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List of Abbreviations and Definitions of Terms

Abbreviation	Definition
bAb	Binding antibodies
BAL	bronchoalveolar lavage
BLA	Biologics License Application
CoV	coronavirus
COVID-19	coronavirus
CSR	clinical study report
CV	coefficient of variation
ECLIA	electrochemiluminescence immunoassay
ELISA	enzyme-linked immunosorbent assay
EUA	Emergency Use Authorization
FRNT	focus reduction neutralization test
IgG	immunoglobulin G
MERS	Middle East respiratory syndrome–related coronavirus
MN	microneutralization
MSD	MesoScale Discovery
N	nucleocapsin
NAb	Neutralizing antibodies
NGS	new generation squencing
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PPD	Pharmaceutical Product Development
PRNT	plaque reduction neutralization test
PsVNA	pseudovirus neutralization assay
RBD	receptor binding domain
RP	respiratory panel
RT-PCR	reverse transcriptase - polymerase chain reaction
S-2P	S-protein modified with 2 proline substitutions within the heptad repeat 1 domain
S	spike
SARS	severe acute respiratory syndrome
SARS-CoV-2	severe acute respiratory syndrome coronavirus-2
SCE	summary of clinical efficacy

Abbreviation	Definition
VAERD	vaccine-associated enhanced respiratory disease
VIP	Vaccine Immunology Program
VRC	Vaccine research center
WGS	whole genome sequencing

2.7.2.1 BACKGROUND AND OVERVIEW

The Sponsor is developing mRNA-1273 as a vaccine to prevent coronavirus disease 2019 (COVID-19), the disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Module 2.7.2 should provide an overall view of clinical pharmacology studies; however, this is a mRNA-based product for which specific immunological reactions have been measured. Therefore, data regarding immunogenicity have been summarized including a brief explanation of the methods used and their performance. An overview of the development program is presented in Module 2.2 of this Biologics License Application (BLA), with greater detail provided in Module 2.5.

The summary of safety data is located in Module 2.7.4. The summary of clinical efficacy (SCE), including immunogenicity, is located in Module 2.7.3.

Three studies analyzing the immunogenicity of mRNA-1273 in the prevention of COVID-19 and one study (Study mRNA-1273-P301) analyzing the clinical efficacy were performed. The Phase 2 and Phase 3 studies were comprised of a Part A and Part B. Part A was a randomized, placebo-controlled, blinded phase of the study, where participants were blinded to their treatment assignment. Part B was an Open-Label Observational Phase of this study designed to offer participants who received placebo in Part A of this study and who met Emergency Use Authorization (EUA) eligibility, an option to request open-label mRNA-1273. The SCE summarizes data from the following 3 studies as evidence of the clinical efficacy and/or immunogenicity of mRNA-1273 in the prevention of COVID-19:

- Study mRNA-1273-P301 (hereafter Study 301; NCT04470427) is a Phase 3 study that provides the clinical evidence of vaccine efficacy (Section 2.7.3.2.1.1) and immunogenicity (Section 2.7.3.2.1.2) and the majority of the clinical safety data (Section 2.7.4) for this BLA. The Study P301 Final Randomized Blinded Phase (Part A) Clinical Study Report (CSR; primary efficacy analysis) includes immunogenicity data through Day 57 and is located in Module 5. The Study 301 CSR Addendum 1 (Safety from Part B) describes safety and COVID-19 case data from the participant decision visit (unblinding) up to the data cutoff date (26 Mar 2021), and is located in Module 5. A tabular overview of Study 301 is provided in Section 2.7.3.1.1 (Table 1). Primary analysis results were reported on 30 Dec 2020 (Baden et al 2020).

- Study mRNA-1273-P201 study (hereafter Study 201; NCT04405076) is a Phase 2a study that provides confirmation of the dose selected from the Phase 1 study, comprising immunogenicity data (Section 2.7.3.2.2) and safety data (Section 2.7.4). The Study P201 Primary Analysis CSR includes the results from the primary analysis of immunogenicity

and safety data through Day 57 (data cutoff date 05 Nov 2020) and is located in Module 5. The Study P201 End of Part A CSR addendum includes safety and immunogenicity results through Day 209, and is located in Module 5. A tabular overview of Study 201 is provided in Section 2.7.3.1.1 (Table 1). A preliminary report of the immunogenicity and safety data through Day 57 were available online on 09 Feb 2021 (Chu et al 2021).

- Study 20-0003 (hereafter Study 101; Study NCT04283461) is a Phase 1 dose-ranging study of immunogenicity (Section 2.7.3.2.3) and safety that provided support for dose selection for Study 201 and Study 301. In addition, Study 101 provides evidence of persistence of immunogenicity through Day 209 (Section 2.7.3.5) and safety (Section 2.7.4) in this BLA. The Study P101 Day 119 CSR and Study P101 Day 209 CSR Addendum are located in Module 5 and a tabular overview is provided in Table 1 of Section 2.7.3.1.1. Durability of responses through 90 days after the second vaccination dose were published on 07 Jan 2021 (Widge et al 2021) and through 180 days after dose 2 on 06 Apr 2021 (Doria-Rose et al 20210). Safety and immunogenicity results in older adults were published on 17 Dec 2020 (Anderson et al 2020) and in adults 18 to 55 years old on 14 Jul 2020 (Jackson et al 2020).

This BLA includes no analysis of pooled data from these 3 studies. Efficacy was only studied in Study 301, which also provided the vast predominance of immunogenicity and safety data.

2.7.2.2 SUMMARY OF RESULTS OF INDIVIDUAL STUDIES

A summary of immunogenicity results from Study 301 is presented in Section 2.7.3.2.1.2. Detailed immunogenicity results from Study 301 are presented in the Study P301 Part A CSR (Section 6.5).

A summary of immunogenicity results from Study 201 is presented in Section 2.7.3.2.2. Detailed immunogenicity results from Study 201 are presented in the Primary Analysis CSR (Section 8) and the Study P201 End Of Part A Addendum CSR (Section 8).

A summary of immunogenicity results from Study 101 is presented in Section 2.7.3.2.3. Detailed immunogenicity results from Study 101 are presented in the Day 119 CSR (Section 10) and the Day 119 CSR Addendum 1 (Day 209 Immunogenicity And Safety; Section 8).

2.7.2.3 COMPARISON AND ANALYSES OF RESULTS ACROSS STUDIES

A comparison of immunogenicity results across studies is presented in Section 2.7.3.3.3.

Study 101, Study 201, and Study 301 all confirmed the high immunogenicity of mRNA-1273. The vaccine efficacy (VE) results of Study 301 (Section 2.7.3.2.1) and the Study 301 safety data (Section 2.7.4.2.1) support the dosing recommendation of an mRNA-1273 vaccine regimen comprising 2 doses of 100 µg mRNA-1273, administered 28 days apart. The full justification for selection of the 100 µg dose of mRNA-1273 is presented in Section 2.7.3.4.

2.7.2.4 SPECIAL STUDIES

Supporting immunogenicity data for clinical development of mRNA-1273 includes an extensive panel of assays to assess SARS-CoV-2 infection and characterize the immune response induced by mRNA-1273.

Good Manufacturing Practice production of mRNA-1273 was initiated in parallel with preclinical evaluation. Prior to vaccination of the first human subject, expression and antigenicity of the S-2P antigen delivered by mRNA was confirmed in vitro, and immunogenicity of mRNA-1273 was documented in several animal models. These nonclinical immunogenicity assays were developed by Vaccine Research Center (VRC) at the National Institutes of Health (NIH) (Corbett et al 2020).

The immunogenicity assay development strategy for clinical samples was phase-dependent and flexible to accommodate for the evolving COVID-19 pandemic and in compliance with regulatory requirements. In Study 101, fit-for-purpose immunoassays were used to assess sera for binding immunoglobulin G (IgG) antibody titers, neutralizing activity and T-cell cytokine response (performed at the National Institute of Allergy and Infectious Diseases [NIAID] VRC) (Section 2.7.3.2.3). Quantitative enzyme-linked immunoassay (ELISA) assay were used to detect IgG antibody to the SARS-CoV-2 virus S-2P protein as well as spike (S)-protein receptor binding domain (RBD) subdomain in human serum. Vaccine-induced neutralizing activity was assessed by pseudotyped lentivirus reporter single-round-of-infection neutralization assay (PsVNA) and by live wild-type SARS-CoV-2 virus focus/plaque-reduction neutralization test (F/PRNT) assay. These experimental assays were developed using a fit-for-purpose approach due to the early phase of study. In addition, in Study 101, convalescent sera obtained from 41 patients who recovered from SARS-CoV-2 infection were used during assay development to generate a relative benchmark (based on levels elicited by natural infection) (Study P101 Day 119 CSR Section 10).

To address concerns about the theoretical risk of enhanced disease after injection with mRNA-1273, an SARS-CoV-2 intracellular cytokine stimulation (ICS) assay was developed using peripheral blood mononuclear cells (PBMCs) isolated from participants in Study 101. The

intent of evaluating the ICS assay was to evaluate if the mRNA-1273 vaccine induced a Th-2 directed response (which has been linked with VAERD) or a Th-1 directed response (which has been linked with lack of VAERD). This was done through the evaluation of CD4+/CD8+ T cells which can be segregated into Th-1/Th-2 directed responses. In the SARS-CoV-2 ICS assay PBMCs were stimulated with custom ordered peptides peptide pools ranging from the N-terminus of SARS-CoV-2 S-protein up to the furin cleavage site (S1 pool) and the C-terminus of the SARS-CoV-2 S-protein up to the furin cleavage site (S2 pool) and antigen-specific cytokine frequencies are reported. This assay is a fit-for-purpose research assay that was developed by VRC in NIH in Study 101 but has not been qualified or validated.

Although, these bioassays were not formally qualified or validated at the time of Study 101, they demonstrated to be scientifically sound, suitable, and reliable for their intended purpose.

The suitability of the immunogenicity analytical methods for clinical use were subsequently demonstrated in the Study 201 study (Section 2.7.3.2.2). In Study 201, the immunogenicity assays were qualified for the primary analysis of immunogenicity data (Day 57) (Study P201 Primary Analysis CSR [Part A]), and validated for subsequent immunogenicity data analysis (Day 209) (Study P201 End Of Part A Addendum CSR [Part B]), with the exception of Live MN which was qualified for all data analyzed in Study 201. In Study 301 study, all analytical assays were validated, following regulatory expectations, except for BioFire® Respiratory Panel qualitative RT-PCR which was qualified at the time.

To evaluate vaccine immunogenicity, an ELISA method for the detection of IgG specific to S-2P protein in human serum was developed, qualified (VSDVAC58), and validated (VSDVAC65) by PPD® Laboratories. This assay was qualified for primary analysis in the Study 201 study and used as a validated assay for subsequent analysis of Study 201 and Study 301 data. For Study 301, an additional assay detecting IgG against S-protein was developed with the aim of increasing automatization of immunogenicity evaluation. This multiplex assay using a MesoScale Discovery (MSD) platform was developed by the NIH VRC Vaccine Immunology Program (VIP) and measured IgG specific S, N, and RBD-protein antibody. The anti-S-protein bAb assay within the multiplex MSD was qualified and validated and was used to evaluate vaccine immunogenicity along with the previously validated immunogenicity ELISA assays for detection of IgG specific to SARS-CoV-2 S-2P protein. Both assays provided evidence of immunogenicity following two doses of vaccine and were used to assess persistence of antibody at Day 209 in Study 201 (Section 2.7.3.2.1.2).

Neutralizing antibodies against SARS-CoV-2 S-protein were analyzed throughout the clinical development of mRNA-1273. A live virus microneutralization (MN) assay was developed to

quantify SARS-CoV-2 neutralizing antibodies in serum or plasma samples from individuals who received a SARSCoV-2 vaccine or were exposed to SARSCoV-2. This assay was qualified in Study 201 and thereafter by Battelle Memorial Institute. These live MN assays are labor intensive and require high biosafety level containment 3 handling by trained personnel and pose challenges for high throughput. A pseudovirus neutralization assay (PsVNA) in 293T/ACE2 cells had been developed by VRC at NIH and used in Study 101. This bioassay was further developed, qualified and validated by the "Neutralizing Antibody Core" Laboratory, at Duke University Medical Center and used in Study 301. The PsVNA was determined to be concordant with the binding antibody assays (ELISA and MSD assays for SARS-CoV-2 IgG antibody quantification) and the critical fold rise value for seroresponse was adjusted based on the concordance analysis (Module 5, Section 5.3.1.4).

In Study 201 and Study 301, baseline SARS-CoV-2 status and asymptomatic/symptomatic SARS-CoV-2 infection were evaluated using a SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) assay (validated by Eurofins-Viracor Laboratories) using nasopharyngeal swabs from individuals suspected of COVID-19. To test for antibodies generated by an intercurrent SARS-CoV2 infection in Study 301 participants, an in-vitro diagnostic assay, Elecsys® anti-SARS-CoV-2 (developed by Roche Diagnostics) was used. This assay evaluated anti-SARS-CoV-2 IgG antibody presence in study participants. The commercially available Elecsys anti-SARS-CoV-2 in vitro diagnostic electrochemiluminescence immunoassay (ECLIA) is a qualitative assay which evaluates the SARS-CoV-2 baseline infection status by detecting anti-IgG specific SARS-CoV-2 Nucleocapsid (N) protein antibodies present in human serum and plasma (Section 2.7.3.2.1.2). This assay was validated by PPD Global Central Labs-US laboratory for its use in Study 301. Serum from study participants in Study 201 and Study 301 were then additionally evaluated using a quantitative ELISA method for the detection of IgG specific to SARS-CoV-2 N-protein. This assay, developed by PPD Laboratories, was qualified for primary analysis in Study 201 (VSDVAC64) and validated for subsequent analysis of Study 201 data and Study 301 (VSDVAC66).

A quantitative RT-PCR analysis of RNA from SARS-CoV-2 virus in swab and saliva was additionally carried out in Study 301. This quantitative RT-PCR from swab and saliva samples was carried out to measure viral load at SARS-CoV-2 infection diagnosis over time to evaluate the effect of mRNA on the viral infection kinetics. This assay was validated by Eurofins-Viracor Laboratories.

During mRNA-1273 clinical development, increasing concerns arose around emerging SARS-CoV-2 variants that may be associated with increased transmission rates and mortality and/or a decrease in the efficacy of the authorized COVID-19 vaccines (Haynes et al 2021). In

order to determine the strain of SARS-CoV-2 which caused COVID-19 cases in Study 301 and to compare with the mRNA-1273 vaccine sequence, a whole SARS-CoV-2 genome analysis (whole genome sequencing [WGS] assay) and a whole SARS-CoV-2 S-protein analysis (New Generation Sequencing [NGS] assay) were performed. These assays were developed by Eurofins Genomics and validated by Eurofins-Viracor, and used in Study 301 (Section 2.7.3.2.1.2).

Additionally, the BioFire® Respiratory Panel (RP) assay for the identification of a variety of respiratory targets, including SARS-CoV-2, was used as a qualitative assay in Study 301. This bioassay can simultaneously detect nucleic acids from 20 viral and bacterial organisms in nasopharyngeal swab samples. BioFire RP, qualified by Eurofins-Viracor was used in Study 301 for the detection of other viral and bacterial organisms associated with respiratory disease (Section 2.7.3.2.1.2).

A summary of the context of use, assay name, development status, supporting available information, and module location of the qualified and validated analytical methods used for the assessment of clinical endpoints in Study 101, Study 201 and Study 301 is provided in Table 1.

The summary of method validation parameters (e.g. sensitivity, specificity, reliability, validity) for each bioassays is located in Module 5, Section 5.3.1.4.

Table 1: Overview of the Main Bioassays for the Assessment of Clinical Endpoints

Context of Use	Assay Name	Methodology (Parameter measured)	Development Status (Vendor)[a]
SARS-CoV-2 status and infection assessments	RT-qPCR (Upper Respiratory Samples)	RT-PCR (RNA)	**STUDY 201:** Validated (Eurofins-Viracor) **STUDY 301:** Validated (Eurofins-Viracor)
	ELECSYS: N-protein IgG	RT-PCR (RNA)	**STUDY 301:** Validated (PPD GCL)
	ELISA: N-protein IgG	ELISA (Anti-N IgG bAb)	**STUDY 201:** Qualified (PPD Laboratories)[b] Validated (PPD Vaccine Laboratories)[b] **STUDY 301:** Validated (PPD Vaccine Laboratories)
Viral infection kinetic assessment	RT-PCR Multiplex	RT-PCR (RNA)	**STUDY 301:** Validated (Eurofins-Viracor)
Genotype variant analysis	RT-PCR NGS	RT-PCR (RNA)	**STUDY 301:** Validated (Eurofins-Viracor)
	RT-PCR WGS	RT-PCR (RNA)	**STUDY 301:** Validated (Eurofins-Viracor)
Detection of other viral and bacterial organisms	RT-PCR BioFire	RT-PCR (RNA)	**STUDY 301:** Qualified (Eurofins-Viracor)
Quantitative bAb	ELISA: S2P-protein IgG	ELISA (Anti S-2P IgG bAb)	**STUDY 101:** Developed (VRC VIP at the NIH) **STUDY 201:** Qualified (PPD Laboratories)[b] Validated (PPD Vaccine Laboratories)[b] **STUDY 301:** Validated (PPD Vaccine Laboratories)
	ELISA: RBD-protein IgG	ELISA (Anti RBD IgG bAb)	**STUDY 101:** Developed (VRC VIP at the NIH)
	MSD Multiplex: S-protein IgG	MSD Multiplex (Anti-S IgG bAb)	**STUDY 301:** Validated (VRC VIP at the NIH)

Context of Use	Assay Name	Methodology (Parameter measured)	Development Status (Vendor)[a]
Quantitative nAb	PRNT	PRNT (Anti-S IgG nAb)	**STUDY 101:** Developed (VRC VIP at the NIH)
	FRNT	FRNT (Anti-S IgG nAb)	**STUDY 101:** Developed (VRC VIP at the NIH)
	Live Virus MN	Live virus MN (Anti-S IgG nAb)	**STUDY 201:** Qualified (Batelle) **STUDY 301:**Validated (Batelle)
	PsVNA	PsVNA (Anti-S IgG nAb)	**STUDY 101:** Developed (VRC VIP at the NIH) **STUDY 301:** Validated (Duke University medical Center)
T-cell response assessment	ICS assay	Intracellular cytokine staining (cytokines)	**STUDY 101:** Developed (VRC VIP at the NIH)

Abbreviations: bAb = binding antibodies; ECLIA = electrochemiluminescence immunoassay; ELISA = enzyme-linked immunosorbent assay; FRNT = focus reduction neutralization test; ICS = intracellular cytokine stimulation; IgG = immunoglobulin G; MSD = MesoScale Discovery; N = nucleocapsid; N/A = ; nAb = neutralizing antibodies; NIH = National Institutes of Health; QSR = quality statistical report; PRNT = plaque reduction neutralization test; PsVNA = pseudotyped virus neutralization assay; RBD = receptor binding domain; RT-PCR = reverse transcriptase polymerase chain reaction; S = spike; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = Severe Acute Respiratory Syndrome coronavirus 2; VIP = Vaccine Immunology Program; VRC = Vaccine Research Center.

[a] Study 201 immunogenicity assays were qualified for the primary analysis of immunogenicity data (05 Nov 2020 data cutoff) . However, this study is ongoing and validated results have been used for subsequent analysis of immunogenicity data.

[b] Refer to Module 5, Section 5.3.1.4 “Summary of Biomarker Assays used in mRNA-1273 Program” for further information.

2.7.2.5 REFERENCE

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2.7.2.6 APPENDIX

Not applicable