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List of Abbreviations and Definition of Terms

Abbreviation	Definition
COVID-19	coronavirus disease of 2019
GMT	geometric mean titer
hACE-2	human angiotensin-converting enzyme 2
mAb	monoclonal antibody
MFI	mean fluorescence intensity
mRNA	messenger ribonucleic acid
PSVNA	pseudovirus neutralization assay
RBD	receptor-binding domain
S-2P	spike protein with 2 proline substitutions within the heptad repeat 1 domain
S-2P.045	Omicron (BA.4/BA.5)-matched S-2P
S-2P.529	Omicron (BA.1)-matched S-2P
WA.1	USA46 WA1/2020
VSV	vesicular stomatitis virus

2.4 Nonclinical Overview – Addendum A

Addendum A: Nonclinical Reports Evaluating mRNA-1273.222

The purpose of this addendum is to provide an overview of nonclinical reports evaluating mRNA-1273.222. The bivalent mRNA-1273.222 vaccine contains 2 mRNAs encoding S-2P mixed in a 1:1 ratio: the mRNA found in the monovalent mRNA-1273 vaccine (Wuhan-Hu-1 S-2P spike sequence) and the mRNA found in the monovalent mRNA-1273.045 vaccine (Omicron subvariants BA.4/BA.5 S-2P spike sequence). The inclusion of the original spike protein sequence in the mRNA-1273 vaccine provides the best strategy to reactivate and promote affinity maturation of existing immunity provided by prior immunization. The inclusion of the BA.4/BA.5 spike protein sequence in the mRNA-1273.045 vaccine diversifies immunity against the historical strain as well as current variants, and potentially against yet-to-emerge variants. This bivalent, variant-matched booster strategy with the mRNA-1273.222 vaccine is likely to further enhance the antibody response against variants compared to the authorized, standard-of-care booster vaccines against COVID-19, as evidenced from preclinical and clinical data from the Sponsor's previous bivalent COVID-19 vaccines mRNA-1273.211 (Wuhan + Beta) and mRNA-1273.214 (Wuhan + BA.1).

Table 1 summarizes the additional nonclinical pharmacology studies performed in support of the development of mRNA-1273.222. The reports for these studies are included in Module 4.

Table 1: Summary of Pharmacology Program for mRNA-1273.222

Study Type/Description	Test Article Dose	Test System (Species, Strain)	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Evaluation of In Vitro Expression of the BA.4/BA.5 mRNA Contained in the Bivalent mRNA-1273.222 Vaccine	Mock mRNA (control), mRNA that encodes the SARS-CoV-2 S-2P antigen of the BA.4/BA.5 subvariants of Omicron mRNA that encodes the NTD and RBD of the Wuhan-Hu-1 spike protein linked together and inserted into the cell membrane with a transmembrane domain (NTD-RBD-HATM) 500 and 100 ng/mL	Expi293F human cell	48 hours after transfection, cells collected, stained, and assessed by flow cytometry for expression of the encoded antigen	No	MOD-045EXP
Evaluation of Immunogenicity of Primary Series mRNA-1273.222 in BALB/c Mice	mRNA-1273 ^a , mRNA-1273.529 ^b , mRNA-1273.045 ^c , mRNA-1273.214 ^d , mRNA-1273.222 ^e 1 µg	Mouse, BALB/c	IM; Day 1, 22 (primary series)	No	MOD-5482

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Study Type/Description	Test Article Dose	Test System (Species, Strain)	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Evaluation of Immunogenicity and Protection From a Booster Dose of the mRNA-1273.222 Vaccine After Primary Series Vaccination With mRNA-1273 in K18 hACE-2 Mice	UNFIX-01 (control) mRNA-1273 ^a , mRNA-1273.214 ^d , mRNA-1273.222 ^e 0.25 µg	Mouse, K18-hAC E2 C57BL/6	IM; Day 0, 21 (primary series), Day 241 (booster)	No	WASHU-K 18-89

Abbreviations: GLP = good laboratory practice; hACE-2 = human angiotensin converting enzyme 2;

HATM = hemagglutinin transmembrane domain; IM = intramuscular; mRNA = messenger RNA;

NTD = N-terminal domain; RBD = receptor-binding domain; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

- ^a mRNA-1273 vaccine contains a single mRNA that encodes the spike protein of the Wuhan-Hu-1 isolate of SARS-CoV-2.
- ^b mRNA-1273.529 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the BA.1 variant of Omicron (S-2P.529).
- ^c mRNA-1273.045 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the BA.4/BA.5 subvariants of Omicron.
- ^d mRNA-1273.214 is a 1:1 bench side mix of separately formulated mRNA-1273 and mRNA-1273.529 vaccines.
- ^e mRNA-1273.222 is a 1:1 bench side mix of separately formulated mRNA-1273 and mRNA-1273.045 vaccines.

A summary of these nonclinical reports are provided in this addendum:

- In Study MOD-045EXP, in the evaluation of in vitro expression with 2 dose concentrations (500 ng/mL and 100 ng/mL) of BA.4/BA.5 mRNA contained in mRNA-1273.222, levels of the SARS-CoV-2 S-2P antigens were seen in Expi293 cells over 48 hours. There was an overall increase in the frequency of cells that express the BA.4/BA.5 SARS-CoV-2 S-2P antigen, evident after staining with the CC40.8 mAb that binds to the S2 subdomain. No CR3022 binding was measured in cells transfected with the BA.4/BA.5 mRNA contained in mRNA-1273.222, while increased frequency and substantial binding was measured in cells transfected with mRNA contained in mRNA-1283. This indicates that the CR3022 mAb is specific to the original Wuhan-Hu-1 RBD and confirms that the S-2P encoded by the BA.4/BA.5 mRNA contained in mRNA-1273.222 does not contain the same binding epitope, as expected. The frequency of cells that express the BA.4/BA.5 mRNA contained in mRNA-1273.222 and the mRNA contained in mRNA-1283 was similar after staining with recombinant hACE-2. A small dose effect was observed overall. The MFI of expression showed similar results.
- The objective of Study MOD-5482 was to evaluate the immunogenicity elicited by a 2-dose primary vaccination series of the bivalent mRNA-1273.222 vaccine in mice. Robust S-2P, S-2P.529, and S-2P.045 IgG GMTs were observed in all mRNA groups at

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2 weeks after the second dose. In general, the monovalent BA.1-matched mRNA-1273.529 and BA.4/BA.5-matched mRNA-1273.045 vaccines had lower titers when assessed against non-matched spike antigens. Individually, the bivalent mRNA-1273.222 and mRNA-1273.214 vaccines potently neutralized the BA.4/BA.5 and BA.1 viruses, respectively, as evident from both neutralization assays used in the study (VSV-based and lentivirus-based PSVNAs). Furthermore, the nAb BA.4/BA.5 and BA.1 titers elicited by the bivalent mRNA-1273.222 and mRNA-1273.214 vaccines, respectively, exceeded or were similar to the WA.1 + D614G nAb titers elicited by the monovalent mRNA-1273 vaccine, thereby offering the best neutralization breadth.

- The objective of Study WASHU-K18-89 was to evaluate immunogenicity and protection elicited by a booster dose of the mRNA-1273.222 vaccine after primary series vaccination with mRNA-1273 in K18-hACE2 transgenic mice. Administering the mRNA-1273.222 vaccine as a booster following a primary series of the mRNA-1273 vaccine enhanced nAbs against both BA.1 and BA.5. Notably, enhanced nAb production after boosting with the mRNA-1273.222 vaccine against BA.1 and BA.5 was observed compared to boosting with the mRNA-1273 vaccine. A similar effect was observed in mice who were boosted with the mRNA-1273.214 vaccine following a primary series of the mRNA-1273 vaccine. Overall, boosting with either mRNA-1273.222 or mRNA-1273.214 vaccine enhanced protection against BA.5 infection compared with the protection elicited by boosting with the mRNA-1273 vaccine.

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