-CoV-2	2019 novel coronavirus
SM-102	heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate
Th	T helper
Tris	tris(hydroxymethyl)aminomethane
WHO	World Health Organization
WT	wild type

2.4.1.   OVERVIEW OF NONCLINICAL TESTING STRATEGY

2.4.1.1   Background

Coronaviruses (CoVs) are part of a large family of viruses that cause illnesses ranging from the common cold to more severe diseases, such as Middle East respiratory syndrome and severe acute respiratory syndrome (SARS).

An outbreak of the CoV disease 2019 (COVID-19) caused by the 2019 novel CoV (2019-nCoV, later designated SARS-CoV-2) began in Wuhan, Hubei Province, China, in Dec 2019 and the disease has since spread globally (WHO 2020a). The WHO declared COVID-19 a pandemic on 11 Mar 2020; however, widespread community transmission was already occurring in many locations. As of Nov 2020, more than 53 million cases and over 1.3 million deaths worldwide have been attributed to the COVID-19 pandemic (WHO 2020a; WHO 2020b). Widespread community transmission of SARS-CoV-2 has been reported in the Americas, Europe, Africa, and Southeast Asia, and clusters of cases continue to be reported throughout Asia and Australia (WHO 2020a). During the winter, the combination of re-opening of schools and increase in indoor activity, because of lower temperatures, is expected to further increases in COVID-19 cases and deaths in some parts of the world.

Current evidence suggests that SARS-CoV-2 is primarily transmitted via direct contact or person-to-person via respiratory droplets by coughing or sneezing from an infected individual (regardless of whether they are symptomatic) (Chen et al 2020; Licciardi et al 2020; Rothan and Byrareddy 2020; Shen et al 2020). Airborne transmission may be possible during certain medical procedures and in indoor, crowded, and poorly ventilated environments (WHO 2020c). Common symptoms of COVID-19 include fever and cough, and other symptoms include shortness of breath or difficulty breathing, muscle aches, chills, sore throat, headache, and loss of taste or smell. Individuals at highest risk of COVID-19 and severe COVID-19 are older adults ($\geq$ 65 years old) and people of any age who have certain underlying medical conditions such as cancer, chronic kidney disease, chronic obstructive pulmonary disease, serious heart conditions, immunocompromised state, obesity, pregnancy, sickle cell disease, and type 2 diabetes mellitus; smokers are also at increased risk for severe COVID-19 disease (CDC 2020).

Currently, there is no FDA-approved vaccine against SARS-CoV-2. Without further advances in the use of nonpharmaceutical interventions, over 2.5 million COVID-19 deaths are projected globally by 01 Mar 2021, with daily deaths peaking at about 15,000/day during this time (IHME 2020). Global efforts to evaluate novel antivirals and therapeutic strategies to treat severe

SARS-CoV-2 infections have intensified, and there is an urgent public health need for rapid development of novel prophylactic therapies, including vaccines, to prevent the spread of this disease.

ModernaTX, Inc. (Sponsor) has developed a rapid-response proprietary vaccine platform based on a messenger RNA (mRNA) delivery system. The platform is based on the principle and observation that cells can take up mRNA, translate it, and then express viral antigen(s) on the cell surface. The delivered mRNA does not enter the nucleus or interact with the genome, is nonreplicating, and is expressed transiently. mRNA vaccines developed with the Sponsor's mRNA-based platform have been used to induce immune responses against infectious pathogens such as cytomegalovirus (CMV; NCT03382405), human metapneumovirus (hMPV) and parainfluenza virus type 3 (PIV3; NCT03392389), Zika virus (NCT04064905), and influenza virus (NCT03076385 and NCT03345043).

The Sponsor has used its mRNA-based platform to develop mRNA-1273, a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine against SARS-CoV-2. mRNA-1273 contains a single mRNA that encodes the full-length SARS-CoV-2 spike (S) protein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilize the S protein into the prefusion conformation. The CoV S protein mediates attachment and entry of the virus into host cells (by binding to the angiotensin converting enzyme 2 [ACE-2] receptor followed by membrane fusion), making it a primary target for neutralizing antibodies that prevent infection (Corti et al 2015; Wang et al 2015; Yu et al 2015; Johnson et al 2016; Chen et al 2017; Wang et al 2018; Kim et al 2019; Widjaja et al 2019). It has been confirmed that the stabilized SARS-CoV-2 S-2P mRNA expresses well in mammalian cells and is in the prefusion conformation (Wrapp et al 2020).

Nonclinical studies have demonstrated that CoV S proteins are immunogenic and that S protein-based vaccines, including those based on mRNA delivery platforms, are protective in animals (Corbett et al 2020a, Corbett et al 2020b, Graham et al 2020, Mercado et al 2020, Tian et al 2020, Tostanoski et al 2020, Vogel et al 2020). Prior clinical studies of vaccines targeting related CoVs and other viruses have assessed the immunogenicity and safety profiles of mRNA-based vaccines (Anderson et al 2020, Folegatti et al 2020, Jackson et al 2020, Keech et al 2020, Mulligan et al 2020, Sadoff et al 2020, Walsh et al 2020).

The clinical development of mRNA-1273 to support its use in the adult population consists of 3 ongoing clinical trials being conducted in the US: a Phase 1, open-label, dose-ranging study (NCT04283461) sponsored by the National Institute of Allergy and Infectious Diseases and a Phase 2a, randomized, observer-blind, placebo-controlled, dose-confirmation study

(NCT04405076) and a Phase 3 randomized, stratified, observer-blind, placebo-controlled study (NCT04470427) conducted by the Sponsor to evaluate the efficacy, safety, and immunogenicity of the vaccine. The development of mRNA-1273 has been accelerated to address the current COVID-19 outbreak, benefiting from the uniquely rapid and scalable manufacturing processes that have been developed for this vaccine.

2.4.1.2   Test Material

mRNA-1273 contains a single mRNA that encodes for SARS-CoV-2 S-2P combined in a mixture of 4 lipids common to the Sponsor's mRNA vaccine platform: SM-102, cholesterol, DSPC, and PEG2000-DMG, respectively. The mRNA-1273 Drug Product is provided as a sterile suspension for injection at a concentration of 0.20 mg/mL in 20 mM Tris buffer containing 87 g/L sucrose and 4.3 mM acetate, at pH 7.5.

The pivotal submission-enabling toxicology studies were conducted with mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs. The development mRNA-1273 lots (Lots AMPDP-200005 and 8520100101) evaluated in the nonclinical pharmacology programs were prepared with a manufacturing process comparable to the GMP mRNA-1273 Drug Product evaluated in the Phase 3 clinical trial (as described in Module 3, Section 3.2.P.2.3 [Scale A process]) and were therefore representative of the clinical presentations. Lots AMPDP-200005 and 8520100101 were manufactured at a concentration of 0.5 mg/mL in 20 mM Tris buffer containing 87 g/L sucrose and 10.7 mM acetate, at pH 7.5.

The distribution, toxicity, and genotoxicity associated with mRNA vaccines formulated in LNPs are driven primarily by the composition of the LNPs and, to a lesser extent, by the biologic activity of the antigen(s) encoded by the mRNA. Therefore, the distribution study, Good Laboratory Practice (GLP)-compliant toxicology studies, and in vivo GLP-compliant genotoxicity study conducted with mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs are considered supportive and BLA-enabling for mRNA-1273. SM-102, the novel lipid used in mRNA-1273, and the commercially available PEG2000-DMG _____(b) (4)_____ were evaluated as individual agents in GLP-compliant in vitro genotoxicity studies. The potential effects of mRNA-1273 on fertility and pre- and postnatal development in pregnant and lactating female Sprague Dawley rats was assessed in a GLP-compliant perinatal/postnatal reproductive toxicity study. Additionally, the immunogenicity and toxicity profiles of mRNA-1273 were assessed in a non-GLP repeat-dose study.

2.4.1.3   Nonclinical Testing Program

The nonclinical testing program was designed to adhere to international regulatory guidelines, the intended clinical development program, and pharmacology and toxicology principles and was consistent with International Council for Harmonisation (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).

The route of administration of mRNA-1273 used in the nonclinical studies was intramuscular (IM), consistent with the clinical route.

Nonclinical primary pharmacology evaluations were conducted in vitro in HEK293T cells and in vivo in young and aged mice (BALB/c, BALB/cJ, C57/BL6J, and B6C3F1/J strains), golden Syrian hamsters, and rhesus macaques (nonhuman primates [NHPs]) animal models to characterize the expression and 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 to promote vaccine-associated enhanced respiratory disease (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 2007; Bolles et al 2011; Corbett et al 2020a). Additionally, the immunogenicity of mRNA-1273 was assessed in a non-GLP repeat-dose pharmacology study in Sprague Dawley rats.

As SARS-CoV-2 is a newly emerged CoV, there were no established animal models for the evaluation of prophylactic vaccines and therapeutics. As a proof of concept, the expression of the mRNA-encoded SARS-CoV-2 S-2P antigen was confirmed in vitro and in vivo, and nonclinical studies were initiated in multiple animal species in order to gain a comprehensive understanding of the effects of mRNA-1273 immunization. Wild-type (WT) mice are a convenient and easy-to-use model to assess vaccine immunogenicity; however, the ACE-2 receptor, the primary route for SARS-CoV-2 binding and entry, differs significantly between mice and humans and, as result, WT SARS-CoV-2 does not infect mice. Therefore, a mouse-adapted SARS-CoV-2 strain, which was developed by the laboratory of Dr. Ralph Baric at the University of North Carolina at Chapel Hill, was used to assess protection of immunized mice from SARS-CoV-2 challenge.

Although this mouse-adapted strain infects young mice and induces mild disease symptoms, more severe symptoms are evident in aged mice (> 12 months) (Dinnon et al 2020). Aged mice were therefore included in the nonclinical pharmacology program to further characterize the immune response and the level of protection from viral challenge. In addition, this model was used to characterize the quality of the immune response to determine if the mRNA-1273-induced immunity would be predicted to promote vaccine-associated ERD. The immunogenicity and protection study in aged mice was designed to directly address this concern through the evaluation of dose levels predicted to drive optimal or suboptimal protection from viral challenge. Golden Syrian hamsters were also selected as a relevant model for evaluation. Wild-type SARS-CoV-2 productively infects hamsters, causing weight loss and moderate to severe lung pathology. This model was selected because it is currently the only animal species in which severe respiratory disease is evident after virus challenge (Chan et al 2020). 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).

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 supports the development of mRNA-1273.

The Sprague Dawley rat was selected as the animal model for the toxicity assessments because it is an accepted rodent species for nonclinical toxicology testing by regulatory agencies and is a relevant species to assess the toxicity and immunogenicity of mRNA vaccines, as evidenced by immunogenic responses.

The toxicological profile associated with mRNA-based vaccines formulated in SM-102-containing LNPs, including mRNA-1273, is driven primarily by the LNP composition and, to a lesser extent, by the biologic activity of the antigen(s) encoded by the mRNA. The safety and tolerability of 5 mRNA-based vaccines that encode 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) have been evaluated in 6 GLP-compliant repeat-dose toxicity studies in Sprague Dawley rats. Additionally, the Sponsor completed a non-GLP repeat-dose

study in Sprague Dawley rats to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses.

Rats were administered doses up to the anticipated maximum tolerated dose of 150 µg/dose, where clinical observations including vocalization (at 100 µg/dose) were accompanied by body weight loss and decrease in food consumption. The number of doses selected for the individual GLP studies was 1 more than the intended number of doses proposed for the individual clinical studies. The number of doses ranged from 3 to 4, and doses were administered every 2 weeks.

SM-102, the novel lipid used in mRNA-1273, and the commercially available PEG200-DMG (b) (4) were evaluated in genotoxicity studies as individual agents using a standard ICH S2 (R1) approach (ICH 2011), including GLP-compliant bacterial reverse mutation (Ames) tests in *Salmonella typhimurium* and *Escherichia coli* and GLP-compliant in vitro micronucleus tests 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 [NPI] luciferase mRNA) formulated in SM-102 LNPs.

Additionally, a GLP-compliant combined developmental and perinatal/postnatal reproductive toxicity study was conducted to assess the potential effects of mRNA-1273 on fertility and pre- and postnatal development in pregnant and lactating female Sprague Dawley rats.

2.4.2.   PHARMACOLOGY

Table 1 summarizes the nonclinical pharmacology program for mRNA-1273. Pharmacology results are fully summarized in Module 2.6.2.

Table 1:   Summary of Pharmacology Program for mRNA-1273

Study Type/Description	Test Article Dose (μg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Evaluation of in vitro expression of SARS-CoV-2 mRNA and in vivo expression of mRNA-1273	SARS-CoV-2 S-2P mRNA: 0.003125 through 0.2 μg mRNA-1273: 2 or 10 μg	HEK293T cells BALB/c mice	In vitro transfection In vivo IM; single injection	No	MOD-4117.1273
Evaluation of immunogenicity, protective capacity, and safety in young mice	mRNA-1273: 0.01, 0.1, 1 or 10 μg SARS-CoV-2 S-2P: 0.01, 0.1, or 1 μg (+ SAS-adjuvant)	Mouse (young), BALB/cJ, C57BL/6J, B6C3F1/J	IM; prime only prime/boost (3-week interval) prime/boost (4-week interval)	No	VRC01
Immunization and protein restimulation in young BALB/c mice with enhanced respiratory disease endpoint monitoring	mRNA-1273: 1 or 10 μg SARS-CoV-2 S-2P: 10 μg (+ alum)	Mouse (young), BALB/c	IM; prime/boost (2-week interval)	No	MOD-3937
Immunogenicity and determination of titer dynamic range in young BALB/c mice	mRNA-1273: 0.0025 through 20 μg	Mouse (young), BALB/c	IM; prime/boost (3-week interval)	No	MOD-3938/ MOD-3940
Immunogenicity and characterization of cellular response in young BALB/cJ mice	mRNA-1273: 0.1, 1, or 10 μg SARS-CoV-1 DIV: 0.2 or 1 μg (+ alum) CDS: 0.2 or 1 μg (+ alum)	Mouse (young), BALB/c	IM; prime/boost (3-week interval)	No	VRC05
Efficacy and enhanced respiratory disease in aged BALB/c mice	mRNA-1273: 0.1 or 1 μg SARS-CoV-1 DIV: 0.1 μg (+ alum)	Mouse (aged), BALB/c	IM; prime/boost (3-week interval)	No	VRC02

Study Type/Description	Test Article Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Five-week (2 doses: prime/boost) repeat-dose immunogenicity with safety endpoints	mRNA-1273: 0, 30, 60, or 100 µg	Rat, Sprague Dawley	IM; prime/boost (3-week interval)	No	2308-123
Protection from WT SARS-CoV-2 in hamsters using optimal and suboptimal doses	mRNA-1273: 1, 5, or 25 µg	Hamster, golden Syrian	IM; prime/boost (3-week interval)	No	UTMB01
Immunogenicity and protective efficacy in NHPs	mRNA-1273: 10 or 100 µg	NHP, rhesus macaque (Indian-origin)	IM; prime/boost (4-week interval)	No	VRC04
Evaluation of immunogenicity and efficacy from expanded dose range in NHPs	mRNA-1273: 2.5, 30, or 100 µg	NHP, rhesus macaque (Indian-origin)	IM; prime/boost (4-week interval)	No	VRC07

Abbreviations: alum = aluminum hydroxide; CDS = conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein; GLP = Good Laboratory Practice; IM = intramuscular; mRNA = messenger RNA; NHP = nonhuman primate; SARS-CoV-1 DIV = double-inactivated severe acute respiratory syndrome coronavirus-1; SARS-CoV-2 = 2019 novel coronavirus; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SAS = Sigma Adjuvant System®; WT = wild-type.

2.4.2.1   Primary Pharmacology

Nonclinical primary pharmacology studies were conducted in vitro in HEK293T cells and in vivo in young and aged mice (BALB/c, BALB/cJ, C57BL/6J, and B6C3F1/J strains), golden Syrian hamsters, and rhesus macaques (NHPs) animal models to characterize the expression and 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 potential to promote vaccine-associated ERD after viral challenge (Module 2.6.2). Additionally, the immunogenicity of mRNA-1273 was evaluated in a non-GLP repeat-dose pharmacology study in Sprague Dawley rats (Module 2.6.6; Section 2.6.6.9).

The expression of the mRNA-encoded SARS-CoV-2 S-2P antigen was confirmed in vitro and in vivo. Sustained levels of antigen expression were observed over 24, 48, and 72 hours in HEK293T cells transiently transfected with a dose range (0.003125 through 0.2 µg) of an mRNA construct that encodes the SARS-CoV-2 S-2P antigen, as demonstrated by an overall increase in the frequency of cells expressing the receptor binding domain (RBD) or the N-terminal domain (NTD) epitopes (using the monoclonal antibody [mAb] clones CR3022 and 4A8, respectively) of

the SARS-CoV-2 S protein; a slight dose effect was observed. Immunization of BALB/c mice with 2 or 10 µg mRNA-1273 induced in vivo expression of the SARS-CoV-2 S-2P antigen in spleen and draining lymph node immune cells (conventional dendritic cells [cDCs] and plasmacytoid dendritic cells [pDCs]), as demonstrated by staining with mAbs specific to the RBD (CR3022) or the NTD (4A8) epitopes of the SARS-CoV-2 S protein. The level of antigen expression measured in cDCs was similar to that of pDCs. The higher dose (10 µg mRNA-1273) induced higher levels of antigen expression in these cells.

Immunogenicity was characterized in young and aged mice, rats, hamsters, and NHPs through the evaluation of the humoral (immunoglobulin [Ig] G binding antibodies), cellular (T-cell cytokines and T helper [Th] 1-directed CD4+ and CD8+ responses), and/or neutralizing antibody responses elicited by prime-only or prime/boost immunization schedule with a range of mRNA-1273 dose levels.

Protection by mRNA-1273 immunization was assessed in young and aged mice, hamsters, and NHPs immunized with a prime-only or prime/boost schedule, followed by viral challenge with a high dose of SARS-CoV-2 (mouse-adapted SARS-CoV-2 strain; hamsters and NHPs: WT SARS-CoV-2 strain, Washington state isolate). mRNA-1273 dose levels and immunization schedules predicted to drive optimal and suboptimal protection were included in these studies to identify immune signatures for each regimen and to assess the level of protection mediated by different dose levels. Suboptimal dose levels that confer only partial protection were also included to evaluate the theoretical risk of disease enhancement. Viral load and replication in the upper (nasal turbinates) and lower (lungs) airways, as well as lung pathology and inflammation, were evaluated after viral challenge.

The potential of mRNA-1273 to promote vaccine-associated ERD was assessed in young and aged mice, hamsters, and NHPs through the evaluation of immunogenicity endpoints (IgG1:IgG2a ratio, Th1/Th2 cytokine profiles, and the ratio of binding to neutralizing antibodies) indicative of a protective versus a disease enhancement phenotype, and through monitoring of viral load, viral replication, and histopathological evaluation of lung tissues after viral challenge. The immune signature of mice immunized with mRNA-1273 was compared to that of vaccines that have been associated with ERD (SARS-CoV-1 DIV and conformationally disrupted spike protein [CDS] in alum adjuvant) included as controls in 2 mouse studies.

These studies demonstrated that mRNA-1273 is immunogenic in all the species assessed, showing a dose-dependent response in IgG binding antibody titers and neutralizing antibody activities. Antigen-specific T-cell responses were observed in mice and NHPs. Direct measurement of Th1-directed responses in mice and NHPs, indirect measurement of

Th1-directed responses (IgG2a/c:IgG1 antibody subclasses) in mice, and the high levels of neutralizing antibody in all species lessen the concerns regarding the risk of ERD associated with mRNA-1273 immunization. Additionally, a robust and dose-dependent CD8+ T-cell response in mice and a low CD8+ T-cell response in NHPs were observed after boosting with a second dose of mRNA-1273.

In addition to measurements of the immune response, mice, hamsters, and NHPs were challenged with a high dose of SARS-CoV-2 (mice: mouse-adapted SARS-CoV-2 strain; hamsters and NHPs: WT SARS-CoV-2 strain, Washington state isolate); mice and hamsters were challenged intranasally and NHPs were challenged intranasally and intratracheally. Dose levels predicted to be optimal (fully protective) and suboptimal (subprotective) were included in these studies. At higher doses, mice, hamsters, and NHPs were fully protected from viral replication in both lungs and nasal passages. At suboptimal dose levels, animals were either fully protected in the lungs or had reduced viral burden after challenge compared to control animals. There were no observations of increased viral load in animals immunized with suboptimal dose levels of mRNA-1273, which further supports that mRNA-1273 immunization does not promote ERD. Lung histopathology assessments were performed to verify reduction of inflammation, immune complex deposition, and immune cell invasion in response to viral challenge in animals immunized with mRNA-1273 compared to control (PBS) animals. In animals immunized with either optimal or suboptimal mRNA-1273 dose levels, histopathological evaluation of the lungs of mice and NHPs confirmed the lack of evidence of ERD, as demonstrated by minimal inflammation and no noteworthy neutrophilic-associated alveolar disease or eosinophil-dominant inflammatory response, which have been historically associated with vaccine-associated ERD. In contrast, moderate to severe inflammation involving the small airways and the adjacent alveolar interstitia was elicited by SARS-CoV-2 infection in PBS-control animals.

Overall, nonclinical pharmacology studies demonstrated that mRNA-1273 is well tolerated, is immunogenic, and provides protection from SARS-CoV-2 challenge. In mice, hamsters, and NHPs, a prime-only immunization schedule induced robust SARS-CoV-2-specific binding and neutralizing antibody responses that significantly increased after boosting with a second dose of mRNA-1273. A prime/boost immunization schedule elicited a substantial dose-dependent binding antibody response in rats. In addition, Th1-directed antigen-specific CD4+ and CD8+ T-cell responses were observed in mice and a Th1-directed antigen-specific CD4+ T-cell response was observed in NHPs. mRNA-1273 was fully protective from viral challenge in immunized mice and hamsters when administered as a prime-only or prime/boost schedule at ≥ 1 µg/dose and in immunized NHPs when administered as a prime/boost schedule at ≥ 30 µg/dose. Furthermore, mRNA-1273 did not promote vaccine-associated ERD in mice,

hamsters, and NHPs as demonstrated by balanced Th1/Th2-directed immune responses to immunization, the absence of increased lung pathology, and controlled viral replication after viral challenge when administered at doses predicted to be fully (optimal dose) or partially (suboptimal dose) protective.

2.4.3.   PHARMACOKINETICS AND TISSUE DISTRIBUTION

Table 2 lists the nonclinical pharmacokinetics and tissue distribution study with mRNA-1647 in support of the development of mRNA-1273. Biodistribution results are fully summarized in Module 2.6.4.

Table 2:   Summary of Pharmacokinetics Program for mRNA-1273

Study Type	Test Article	Species, Strain	Method of Administration, Dose	GLP	Report Number
Single-dose tissue distribution study	mRNA-1647[a]	Rat, Sprague Dawley	IM injection dose of 100 μg on Day 1	No	5002121 Amendment 1

Abbreviations: CMV = cytomegalovirus; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; GLP = Good Laboratory Practice; IM = intramuscular; 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.

2.4.3.1   Pharmacokinetics and Tissue Distribution

The results of a biodistribution, non-GLP, single dose, IM injection study of mRNA-1647 in male Sprague Dawley rats (Table 2.6.5.5 [Module 2.6.5] and Report 5002121 Amendment 1) support the development of mRNA-1273. mRNA-1647 is a novel mRNA-based CMV vaccine that contains 6 distinct mRNA sequences (1 that encodes the full-length CMV glycoprotein B [gB] and 5 that encode the pentameric glycoprotein H [gH]/glycoprotein L [gL]/UL128/UL130/UL131A glycoprotein complex) combined at a target mass ratio of 1:1:1:1:1:1 in the Sponsor's standard proprietary SM-102–containing LNPs.

After a single IM dose of mRNA-1647 in male rats, concentrations of the 6 mRNA constructs of mRNA-1647 (ie, gB, gH, gL, UL128, UL130, and UL131A) were detectable in plasma and tissues in a 1:1:1:1:1:1 ratio. The time after dosing at which the maximum concentration was observed in plasma (T_{max}) was 2 hours for all constructs and was followed by a rapid elimination phase with a half-life ($T_{1/2}$) estimated to range from 2.7 to 3.8 hours. The maximum plasma concentration (C_{max}) ranged from 1.60 to 2.30 ng/mL, and the area under the concentration versus time curve (AUC) from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed ($AUC_{[0-t]}$) ranged from 22.7 to 25.5 ng·h/mL.

Concentrations of the 6 mRNA constructs of mRNA-1647 were detected at levels above the lower limit of quantitation (LLOQ) in most tissues analyzed, except for the kidney, where all levels were below the LLOQ. For highly exposed tissues (injection site [muscle], lymph nodes

[proximal and distal], and spleen), the C_{max} was observed between 2 and 24 hours post dose. The $T_{1/2}$ was calculated using the average tissue $T_{1/2}$ values for the 6 mRNA constructs; the results were 14.9 hours for injection site (muscle), 34.8 hours for proximal (popliteal) lymph nodes, 31.1 hours for distal (axillary) lymph nodes, and 63.0 hours for spleen.

As observed with other IM-delivered vaccines, the highest mRNA concentrations were observed at the injection site followed by the proximal (popliteal) and distal (axillary) lymph nodes, consistent with distribution via the lymphatic system. These tissues, as well as spleen and eye, had tissue-to-plasma AUC ratios > 1.0. Only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for all constructs ranged from 14.9 to 63.0 hours.

2.4.4.   TOXICOLOGY

Table 3 summarizes the nonclinical toxicology program used in support of the development of mRNA-1273. Toxicology results are fully summarized in Module 2.6.6.

Table 3:   Summary of 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
In Vitro Genotoxicity					
Bacterial reverse mutation test	SM-102	*Salmonella typhimurium, Escherichia coli*	Incubation for 67 hours 29 minutes 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
Bacterial reverse mutation test	PEG2000-DMG (b) (4)	*Salmonella typhimurium, Escherichia coli*	Incubation for 67 h and 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 hours with 0, 163, 286, 500 μg/mL SM-102 with or without supplemented rat liver fraction	Yes	9601568
Mammalian cell micronucleus test	PEG2000-DMG (b) (4)	Human peripheral blood lymphocytes	Incubation for 4 h and/or 24 hours with 0, 0, 53.3, 93.3, 163, 286, 500 μg/mL PEG2000-DMG with or without supplemented rat liver fraction	Yes	9601036
In Vivo Genotoxicity					

Study Type	Test Article	Species, Strain	Method of Administration; Dose	GLP	Report Number
In vivo mammalian erythrocyte micronucleus test	mRNA-1706[a]	Rat, Sprague Dawley	Single IV; 0, 0.6/6.2 (F), 1.3/13.5, 2.6/27.0, 5.2/54.1 (M) mg/kg mRNA-1706/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 mRNA/SM-102	No	AF87FU.125012 NGLPICH.BTL
Reproductive and Developmental Toxicity					
Combined developmental and perinatal/postnatal developmental and 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; IM = intramuscular; IV = intravenous; M = male; min = minute; mRNA = messenger RNA; NPI = nascent peptide imaging; 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 human metapneumovirus and parainfluenza virus type 3. The 2 mRNAs are combined at a target mass ratio of 1:1 in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 93 mM Tris, 7% PG, 1 mM DTPA, pH 7.4.

[d] mRNA-1893 contains a single mRNA sequence that encodes the prME structural proteins of Zika virus in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 100 mM Tris, 7% PG, 1 mM DTPA, pH 7.5.

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

[f] The original dose levels selected were 0, 10, 30, and 100 µg/dose, respectively (SoA issued on 16 Mar 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
        phosphoprotein 65 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 Mar 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   (b) (4)   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 IV injection (doses 2.6/27.0, 3.9/40.6, and 5.2/54.1 mg/kg
        mRNA-1706/SM-102 for females, and 2.6/27.0, 5.2/54.1, and 10.3/107.1 mg/kg mRNA-1706/SM-102 for
        males).

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%.

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 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.

2.4.4.1   Repeat-Dose Toxicity

The safety and tolerability of mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs have been evaluated in multiple GLP-compliant, repeat-dose toxicity studies in Sprague Dawley rats at doses up to 150 µg administered every 2 weeks for up to 6 weeks followed by a 2-week recovery period. Additionally, the Sponsor completed a repeat-dose non-GLP study in Sprague Dawley rats to characterize the immunogenic response and potential toxicity of mRNA-1273 at clinically relevant doses (Section 2.4.4.3).

Rats were administered mRNA-based vaccines (mRNA-1706, mRNA-1653, or mRNA-1893) IM once every 2 weeks for 1 month (3 doses) at doses up to 150 µg followed by a 2-week recovery period (Table 2.6.7.7A [Module 2.6.7] and Report 5002045; Table 2.6.7.7B [Module 2.6.7] and Report 5002231; Table 2.6.7.7C [Module 2.6.7] and Report 5002033; Table 2.6.7.7D [Module 2.6.7] and Report 5002400). In addition, rats were administered mRNA-based vaccines (mRNA-1647 or mRNA-1443) IM once every 2 weeks for 6 weeks (4 doses) at doses up to 96 µg followed by a 2-week recovery period (Table 2.6.7.7E [Module 2.6.7] and Report 5002034; Table 2.6.7.7F [Module 2.6.7] and Report 5002158).

The aggregate rat repeat-dose toxicity profile from the GLP studies for mRNA-based vaccines formulated in SM-102-containing LNPs consisted of IM doses ranging from 8.9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks. All doses administered were tolerated. Test article-related in-life observations at ≥ 8.9 µg/dose included reversible or reversing erythema and edema at the injection site and transient increases in body temperature at 6 hours post-dose returning to baseline 24 hours post-dose.

Test article-related, generally dose-dependent clinical pathology changes were observed at ≥ 8.9 µg/dose. Hematology changes included increases in white blood cells, neutrophils, and eosinophils and decreased lymphocytes; coagulation changes included increases in fibrinogen and activated partial thromboplastin time; and clinical chemistry changes included decreases in albumin, increases in globulin, and a corresponding decrease in albumin/globulin ratio. Clinical pathology changes generally reversed or were reversing by the end of the 2-week recovery period. Test article-related, transient cytokine increases were observed at ≥ 8.9 µg/dose at 6 hours post-dose, including in IP-10, MCP-1, and MIP-1-α. Cytokine changes were generally reversing by the end of the 2-week recovery period.

Post-mortem test article-related and generally dose-dependent changes in organ weights and macroscopic and microscopic findings were observed at ≥ 8.9 µg/dose. Organ weight increases were observed in the spleen, liver, and adrenal gland. Organ weight changes were generally reversing by the end of the 2-week recovery period. Macroscopic changes included skin thickening at the injection site and enlarged lymph nodes. Injection site changes completely recovered, and lymph node changes were recovering by the end of the 2-week recovery period. Microscopic changes included mixed cell inflammation at the injection site; increased cellularity and mixed cell inflammation in the inguinal, iliac, and popliteal lymph nodes; decreased cellularity in the splenic periarteriolar lymphoid sheath; increased myeloid cellularity in the bone marrow; and hepatocyte vacuolation and Kupffer cell hypertrophy in the liver. Microscopic changes were generally reversing by the end of the 2-week recovery period.

2.4.4.2   Genotoxicity

SM-102, the novel lipid used in mRNA-1273, and the commercially available PEG2000-DMG (b) (4) were evaluated in genotoxicity studies as individual agents using a standard ICH S2 (R1) approach (ICH 2011), including GLP-compliant in vitro bacterial reverse mutation (Ames) tests in *S typhimurium* and *E coli* (Table 2.6.7.8A [Module 2.6.7] and Report 9601567; Table 2.6.7.8B [Module 2.6.7] and Report 9601035) and GLP-compliant in vitro micronucleus tests in human peripheral blood lymphocytes

(Table 2.6.7.8C [Module 2.6.7] and Report 9601568; Table 2.6.7.8D [Module 2.6.7] and
Report 9601036).

In addition, SM-102 was evaluated for in vivo genotoxicity risk in a GLP-compliant in vivo rat
micronucleus test using a similar mRNA-based vaccine formulated in SM-102 LNPs
(Table 2.6.7.9A [Module 2.6.7] and Report 9800399) and in a non-GLP-compliant in vivo rat
micronucleus test using a reporter mRNA (NPI luciferase mRNA) formulated in SM-102 LNPs
(Table 2.6.7.9B [Module 2.6.7] and Report AF87FU.125012NGLPICH.BTL).

Genotoxicity assessments of the SM-102 lipid concluded that the lipid is not genotoxic in the
bacterial mutagenicity and human peripheral blood lymphocytes chromosome aberration assays.
Two intravenous in vivo micronucleus assays were conducted with mRNA-based vaccines
formulated in the SM-102-containing LNPs. Results from
Report AF87FU.125012NGLPICH.BTL were negative up to 3.21/60 mg/kg NPI luciferase
mRNA/SM-102, while results from Report 9800399 were positive at 2.6/27.0 mg/kg
mRNA-1706/SM-102 in females and at 5.2/54.1 mg/kg mRNA-1706/SM-102 in males. The
equivocal results are likely driven by micronuclei formation secondary to increased cytokines
and/or body temperature induced by LNP-driven systemic inflammation at high systemic
(intravenous) doses. 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.

Genotoxicity assessments of the PEG2000-DMG lipid concluded that the lipid is not genotoxic
in the bacterial mutagenicity and the human peripheral blood lymphocytes chromosome
aberration assays.

2.4.4.3   Reproductive and Developmental Toxicity

A GLP-compliant combined developmental and perinatal/postnatal developmental and
reproductive toxicity study was conducted to assess the potential effects of mRNA-1273 on
fertility and pre- and postnatal development in pregnant and lactating female Sprague Dawley
rats following IM administration of the human dose of 100 µg to dams on Study Days 1 and 15
(28 and 14 days prior to mating, respectively) and Gestation Days (GDs) 1 and 13
(Table 2.6.7.11 [Module 2.6.7], and Report 20248897).

Female rats assigned to Cohort 1 were euthanized on GD 21 for Caesarean-sectioning and fetal
examinations. Female rats assigned to Cohort 2 were allowed to deliver their litters naturally and
dams and pups were followed through Lactation Day (LD) 21.

Robust IgG response to S-2P antigen was observed in both the F_0 rats and F_1 generation rats following immunization of F_0 rats with mRNA-1273. In the F_0 rats, peak titer of 442,138 antibody units/mL was reached on GD 13. Titers subsequently plateaued at parturition (GD 21) and stayed relatively constant through LD 21. High IgG antibodies to SARS-CoV-2 S-2P were also observed in GD 21 F_1 fetuses and LD 21 F_1 pups, indicating strong transfer of antibodies from dam to fetus and from dam to pup.

In the F_0 generation, there were no mRNA-1273-related effects or changes in the following parameters: mortality, body weight, body weight gain, food consumption, macroscopic observations, estrous cycling during pre-cohabitation, mating and fertility, ovarian/uterine examinations, or natural delivery or litter observations. mRNA-1273-related clinical observations included transient thin fur cover, swollen hindlimbs, and limited usage of the hindlimb during the premating, gestation, and/or lactation phases of the study, with the most observations noted following dose administration on GD 13. These mRNA-1273–related observations were not considered adverse as effects did not significantly impair the animal's mobility, access to food, or ability to thrive. Only thin fur cover was still present during the lactation phase and was resolved by LD 18.

In the F_1 generation, there were no mRNA-1273-related effects or changes in the following parameters: mortality, body weight, clinical observations, macroscopic observations, gross pathology, external or visceral malformations or variations, skeletal malformations, and mean number of ossification sites per fetus per litter. mRNA-1273-related variations in skeletal examination included statistically significant increases in the number of F_1 rats with 1 or more wavy ribs and 1 or more rib nodules. Wavy ribs appeared in 6 fetuses and 4 litters with a fetal prevalence of 4.03% and a litter prevalence of 18.2%. Rib nodules appeared in 5 of those 6 fetuses. Skeletal variations are structural changes that do not impact development or function of a developing embryo, are considered reversible, and often correlate with maternal toxicity and/or lack of other indicators of developmental toxicity (Carney and Kimmel 2007). Maternal toxicity in the form of clinical observations was observed for 5 days following the last dose (GD 13), correlating with the most sensitive period for rib development in rats (GDs 14 to 17). Furthermore, there were no other indicators of mRNA-1273-related developmental toxicity observed, including delayed ossification; therefore, these common skeletal variations were not considered adverse.

Overall, maternal administration of mRNA-1273 on Study Day 1 (28 days prior to mating), Study Day 15 (14 days prior to mating) and GD 1, and GD 13 did not have any adverse effects on the F_0 or F_1 generations. The mRNA-1273-related, non-adverse effects were limited to an increase in the number of fetuses with common skeletal variations of 1 or more rib nodules and 1

or more wavy ribs with no effect on viability or growth and development of the F_1 generation. Robust IgG titers were observed in the rats following 4 immunizations of mRNA-1273 vaccine. Peak titer was reached on GD 13 and plateaued at the time of parturition GD 21 and stayed constant through LD 21. Strong maternal-to-fetal and maternal-to-pup transfer of antibodies was observed with mRNA-1273.

2.4.4.4   Other Toxicity

A non-GLP study in Sprague Dawley rats was conducted to characterize the immunogenic response and potential toxicity of mRNA-1273 at IM doses levels of 30, 60, and 100 µg/dose administered on Days 1 and 22 (Section 2.6.2.2.6 [Module 2.6.2], Table 2.6.7.17 [Module 2.6.7], and Report 2308-123).

A strong immunogenic response against SARS-CoV-2 S-2P was observed on Day 35, with measured IgG antibody titers above 10^6 at all dose levels. mRNA-1273 had no effect on body weights and limited, transient clinical signs starting at 30 µg/dose consisting of transient dose-dependent injection site edema with or without hindlimb impairment. Clinical pathology findings consisted, in part, of changes associated with inflammation starting at 30 µg/dose. In general, the changes observed are consistent with the results from the previous GLP rat toxicity studies conducted with other mRNA-based vaccines formulated with SM-102-containing LNPs.

2.4.4.5   Summary of Nonclinical Safety Margins

Pending the outcome of the Phase 3 clinical trial with mRNA-1273, a human dose of 100 µg/dose is anticipated to be safe and to provide protective immunization against SARS-CoV-2 infection.

In the rat repeat-dose toxicity studies in which up to 100 µg/dose of mRNA-1273 administered on Day 1 and Day 22, up to 150 µg/dose of mRNA-1706, mRNA-1653, or mRNA-1893 administered once every 2 weeks for 1 month (3 doses), or up to 96 µg/dose of mRNA-1647 and mRNA-1443 administered once every 2 weeks for 6 weeks (4 doses) were evaluated, the administered mRNA/LNP vaccines were well tolerated. In addition, results from the combined developmental and perinatal/postnatal reproductive toxicity study showed that a 100 µg/dose of mRNA-1273 administered once every 2 weeks for 6 weeks (4 doses) to Sprague Dawley rats did not result in any adverse effects on the F_0 or F_1 generations. Typical vaccine-associated findings included increases in body temperature and spleen weight, changes in cytokine profile reflecting an inflammatory pattern, and injection site reaction characteristics for vaccines, with all findings

showing reversibility. In addition, no exaggerated immune reactions were observed in the rat toxicity studies or in the completed immunogenicity studies in mice (young and old), rats, hamsters, and NHPs.

If a 100 µg/dose of mRNA-1273 is well tolerated in a rat with a conservative body weight estimate of 0.30 kg as compared to a human subject with a conservative body weight of 60.0 kg, there is a 200-fold safety margin for the human dose as compared to the rat dose based on body weight. The efficacy and safety profile of the mRNA-1273 vaccine in the Phase 3 clinical trial will be the ultimate determinant in identifying the approved dose for human subjects.

2.4.5.   INTEGRATED OVERVIEW AND CONCLUSIONS

In support of the development of mRNA-1273 against SARS-CoV-2, nonclinical pharmacology, biodistribution, and toxicology studies have been completed using mRNA-1273 or other mRNA vaccines that encode various antigens developed with the Sponsor's mRNA-based platform using SM-102-containing LNPs.

Data from the nonclinical testing program presented in this submission support the clinical efficacy and safety of mRNA-1273 at doses up to 100 μg administered twice IM 28 days apart.

- The expression of the mRNA-encoded SARS-CoV-2 S-2P antigen was confirmed in vitro and in vivo. HEK293T cells transfected with a dose range (0.003125 through 0.2 μg) of mRNA expressed the encoded SARS-CoV-2 S-2P antigen, as demonstrated by surface-protein staining with mAbs specific to the RBD (CR3022) or NTD (4A8) epitopes of the SARS-CoV-2 S protein. The expression of these epitopes was similarly confirmed in spleen and draining lymph node immune cells (cDCs and pDCs) in BALB/c mice administered a single IM dose (2 or 10 μg) of mRNA-1273.

- Nonclinical pharmacology studies of mRNA-1273 vaccination were performed in young and aged mice, golden Syrian hamsters, and rhesus macaques (NHPs). These studies demonstrate that mRNA-1273 is well tolerated, is immunogenic, and drives robust SARS-CoV-2-specific antibody and T-cell responses. Nonclinical viral challenge studies in animal models demonstrated that mRNA-1273 fully protects immunized animals from viral challenge when administered on a prime only or prime/boost schedule at dose levels ≥ 1 μg/dose in mice and hamsters and when administered on a prime/boost schedule at ≥ 30 μg/dose in nonhuman primates. In addition, these studies have shown that mRNA-1273 does not promote vaccine-associated ERD at any dose level evaluated, including those that only provided partial protection from challenge.

- The biodistribution of mRNA-based vaccines formulated in LNPs is predicted to be driven by the characteristics of the LNPs. mRNAs that are within similar LNPs (eg, mRNA-1273 and mRNA-1647) are therefore expected to distribute similarly, and the biodistribution study of mRNA-1647 supports the clinical development of mRNA-1273. This study demonstrated that mRNA constructs do not persist past 1 to 3 days in tissues other than muscle (injection site), proximal popliteal and distal axillary lymph nodes, and spleen, in which the average $T_{1/2}$ values for the 6 mRNA constructs of mRNA-1647 ranged from 14.9 to 63.0 hours.

- The aggregate GLP repeat-dose toxicity profile of mRNA vaccines that were developed with the Sponsor's mRNA-based platform in rats at IM doses ranging from 8.9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks was similar and consistent despite the fact that the different mRNA constructs encode different antigens. Therefore, the Sponsor proposes that the toxicity associated with mRNA vaccines formulated in similar LNPs is driven primarily by the LNP composition and, to a lesser extent, by the biologic activity of the antigens encoded by the mRNA and the aggregate GLP repeat-dose rat data, together with results from a mRNA-1273 non-GLP repeat dose rat study at clinically relevant doses, supports the clinical development of mRNA-1273.

- Results of a developmental and reproductive toxicity study showed that administration of a 100 µg/dose mRNA-1273 to Sprague Dawley rats at 28 and 14 days prior to mating and again at GDs 1 and 13 did not result in any adverse effects on the F_0 and F_1 generations; a small increase in the common non-adverse malformation of wavy ribs/nodules was observed in mRNA-1273-treated animals at this dose. Additionally, a robust IgG response to the S-2P antigen was observed in the dams, fetuses, and pups suggesting a strong transfer of antibodies from dam to fetus and from dam to pup.

Overall, the nonclinical studies demonstrate that mRNA-1273 is safe and well tolerated, is immunogenic, fully protects animals from viral challenge, and does not promote ERD at either optimal or suboptimal dose levels.

2.4.6.   REFERENCES

Anderson EJ, Rouphael NG, Widge AT, Jackson LA, Roberts PC, Makhene M, et al; mRNA-1273 Study Group. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. N Engl J Med. In press 2020.

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

Carney EW, Kimmel CA. Interpretation of skeletal variations for human risk assessment: delayed ossification and wavy ribs. Birth Defects Res B Dev Reprod Toxicol. 2007;80(6):473-96.

Centers for Disease Control and Prevention (CDC). Coronavirus disease 2019 (COVID-19): people with certain medical conditions [Internet]. Atlanta (GA): CDC; 2020 Oct 30 [cited 2020 Oct 30]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html.

Chan JF-W, Zhang AJ, Yuan S, Poon VK-M, Chan CC-S, Lee AC-Y, et al. Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility. Clin Infect Dis. In press 2020.

Chen Y, Lu S, Jia H, Deng Y, Zhou J, Huang B, et al. A novel neutralizing monoclonal antibody targeting the N-terminal domain of the MERS-CoV spike protein. Emerg Microbes Infect. 2017;6(5):e37.

Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus in Wuhan, China: a descriptive study. Lancet. 2020;395(10223):507-13.

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

Corbett KS, Flynn B, Foulds KE, Francica JR, Boyoglu-Barnum S, Werner AP, et al. Evaluation of the mRNA-1273 vaccine against SARS-CoV-2 in nonhuman primates. N Engl J Med. 2020b;383(16):1544-55.

Corti D, Zhao J, Pedotti M, Simonelli L, Agnihothram S, Fett C, et al. Prophylactic and postexposure efficacy of a potent human monoclonal antibody against MERS coronavirus. Proc Natl Acad Sci U S A. 2015;112(33):10473-8.

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

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

Dinnon KH III, Leist SR, Schäfer A, Edwards CE, Martinez DR, Montgomery SA, et al. A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures. Nature. 2020;586(7830):560-6.

Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet. 2020;396(10249):467-478. Correction in: Lancet. 2020;396(10249):466.

Graham SP, McLean RK, Spencer AJ, Belij-Rammerstorfer S, Wright D, Ulaszewska M, et al. Evaluation of the immunogenicity of prime-boost vaccination with the replication-deficient viral vectored COVID-19 vaccine candidate ChAdOx1 nCoV-19. NPJ Vaccines. 2020;5:69.

Institute for Health Metrics and Evaluation (IHME). Seattle, WA: IHME, University of Washington, 2020; [cited 15 Nov 2020]. Available from: http://covid19.healthdata.org/global?view=total-deaths&tab=trend.

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 2019 Apr 16] [29 screens]. Available from: https://database.ich.org/sites/default/files/S2%28R1%29%20Guideline.pdf.

Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA vaccine against SARS-CoV-2 - preliminary report. N Engl J Med. In press 2020.

Johansen MD, Irving A, Montagutelli X, Tate MD, Rudloff I, Nold MF, et al. Animal and translational models of SARS-CoV-2 infection and COVID-19. Mucosal Immunol. 2020;13(6):877-91.

Johnson RF, Bagci U, Keith L, Tang X, Mollura DJ, Zeitlin L, et al. 3B11-N, a monoclonal antibody against MERS-CoV, reduces lung pathology in rhesus monkeys following intratracheal inoculation of MERS-CoV Jordan-n3/2012. Virology. 2016;490:49-58.

Keech C, Albert G, Cho I, Robertson A, Reed P, Neal S, et al. First-in-human trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. medRxiv. In press 2020.

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

Kim Y, Lee H, Park K, Park S, Lim J-H, So MK, et al. Selection and characterization of monoclonal antibodies targeting Middle East respiratory syndrome coronavirus through a human synthetic Fab phage display library panning. Antibodies (Basel). 2019;8(3):42.

Licciardi F, Giani T, Baldini L, Favalli EG, Caporali R, and Cimaz R. COVID-19 and what pediatric rheumatologists should know: a review from a highly affected country. Pediatr Rheumatol Online J. 2020;18(1):35.

Lu S, Zhao Y, Yu W, Yang Y, Gao J, Wang J, et al. Comparison of nonhuman primates identified the suitable model for COVID-19. Signal Transduct Target Ther. 2020;5(1):157.

Mercado NB, Zahn R, Wegmann F, Loos C, Chandrashekar A, Yu J, et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature. 2020;586(7830):583-8.

Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature. 2020;586(7830):589-93.

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

Rothan HA and Byrareddy NS. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020;109:102433.

Sadoff J, Le Gars M, Shukarev G, Heerwegh D, Truyers C, Marit de Groot A, et al. Safety and immunogenicity of the Ad26.COV2.S COVID-19 vaccine candidate: interim results of a phase 1/2a, double-blind, randomized, placebo-controlled trial. medRxiv. In press 2020.

Shen KL, Yang YH, Jiang RM, Wang TY, Zhao DC, Jiang Y, et al. Updated diagnosis, treatment and prevention of COVID-19 in children: experts' consensus statement (condensed version of the second edition). World J Pediatr. 2020;16(3):232-9.

Tian J-H, Patel N, Haupt R, Zhou H, Weston S, Hammond H, et al. SARS-CoV-2 spike glycoprotein vaccine candidate NVX-CoV2373 elicits immunogenicity in baboons and protection in mice. bioRxiv. In press 2020.

Tostanoski LH, Wegmann F, Martinot AJ, Loos C, McMahan K, Mercado NB, et al. Ad26 vaccine protects against SARS-CoV-2 severe clinical disease in hamsters. Nat Med. In press 2020.

Vogel AB, Kanevsky I, Che Y, Swanson KA, Muik A, Vormehr M, et al. A prefusion SARS-CoV-2 spike RNA vaccine is highly immunogenic and prevents lung infection in non-human primates. bioRxiv. In press 2020.

Walsh EE, Frenck R, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. RNA-based COVID-19 vaccine BNT162b2 selected for a pivotal efficacy study. medRxiv. In press 2020.

Wang L, Shi W, Joyce MG, Modjarrad K, Zhang Y, Leung K, et al. Evaluation of candidate vaccine approaches for MERS-CoV. Nat Commun. 2015;6:7712.

Wang L, Shi W, Chappell JD, Joyce MG, Zhang Y, Kanekiyo M, et al. Importance of neutralizing monoclonal antibodies targeting multiple antigenic sites on the Middle East respiratory syndrome coronavirus spike glycoprotein to avoid neutralization escape. J Virol. 2018;92(10):e02002-17.

Widjaja I, Wang C, van Haperen R, Gutiérrez-Álvarez J, van Dieren B, Okba NM, et al. Towards a solution to MERS: protective human monoclonal antibodies targeting different domains and functions of the MERS-coronavirus spike glycoprotein. Emerg Microbes Infect. 2019;8(1):516-30.

World Health Organization (WHO). Weekly Operational Update on COVID-19 - 13 November 2020 [Internet]. Geneva, Switzerland: WHO; 2020a Nov 13 [cited 16 Nov 2020]. Available from: https://www.who.int/publications/m/item/weekly-operational-update-on-covid-19---13-november-2020.

World Health Organization (WHO). Dashboard [Internet]. Geneva, Switzerland: WHO; 2020b 16 Nov [cited 16 Nov 2020]. Available from: https://covid19.who.int/.

World Health Organization (WHO). Transmission of SARS-CoV-2: implications for infection prevention precautions, Scientific Brief [Internet]. Geneva, Switzerland: WHO; 2020c 09 Jul [cited 15 Nov 2020]. Available from: https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions.

Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hseih C-L, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020;367(6483):1260-3.

Yu X, Zhang S, Jiang L, Cui Y, Li D, Wang D, et al. Structural basis for the neutralization of MERS-CoV by a human monoclonal antibody MERS-27. Sci Rep. 2015;5:13133.