EMA/896245/2022
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Spikevax

Common name: COVID-19 mRNA vaccine (nucleoside-modified)

Procedure No. EMEA/H/C/005791/II/0075/G

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Status of this report and steps taken for the assessment			
Current step	**Description**	**Planned date**	**Actual Date**
☐	Start of procedure	17 Aug 2022	17 Aug 2022
☐	PRAC Rapporteur Assessment Report	22 Aug 2022	22 Aug 2022
	CHMP Rapporteur Assessment Report	22 Aug 2022	22 Aug 2022
☐	PRAC members comments	24 Aug 2022	24 Aug 2022
☐	CHMP members comments	24 Aug 2022	24 Aug 2022
☐	Updated CHMP Rapporteur Assessment Report	26 Aug 2022	26 Aug 2022
☐	PRAC endorsed relevant sections of the assessment report	29 Aug 2022	29 Aug 2022
☐	Updated PRAC Rapporteur Assessment Report	30 Aug 2022	30 Aug 2022
☒	Opinion	01 Sep 2022	01 Sep 2022

Procedure resources	
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1. Background information on the procedure

1.1. Type II variation

Pursuant to Article 7.2 of Commission Regulation (EC) No 1234/2008, Moderna Biotech Spain, S.L. submitted to the European Medicines Agency on 19 July 2022 an application for a group of variations.

The following changes were proposed:

Variations requested		**Type**	**Annexes affected**
B.I.a.6.a	B.I.a.6.a - Changes to the active substance of a vaccine against human coronavirus - Replacement or addition of a serotype, strain, antigen or coding sequence or combination of serotypes, strains, antigens or coding sequences for a human coronavirus vaccine	Type II	I, II, IIIA, IIIB and A

B.I.a.6.a (Type II): Addition of a new strain (Omicron BA.1) resulting in two new Spikevax bivalent Original/Omicron (25 µg elasomeran / 25 µg imelasomeran per dose) 0.1 mg/mL dispersion for injection presentations. The SmPC, the Package Leaflet and Labelling are updated accordingly. The submission includes a revised RMP version 4.2. The variation also includes a number of quality scopes.

The requested group of variations proposed amendments to the Summary of Product Characteristics, Labelling, Package Leaflet, Annex II and Annex A and to the Risk Management Plan (RMP).

The information between these lines is considered commercially confidential and may not be disclosed to third parties in accordance with the ''HMA/EMA guidance on the identification of commercially confidential information and personal data'.

Variations requested		**Type**	**Annexes affected**
B.II.b.3.c	B.II.b.3.c - Change in the manufacturing process of the finished or intermediate product - The product is a biological/immunological medicinal product and the change requires an assessment of comparability	Type II	I, IIIA and IIIB
B.II.b.1.c	B.II.b.1.c - Replacement or addition of a manufacturing site for the FP - Site where any manufacturing operation(s) take place, except batch release/control, and secondary packaging, for biol/immunol medicinal products or pharmaceutical forms manufactured by complex manufacturing processes	Type II	I, IIIA and IIIB
B.I.a.4.b	B.I.a.4.b - Change to in-process tests or limits applied during the manufacture of the AS - Addition of a new in-process test and limits	Type IA	I, IIIA and IIIB
B.I.a.2.c	B.I.a.2.c - Changes in the manufacturing process of the AS - The change refers to a [-] substance in the manufacture of a biological/immunological substance which may have a significant impact on the medicinal product and is not related to a protocol	Type II	I, IIIA and IIIB

B.I.a.2.a	B.I.a.2.a - Changes in the manufacturing process of the AS - Minor change in the manufacturing process of the AS	Type IB	I, IIIA and IIIB
B.I.d.1.b.2	B.I.d.1.b.2 - Stability of AS - Change in the storage conditions - Change in storage conditions of biological/immunological ASs, when the stability studies have not been performed in accordance with a currently approved stability protocol	Type II	I, IIIA and IIIB
B.I.a.4.a	B.I.a.4.a - Change to in-process tests or limits applied during the manufacture of the AS - Tightening of in-process limits	Type IA	I, IIIA and IIIB
B.II.b.3.a	B.II.b.3.a - Change in the manufacturing process of the finished or intermediate product - Minor change in the manufacturing process	Type IB	I, IIIA and IIIB
B.I.d.1.c	B.I.d.1.c - Stability of AS - Change in the re-test period/storage period or storage conditions - Change to an approved stability protocol	Type IA	I, IIIA and IIIB
B.II.e.5.c	B.II.e.5.c - Change in pack size of the finished product - Change in the fill weight/fill volume of sterile multidose (or single-dose, partial use) parenteral medicinal products, including biological/immunological medicinal products	Type II	I, IIIA and IIIB
B.I.a.6.a	B.I.a.6.a - Changes to the active substance of a vaccine against human coronavirus - Replacement or addition of a serotype, strain, antigen or coding sequence or combination of serotypes, strains, antigens or coding sequences for a human coronavirus vaccine	Type II	I, II, IIIA, IIIB and A
B.II.d.2.d	B.II.d.2.d - Change in test procedure for the finished product - Other changes to a test procedure (including replacement or addition)	Type IB	I, IIIA and IIIB
B.II.d.1.e	B.II.d.1.e - Change in the specification parameters and/or limits of the finished product - Change outside the approved specifications limits range	Type II	I, IIIA and IIIB
B.I.b.1.f	B.I.b.1.f - Change in the specification parameters and/or limits of an AS, starting material/intermediate/reagent - Change outside the approved specifications limits range for the AS	Type II	I, IIIA and IIIB
B.I.d.1.a.3	B.I.d.1.a.3 - Stability of AS - Change in the re-test period/storage period - Extension of storage period of a biological/immunological AS not in accordance with an approved stability protocol	Type II	I, IIIA and IIIB
B.II.b.3.b	B.II.b.3.b - Change in the manufacturing process of the finished or intermediate product - Substantial changes to a manufacturing process that may have a significant impact on the quality, safety and efficacy of the medicinal product	Type II	I, IIIA and IIIB

2. Scientific discussion

2.1. Introduction

2.1.1. Problem statement

Disease or condition

COVID-19 is the respiratory disease caused by the coronavirus SARS-CoV2. The virus first emerged as a human pathogen in Wuhan province in China and has spread world-wide causing a pandemic. The WHO declared the COVID-19 outbreak as a pandemic in March 2020. The virus infects the airways and causes a broad spectrum of respiratory infection from asymptomatic infection to Severe Acute Respiratory Syndrome (SARS).

The SARS-CoV-2 virus has repeatedly evolved and appeared in several variants causing new waves of infection. The variants have so far shown cross-reactivity with the original strain, which was the base for the currently approved vaccines. However, there is a concern that presently circulating virus variants are less cross-reactive with the original strain. The variant causing the latest waves of disease at the time of this application has been the Omicron variant, with several subvariants beginning with BA.1. Currently BA.5 is dominating in the EU.

2.1.2. About the product

Spikevax (also referred to COVID-19 Vaccine Moderna or mRNA-1273) is a vaccine developed for prevention of COVID-19 caused by SARS-CoV-2. It is based on nucleoside-modified mRNA encoding for the full-length SARS-CoV-2 spike (S) protein of the prototype Wuhan-Hu-1 virus isolate. The SARS-CoV-2 S-protein is modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilise the spike protein into a prefusion conformation. The mRNA is encapsulated in lipid nanoparticles (LNP). Spikevax has received conditional marketing authorisation in the EU according to Art. 14-a of Regulation (EC) No 726/2004. Following Emergency Use Authorization of mRNA-1273, the phase 3 trial protocol (COronavirus Vaccine Efficacy [COVE]; mRNA-1273-P301) was amended (23 December 2020) from the observer-blind part of the study to an open-label part that is ongoing.

Spikevax is currently indicated for active immunisation to prevent COVID-19 caused by SARS-CoV-2 in individuals 6 years of age and older (conditional marketing authorisation on 06-01-2021).

Spikevax is administered as a course of two 100 µg doses to individuals 12 years of age and older and to children 6 through 11 years of age as a course of two 50 µg doses, which is half of the primary dose for individuals 12 years and older. A third dose of Spikevax may be given at least 28 days after the second dose to individuals 12 years of age and older (100 µg) and children 6 through 11 years (50 µg) who are severely immunocompromised.

A booster dose of Spikevax at a dose of 50 µg mRNA-1273 given at least 3 months after completion of the primary series has been approved for adults 18 years of age and older and for adolescents aged 12 to <18 years.

Due to the emergence of SARS-CoV-2 variants the MAH has developed modified, variant-matched bivalent COVID-19 mRNA vaccines that contain equal amounts of two mRNAs that encode for the Spike protein of the ancestral SARS-CoV-2 (Wuhan-Hu-1) and antigenically divergent variants of concern, each encapsulated into individual LNPs, and co-formulated into a single drug product.

The inclusion of both the original and the variant spikes in the vaccine are intended to broaden immunity as significantly as possible. To that end, inclusion of the Wuhan spike is intended for reactivation and boosting of memory immune cell populations, increasing immunity that was previously present. Inclusion of the variant spike, which has novel epitopes present primarily on the receptor binding site (RBD) and the N-terminal domain (NTD), is intended to engage new naïve immune populations and to elicit new memory responses.

The purpose of this submission is to request an amendment to expand booster vaccination for individuals ≥12 years of age to permit the use of the variant-modified bivalent mRNA-1273.214 (Original + Omicron) vaccine, 50 µg dose, for the prevention of coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2. The dosing regimen is proposed to be an interval of at least 3 months following a primary series or previous booster dose with Spikevax or another authorised/approved COVID-19 vaccine.

The MAH provided results from study P205 in order to infer vaccine efficacy from immunogenicity endpoints. Study mRNA-1273-P205 is an ongoing open label Phase 2/3 study with multiple, sequentially-enrolled cohorts to evaluate the immunogenicity and safety of variant-modified booster candidate vaccines. This submission includes the Day 29 interim results analysing the safety and immunogenicity of the mRNA-1273.214 50 µg booster vaccine as well as supportive safety and immunogenicity data of the Day 181 interim analysis results of the mRNA-1273.211 50 µg booster vaccine. The mRNA-1273.211 Day 181 interim analysis also includes Day 29 immunogenicity data.

Study P205 Part G evaluated the safety, reactogenicity, and immunogenicity of 50 µg of mRNA 1273.214 when administered as a second booster dose in adults who previously received 2 doses of 100 µg mRNA-1273 as a primary series and a single booster dose of 50 µg mRNA-1273.

Study P205 Part F (cohort 2) evaluated the safety, reactogenicity, and immunogenicity of 50 µg of mRNA-1273 when administered as a second booster dose in adults who previously received 2 doses of 100 µg mRNA-1273 as a primary series and a single booster dose of 50 µg mRNA-1273. P205 Part F (cohort 2) serves as the within-study, non-contemporaneous comparator group for the P205 Part G in the comparison between the two booster vaccines, mRNA-1273.214 and mRNA-1273, when administered as second booster doses. The mRNA-1273.214 bivalent vaccine safety and immunogenicity data are summarised as the primary data in this submission.

Study P205 Part A evaluated the safety, reactogenicity and immunogenicity of the mRNA-1273.211 50 µg when administered as a first booster dose in adults who previously received 2 doses of mRNA-1273 as a primary series.

Note: In his submission, the MAH expressed the strength of the bivalent formulation as 0.1 mg/mL. This was not considered acceptable by the CHMP. The MAH was requested to express the strength to reflect each of the active substances. Therefore, the approved strength of the product is: (50 micrograms/50 micrograms)/mL.

2.2. Quality aspects

With this group of variations, the MAH proposes the authorisation of a bivalent COVID-19 vaccine, consisting of mRNA encoding the parental Wuhan strain and mRNA encoding the Omicron BA.1 variant in two presentations: 5 dose and 10 dose multidose vials. Additionally, changes were submitted to reflect adaptations in the manufacturing process, change in storage conditions, to change the identity test, to include a new manufacturing kit line (Kit 8) at Moderna TX, two alternative manufacturing sites for the bivalent Original/Omicron BA.1 finished product (Patheon Italia S.p.A. and Recipharm Monts) and the introduction of a new purity test. The purity assay is changed from an RP-HPLC to an RP-IP-HPLC method.

To note, in his original submission the MAH expressed the strength of the bivalent product presentations applied for in this variation as 0.1 mg/mL. This figure reflects the sum of the quantity of the two active substances it contains (elasomeran and imelasomeran). This was not considered acceptable by the CHMP, and the MAH was requested to change it to (50 micrograms/50 micrograms)/mL to reflect both active substances. As a result, throughout this report the strength of the product is referred to as 0.1 mg/mL and (50 micrograms/50 micrograms)/mL. However, the approved strength for the two multidose vial presentations for the bivalent Original/Omicron BA.1 variant authorised within this procedure (EU/1/20/1507/004and EU/1/20/1507/005) is: (50 micrograms/50 micrograms)/mL.

The main scope of this variation is B.I.a.6.a (Type II): Addition of a new strain (Omicron BA.1) resulting in two new Spikevax bivalent Original/Omicron BA.1 (25 µg elasomeran / 25 µg imelasomeran per dose) (50 micrograms/50 micrograms)/mL dispersion for injection presentations. The text below corresponds to the assessment of this scope.

The format of the assessment of the additional scopes submitted simultaneously within this variation application follows the standard variation/ RSI/ assessment format. The final overall conclusion of the main change (addition of new strain) and additional scopes is presented at the end of the report.

2.2.1. Introduction

The finished product is presented as a dispersion for injection containing 50 micrograms/mL of elasomeran and 50 micrograms/mL of imelasomeran as active substance, embedded in lipid nanoparticles.

Elasomeran is a single-stranded, 5'-capped messenger RNA (mRNA) produced using a cell-free *in vitro* transcription from the corresponding DNA templates, encoding the viral spike (S) protein of SARS-CoV-2 (Original).

Imelasomeran is a single-stranded, 5'-capped messenger RNA (mRNA) produced using a cell-free *in vitro* transcription from the corresponding DNA templates, encoding the viral spike (S) protein of SARS-CoV-2 (Omicron BA.1).

Other ingredients are: SM-102 (heptadecan-9-yl 8-{(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino}octanoate), cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC, 1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2000- DMG), trometamol, trometamol hydrochloride, acetic acid, sodium acetate trihydrate, sucrose and water for injections

The product is available in two presentations with fill volumes of 3.2 mL or 6.3 mL in type 1 glass or type 1 equivalent glass or cyclic olefin polymer with inner barrier coating multidose vials with a stopper (chlorobutyl rubber) and a blue flip-off plastic cap with seal (aluminium seal), containing 5 or 10 doses, respectively. Pack size: 10 vials.

2.2.2. Active substance: elasomeran

The active substance elasomeran (also known as CX-024414) is already approved in the existing Spikevax conditional marketing authorisation. Several changes to the information related to elasomeran were included within this variation application, e.g. change in purity method (from RP-HPLC to RP-IP-HPLC), extension of the storage period, change of the storage conditions, tightening of the bioburden in-process limits. These changes are described below under the section 'additional scopes'.

2.2.3. Active substance: imelasomeran

General information

To introduce the bivalent vaccine the MAH submitted data to support implementation of the Omicron variant mRNA (CX-031302) manufacture.

The generic name of CX-031302 is imelasomeran and it encodes for the pre-fusion stabilised Spike protein of 2019-novel Coronavirus (SARS-CoV-2) B.1.1.529 Omicron Variant.

B.1.1.529 has multiple mutations in the S-protein including several that increase the likelihood of transmissibility and cause a reduction in susceptibility to neutralisation. The mutations in the S-2P protein of the B.1.1.529 variant encoded by CX-031302 include the following: A67V, Δ69-70, T95I, G142D/Δ143-145, Δ211/L212I, ins214EPE, G339D,

S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F. The MAH has designed mRNA-1273.529 containing CX-031302 which encodes for the S-2P of B.1.1.529.

CX-031302 mRNA is chemically identical to naturally-occurring mammalian mRNA with the exception of the uridine nucleoside normally present in mammalian mRNA. This is fully replaced with N1-methylpseudouridine, a naturally-occurring pyrimidine base present in mammalian tRNAs. This nucleoside is included in the CX-031302 mRNA in place of the normal uridine base to minimise the indiscriminate recognition of CX-031302 mRNA by pathogen-associated molecular pattern (PAMP) receptors (e.g., Toll-like receptors). The molecular sequence of CX- 031302, including the 5’ cap, the 5’ untranslated region (UTR), the Open Reading Frame (ORF), the 3’ UTR, and the 3’ polyA tail, is provided.

The amino acid sequence alignment between the Prototype (the construct for the currently approved product) and B.1.1.529 S-2P is also presented; with the differences highlighted.

The corresponding mRNA sequence alignment between CX-024414 (Prototype) and CX-031302 (B.1.1.529) is also presented; with the differences highlighted.

The information provided is considered adequate.

Manufacture

Manufacturers

No new active substance manufacturing sites have been introduced with this submission. However, unlike CX-024414 (the active substance for the currently approved product), CX-031302 will not be manufactured at Lonza Biologics, Inc., US.

The GMP compliance of these sites has been previously confirmed.

Description of manufacturing process and process controls

The manufacturing process and process controls are the same as currently approved for the manufacture of elasomeran, including the additional changes included within this variation application described below under the section ‘additional scopes’ which apply to both active substances.

Control of materials

Raw materials:
No changes compared to the CX-024414 raw material.

Starting material:
The starting materials in the manufacture of CX-031302 mRNA are the linearised plasmid template and the nucleotides ATP, CTP, GTP, N1-Me-ΨTP. The nucleotides are the same nucleotides as used for manufacture of CX-024414 mRNA.

Linearised Plasmid Template:
A unique linearised DNA plasmid template specific for CX-031302 mRNA was manufactured at ModernaTX, Inc. (Norwood, MA, USA). The features of the plasmid template specific for CX-031302 mRNA are consistent with CX-024414 mRNA, with the exception of the specific sequence of the coding region. The full plasmid DNA sequence and the plasmid map are provided. The host cell line used for manufacture of PL-028274 for CX-031302 mRNA is the same as described for CX-024414 mRNA.

The cell banking system is two-tiered, including a master cell bank (MCB) and a working cell bank (WCB). The manufacturers involved in cell bank production are listed. Manufacture and testing of MCB and WCB was conducted as for the original CX-024414 containing plasmid.

Release results for MCB and WCB are provided including for culture purity, lytic and lysogenic bacteriophages, viability, marker retention, strain identity (for MCB only), plasmid identify, plasmid integrity and plasmid copy number. The analytical procedures used to perform release are also described. Qualification of MCB and WCB have also been described.

The MCB and WCB stability protocol and all available data are provided. The test methods and acceptance criteria are the same as for release testing. All available data show compliance to specification.

The CX-031302 mRNA plasmid, PL-028274, is manufactured for CX-031302 mRNA using the same procedure as described for CX-024414 mRNA. The same approach to characterisation testing and kanamycin risk assessment described for CX- 024414 mRNA was taken for CX-031302 mRNA.

The specification for the linearised plasmid includes: appearance, concentration, plasmid identity, %linear plasmid, residual genomic DBA, residual RBNA, residual protein, bacterial endotoxin and bioburden.

The final filtered bulk long-term storage condition for the linearised plasmid is -20°C ± 5°C, with a formal shelf-life of three years.

A shelf life of 3 years under long-term storage condition of -20°C ± 5°C is requested for the linearised plasmid based on the prototype vaccine and supported by limited data collected in an on-going stability study that have been initiated linearised plasmid. Considering that no changes are included in the manufacturing process of the DNA template as compared to the original variant, the shelf-life is considered sufficiently supported by the original data.

Control of critical steps and intermediates

The control of critical steps and intermediates are the same as currently approved for the manufacture of elasomeran.

Process validation and/or evaluation

For manufacture of this and all future variants which use a manufacturing process and control strategy equivalent (except for specific variant sequence) to the prototype mRNA-1273, the MAH proposes to produce one confirmatory process verification lot to demonstrate process consistency per site per the requirements specified in the Process Validation Master Plan. The introduction of CX-031302 mRNA also included the qualification of an additional identical Train 8 (see relevant scope under the section

'additional scopes') and enabling Train 8 for prototype mRNA-1273 and all variants using a mRNA-1273 equivalent process and control strategy at Moderna Norwood. The MAH has successfully completed process verification of the CX-031302 mRNA manufacturing process as a means of demonstrating that the commercial-scale manufacturing process is capable of consistently delivering quality product.

The process performance qualification (PPQ) verification has generated data at commercial scale to support and complement laboratory-scale studies. This can be accepted since the manufacturing process is identical to that used for the original variant with some changes described and justified under the section 'additional scopes'.

Manufacturing process development

The development of additional mRNA-1273 vaccines was initiated by the MAH in response to the emergence of SARS-CoV-2 variants of concern. Sequences for mRNA-1273 vaccines are designed based upon the prefusion-stabilised two-proline (S-2P) encoding sequence for CX-024414 mRNA. Changes relative to this sequence are made only to incorporate the specific mutations of the variant S protein sequence encoded by the sequence of the specific mRNA-1273 RNA. The manufacturing process and analytical control strategies established for the CX-024414 mRNA are applied directly to all mRNA-1273 RNAs.

Subsequent process characterisation beyond that described for CX-024414 was performed for mRNA-1273 RNAs, using the same scale down models used in CX-024414 mRNA process characterisation, with the intent of verifying the applicability of the process description and process control strategy defined in Section 3.2.S.2.2 {mRNA-1273 RNA} and Section 3.2.S.2.4 {mRNA-1273 RNA}, respectively, to all mRNA-1273 RNAs.

The critical process parameters (CPPs) and critical in process controls (CIPCs) for the mRNA-1273 RNA manufacturing process are provided. CPPs and the CIPC are consistent for all mRNA-1273 RNAs, including CX-024414 mRNA.

A comparability study between CX-024414 to CX-031302 was conducted.

The following three elements were included in the comparability study:

- Evaluation of process performance with respect to critical process parameters (CPPs) and in-process controls (IPCs).
- Statistical evaluation of comparability of release testing results.
- Statistical evaluation of selected extended characterisation results.

The mRNA sequence was the only change represented in this comparability exercise. All CIPC and IPC results met the acceptable ranges. Release results met both specification and comparability acceptance criteria and extended characterisation results met the comparability expected ranges established from development, clinical, Scale A, preliminary Scale B, and Scale B CX-024414 data. Therefore, the results from the commercial Scale B (75 L in vitro transcription -IVT- scale) PPQ lot of CX-031302 manufactured at Lonza (Visp, Switzerland) demonstrated that the manufacturing processes and quality attributes were comparable.

Characterisation

The structure and physicochemical properties of CX-031302 mRNA were studied using a variety of techniques applicable to mRNAs, including: determination of UV extinction coefficient, circular dichroism

spectrum, reverse transcription followed by Sanger di-deoxynucleotide sequencing, oligonucleotide mapping, N1-methyl-pseudouridine (N1-MeΨU) ID and content, cap identity, Poly A tail length and dispersity, sequence homogeneity of the CX-031302 mRNA coding region, melting profile by Differential Scanning Calorimetry (DSC).

Process impurities were evaluated. These included double stranded RNA (dsRNA) and residual protein.

There are no new impurities as compared to parental mRNA.

The characterisation of the new mRNA CX-031302 is considered acceptable

Specification

The specification for imelasomeran is presented and contains tests for appearance, identity (reverse transcription/Sanger sequencing), total RNA content (UV), purity (RP- IP-HPLC), product related impurities (RP-IP-HPLC), % 5'- Capped (RP-HPLC), % Poly(A) tailed RNA (RP-HPLC), pH (Ph. Eur.), bacterial endotoxin (Ph. Eur.) and bioburden (Ph. Eur.).

The proposed specification for imelasomeran is identical to the specification for elasomeran and is considered adequate. The analytical methods used for release testing of CX-031302 and prototype CX-024414 are identical with the exception of the identity method, since this is the only method that is sequence-specific. Confirmation of mRNA Sequence by RT-PCR and Sanger Sequencing has been validated and shown to be suitable for the purpose of determining the mRNA identity of CX-031302. The validation characteristics evaluated were specificity and intermediate precision.

To note, a new analytical procedure for determination of purity (RP-IP-HPLC) was introduced within this variation application (see section 'additional scopes'). During the evaluation the CHMP raised a Major Objection due to a shift in purity values obtained with the new method and the MAH proposal to keep the existing acceptance criteria. To address this concern, the MAH adapted the finished product release and end of shelf life specification to account for this shift. Nonetheless, the MAH is recommended to reassess the need to adjust the purity specification limits at the level of the active substance (REC10).

Batch results are presented for two PPQ commercial scale batches from each manufacturer. All batches met the specification acceptance criteria in place at the time of release and confirm consistency of the manufacturing process.

Reference standards of materials

The reference material as described for elasomeran serves as the reference material for imelasomeran. During the review a question was raised to ask the MAH to clarify this. The MAH indicated that the RNA reference material is used as a system suitability standard for several release tests and is also used as a reference standard for measurement of total RNA content. As a system suitability standard, this material is used to assess the system suitability of the analytical testing, for example the consistency of retention time or peak area response in an HPLC-UV method.

Since the mRNA-1273 RNAs all have similar lengths, they have very similar molar extinction coefficients. For example, the calculated sequence-corrected coefficient for CX-031302 mRNA (33.99 µg/mL) and CX-024414 mRNA (34.01 µg/mL) are within 0.06% of each other. Thus, the use of a single RNA standard is suitable for total RNA content measurement across different variant mRNA-1273 materials. The justification presented is acceptable.

Container closure system

The container closure system is the same as for the currently approved active substance, elasomeran.

Stability

An initial shelf-life of 36 months is proposed for CX-031302 mRNA material stored in the commercial container closure system (gamma irradiated, single-use storage bags), when stored at the recommended long-term storage condition of -60°C to -90°C.

The CX-031302 registration stability program was executed according to ICH Q1A (R2), Stability Testing of new Drug Substances and Products, and ICH Q5C, Stability Testing of Biotechnological/Biological Products.

The CX-031302 mRNA is stored at -60 to -90°C, after an optional interim storage at -15 to 25°C of maximum 3 months.

The properties of CX-031302 mRNA with respect to the attributes that affect product potency have been systematically and thoroughly assessed. These attributes include fidelity of the RNA sequence including cap, tail, and open reading frame and integrity of the RNA. Direct measurements of those attributes have been established and are included in the routine release panel for CX-031302 mRNA. The stability of CX-031302 mRNA will be evaluated against a broad set of stability data and modelling data encompassing both CX-024414 mRNA registration lots and additional variant RNAs associated with the mRNA-1273 program.

As discussed at the time of the CMA, the product quality attribute expected to change most during the manufacture and distribution of the product is mRNA purity, which represents the fraction of intact mRNA. The degradation of RNA in the product has been extensively studied by applying a sensitive chromatographic assay to assess the formation of RNA degradants. The principal route of degradation for RNA is hydrolytic chain scission to species that elute prior to the main peak (RNA fragments). mRNA purity correlates with protein levels measured in the in vitro relative protein expression assay. Direct measurement of RNA degradation utilising the RNA purity assay by RP-HPLC is precise, accurate and the most stability-indicating measure of product activity. The MAH indicated that the stability profiles for all the stability-indicating attributes are being evaluated and monitored but purity is the primary determinant of shelf-life, since it is the most stability-indicating attribute.

The shelf-life has been justified from a purity statistical model. The lots used in the purity modelling analysis were manufactured using a development process, small-scale Personalised Vaccine Unit (PVU) scale process, the initial Scale B process, and the Scale B process. Modelling included data from development lots from additional variant RNAs to incorporate sequence differences into the statistical model.

Stability and characterisation studies were designed to evaluate product stability under various stressed and long-term storage conditions. All lots were manufactured using the commercial manufacturing process. Stability samples were stored at -60°C to -90°C (data up to 24 months available), -20 ± 5°C (data up to 24 months available) -and 5 ± 3°C (data up to 3 months available) in containers made of the same materials as the commercial closure system. In the initial submission the MAH provided limited stability data and did not justify the mRNA characteristics to justify the comparability between the different mRNAs and this raised a major objection. In response the MAH provided 24 month stability data at -60°C to -90°C and described the characteristics of the CX-027367 mRNA that is used in the supportive stability study at-60°C to -90°C. This is acceptable.

Size-based RNA purity and polyA tailed RNA, as determined by reverse-phase high-performance liquid

chromatography (RP-HPLC), were demonstrated to be stability indicating.

A stability study was also initiated for CX-031302 mRNA manufactured at ModernaTX, Inc. (Norwood, MA) (refer to the section 'additional scopes'). Additional confirmatory stability studies for the proposed long term storage condition for CX-031302 mRNA have been included. These studies are currently ongoing.

The claimed shelf-life is considered sufficiently justified based on data from the original variant and modelling results. The modelling confirmed similar degradation rates for CX-024414 mRNA and variant RNA supporting the same shelf life for CX-024414 and CX-031302 mRNA. These results therefore support the proposed shelf-life of 36 months for CX-031302 mRNA when stored at -60°C to -90°C.

The MAH is recommended to submit the confirmatory stability data when available (REC3).

2.2.4. Finished product

Description and composition of the drug product

The bivalent vaccine finished product Original/ Omicron BA.1 is a white to off-white sterile dispersion for injection in a preservative-free buffer containing 20 mM Trometamol (Tris), 2.1 mM acetate, 87 g/L sucrose at pH 7.5 for intramuscular administration.

The mRNA-1273.214 bivalent vaccine combines two mRNA sequences (equal mass) encoding for the pre-fusion stabilised Spike glycoprotein of:

- the prototype Wuhan-Hu-1 2019-novel Coronavirus (SARS-CoV-2) (as mRNA CX-024414), and
- the B.1.1.529 (Omicron) Variant-of-Concern (as mRNA CX-031302)

The finished product (also referred to as mRNA-1273.214) is an mRNA- lipid nanoparticle (LNP) dispersion that contains the two mRNAs (CX-024414 and CX-031302) that encode for the pre-fusion stabilised Spike glycoproteins described above, and four lipids which act as protectants and carriers of the mRNA: SM-102 (a custom-manufactured, ionisable lipid), cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1, 2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2000-DMG).

The qualitative and quantitative composition of the product, including amounts per vial, function and quality standard applicable to each component, was provided. Each 0.50 mL dose of the vaccine contains 25 micrograms of each active substance.

All excipients except the lipid excipients SM-102, DSPC and PEG2000-DMG and trometamol-HCl comply to Ph. Eur. grade. The excipients are the same as used in the currently approved formulations of Spikevax (EU/1/20/1507/001-003).

The container closure system is a type 1 glass or type 1 equivalent glass or cyclic olefin polymer (with inner barrier coating) multidose vial with a stopper (chlorobutyl rubber) and a blue flip-off plastic cap with seal (aluminium seal). It is the same container closure system as for the prototype vaccine (0.2 mg/mL: EU/1/20/1507/001).

Pharmaceutical development

This type II-variation introduces a bivalent finished product of Spikevax that is a preservative-free, sterile dispersion of RNA-containing lipid nanoparticles in an aqueous cryoprotectant buffer for intramuscular

administration. The bivalent finished product contains a 1:1 ratio of the original and Omicron BA.1 variant strains at a concentration of (50 micrograms/50 micrograms)/mL.

Briefly, the bivalent Original/ Omicron BA/.1 finished product is manufactured by pooling mRNA-1273 LNP-B (containing mRNA CX-024414) and mRNA-1273.529 LNP-B (containing mRNA CX-031302), followed by a dilution with a dilution buffer containing 20 mM Tris and 87 mg/mL sucrose, pH 7.5.

The mRNA-1273.214 FP shares the same compositional platform as the authorised 0.1 mg/mL formulation ((EU/1/20/1507/002-003). Therefore, all aspects of Spikevax 0.1 mg/mL development can be extrapolated to the bivalent mRNA-1273.214 DP.

The Quality Target Product Profile (QTPP) of Spikevax, which is generally applicable to all strengths of the finished product is presented.

No changes have been made compared to the QTPP for the original vaccine.

According to the MAH, no change in physicochemical properties, processability and stability is expected for the bivalent vaccine compared to the 0.1 mg/mL formulation. This is agreed to.

In accordance with ICH Q9, a systematic assessment of the potential risk to mRNA-1273.214 finished product quality was performed with respect to the manufacturing process.

*CX-024414 mRNA loaded LNP intermediate (*referred to by the MAH as *mRNA-1273 LNP-B)*

This is the same intermediate used to prepare the 0.1 mg/mL formulation ((EU/1/20/1507/002-003).

*CX-031302 mRNA loaded LNP intermediate (*referred to by the MAH as *mRNA-1273.529 LNP-B)*

The only difference between mRNA-1273.529 LNP-B and mRNA-1273 LNP-B is the mRNA sequence. mRNA-1273.529 LNP-B is produced in a 200 g nominal batch size, according to the same manufacturing process described for mRNA-1273 LNP-B.

Process characterisation beyond those conducted for previous formulations, was performed for multiple mRNA-1273 LNPs, including mRNA-1273.529 LNP-B.

The CPPs for the mRNA-1273.529 LNP-B manufacturing process are provided in Table 10. No CIPCs were identified for the mRNA-1273.529 LNP-B manufacturing process.

Across process characterisation studies including multiple mRNA-1273 LNPs, relationships between the studied process parameters and the evaluated CQAs were consistent, and any risk to product quality resulting from change in sequence is not expected based on the observed results. The control strategy verification approach confirmed the applicability of a consistent set of critical process parameter designations and corresponding proven acceptable ranges (PARs) from the prototype mRNA-1273 LNP to mRNA-1273.529 LNP-B.

A conducted failure modes and effects analysis (FMEA) concluded that due to the commonality, the mRNA-1273 LNP manufacturing process and process control strategy is applicable to mRNA-1273.529 LNP. Specifically, this includes the classification of process parameter criticality and the CQAs determined to be impacted by variations in critical process parameters. Leveraging prior process characterisation knowledge established for the prototype mRNA-1273, a streamlined approach focused on process control strategy verification for process steps where critical process parameters were identified for the prototype mRNA-1273 LNP.

Process characterisation and control strategy verification studies for mRNA-1273.529 LNP-B (Omicron) employed the same scale down model developed and assessed for suitability as part of the prototype mRNA-1273 LNP process characterisation.

A development history, lot genealogy and usage of mRNA-1273.529 LNP-B finished product intermediate has been provided.

Comparability of mRNA-1273.529 LNP-B versus mRNA-1273 LNP-B

The following three elements were included in the comparability study:

1. Evaluation of process performance with respect to critical process parameters (CPPs) and in-process controls (IPCs).
2. Statistical evaluation of comparability of release testing results.
3. Statistical evaluation of extended analytical characterisation testing results.

All IPC results met the acceptable ranges. Release results met both specification and comparability acceptance criteria and extended characterisation results met the comparability expected ranges established, demonstrating comparability for all manufacturing sites (Moderna TX, Lonza Visp and Rovi Granada).

Finished product (referred to by the MAH as mRNA-1273.214)

The mRNA-1273.214 Drug Product is manufactured by combining two mRNA-LNP finished product intermediates (mRNA-1273 LNP-B and mRNA-1273.529 LNP-B and further dilution.

Based on the demonstrated control of RNA content in the mRNA-LNP established through the extensive manufacturing history of the mRNA-1273 prototype vaccine, a process control strategy employing direct gravimetric combination of the mRNA-1273 LNP-B and mRNA.1273.529 LNP-B followed by dilution based on the nominal mRNA-LNP RNA concentration of 0.82 mg/mL has been defined. This was supported by a Monte Carlo statistical model and the RNA content data from release of 682 mRNA-1273 LNP commercial batch certificates of analysis across three manufacturing sites (Moderna MTC-S, Lonza Portsmouth, and Lonza Visp).

Pooling and dilution operations have been adequately characterised and validated, demonstrating the ability to apply the existing validated manufacturing process parameters from the original vaccine to the bivalent drug product giving a homogeneous drug product with the desired quality attributes

Characterisation of the clarification / bioburden reduction filtration and sterile filtration operations and the conditioning freeze step are the same as for the mRNA-1273 prototype vaccine.

A science-based approach was used to identify CQAs, CPPs and CIPC to inform process design studies, and to establish the manufacturing control strategy for the finished product. The proposed CQAs for the finished product are listed in the specifications section.

A FMEA was performed to identify potential failure modes for the manufacturing process and to evaluate the impact these failures might have on product quality and/or process performance.

Proven acceptable Ranges (PARs) for CPPs were characterised using a science- and risk-based approach that leveraged current process understanding and historical knowledge from platform unit operations for similar products.

The studies were designed to mitigate potential risks for CPPs identified during the FMEA and establish PARs for the associated process parameters to maintain CQAs.

A development history, lot genealogy and usage of the bivalent vaccine has been provided.

The changes in the manufacturing process implemented to support the bivalent finished product manufacturing have been discussed. They include changes in mRNA sequence, pooling of LNPs and filtration (combination of batch filtration processed to reduce risk of filter fouling).

Comparability of mRNA-1273.214 versus mRNA-1273 FP (prototype vaccine).

The pooling of mRNA-1273 LNP-B (containing mRNA CX-024414) and mRNA-1273.529 LNP-B (containing mRNA CX-031302) and blending step to produce a bivalent finished product and implementation of 3X filtration are represented in this comparability exercise.
The following two elements were included in the comparability study:

- Evaluation of process performance with respect to CPPs and IPCs
- Statistical evaluation of comparability of release testing results.

Extended analytical characterisation and forced degradation testing were not performed for mRNA-1273.214 DP as part of comparability studies since the mRNA-1273 finished product characteristics are the same as the mRNA-1273 LNP finished product intermediates. Therefore, the extended characterisation results for mRNA-1273 LNPs finished product intermediate are considered representative of mRNA-1273 finished product. This approach was used in the development of the authorised formulations and is considered acceptable. The complete comparability methodology and statistical analysis have been described. All CIPC and IPC results met the acceptable ranges and release results met both specification and comparability acceptance criteria established from development, clinical, Scale A, and Scale B mRNA-1273 FP data for all the manufacturing sites (vial line 3 at Catalent, Rota line at Recipharm, Dara and Marchesini Lines at Rovi SSRR and the Xtrema Line at Patheon Monza).

The LNP, finished product formulations and processes have remained the same throughout development of the original vaccine except for necessary changes to the scale as development progressed from initial clinical supplies to commercial manufacture and, changes related to the mRNA-loaded LNP intermediate mRNA-1273 LNP-B made for the 0.1 mg/mL formulation.

Comparability has previously been acceptably demonstrated between clinical and commercial scale original finished product, between various manufacturing sites via comprehensive studies including both release testing and extended characterisation testing. Due to the application of the same formulation, manufacturing process and the use of the same manufacturing sites as the original finished product, extensive prior experience is leveraged. It is found acceptable and sufficient that comparability has been established between the bivalent vaccine finished product to the original finished product based on an evaluation of release testing results against the acceptance criteria in the finished product specification.

For the bivalent vaccine, batch analysis data are provided in section 3.2.P.5.4 for 3 PPQ batches from each finished product manufacturing site (Catalent, Indiana; Patheon, Monza; Rovi, San Sebastian de los Reyes; and Recipharm, Monts). All these batches met the specification acceptance criteria in place at the time of testing. This is found acceptable.

The control strategy for bivalent finished product is based upon the control strategy for the prototype vaccine.

All quality attributes and controls described for the original vaccine are still applicable to the bivalent finished product. In addition, the weight ratio of pooled LNP is introduced as CIPC specific to the bivalent finished product to ensure that an appropriate amount of LNP of each type (containing CX-024414 or CX-03130 has been pooled).

No new information has been provided on microbiological attributes and compatibility studies claiming that the data provided for the prototype vaccine applies to the bivalent product. This is acceptable.

In conclusion, the information provided on the pharmaceutical development of the bivalent vaccine is found sufficient and acceptable.

Manufacture

The manufacturing process consists of preparation of mRNA loaded-LNP finished product intermediates, dilution buffer preparation, LNP thawing, LNP pooling, dilution, clarification/bioburden reduction filtration, sterile filtration, aseptic filling, stoppering and capping.

mRNA loaded LNP intermediate containing mRNA CX-024414 (referred to by the MAH as mRNA-1273 LNP-B)

The manufacturing process is identical to the one used for the 0.1 mg/mL strength.

mRNA loaded LNP intermediate containing mRNA CX-031302 (referred to by the MAH as mRNA-1273.529 LNP-B)

mRNA-1273.529 LNP-B are produced in a 200 g nominal batch size, according to the same manufacturing process and in-process controls described for mRNA-1273 LNP-B. The only difference between these finished product intermediates is the mRNA sequence loaded.

Since the same mRNA manufacturing process and equivalent control strategy is used (mRNA sequence specific control elements); one single Process Verification / Comparability batch was performed to introduce the mRNA-1273 LNP manufacturing at each site. All PPQ verification lot release testing results encompassing all CQAs, met the predefined acceptance criteria demonstrating consistent, robust and well-controlled process performance and product quality.

Qualification of all process hold times was performed independently from PPQ verification.

Finished product

The finished product is manufactured at the manufacturing sites, and using the same platform manufacturing process, as currently approved for Spikevax 0.2 mg/mL and 0.1 mg/mL in vials, namely Catalent, Rovi, and Recipharm, with the addition of Patheon, Monza which is a new manufacturing site being introduced within this variation application (see section on additional scopes). The GMP compliance of these sites has been confirmed.

As described above, there are no changes in the manufacturing process with respect to the existing 0.1 mg/mL formulation except for the pooling of the two mRNA-loaded LNP finished product intermediates containing either CX-024414 or CX-031302), with the addition of an IPC to achieve equal mass of the two RNA sequences by controlling the weights of mRNA-1273 LNP-B and mRNA- 1273.529 LNP-B pooled and adjustment of the dilution step to the target concentration of 0.1 mg/mL (sum of both mRNAs). The diluted bulk product solution is mixed to obtain a homogeneous solution. The updated process parameters and controls for the dilution step for bivalent mRNA-1273.214 finished product have been described.

The manufacturing process is described for all four sites separately.

As for the existing 0.1 mg/mL formulation, two alternative manufacturing process flows have been designed for mRNA-1273.214 DP.

Similar controls during manufacture and similar hold times are applied for both original and bivalent finished product. The manufacturing process is considered sufficiently described including acceptable in-process controls (IPCs) and hold times.

The batch formula for the manufacture of mRNA-1273.214 DP at Rovi, Catalent, Patheon (Monza) and Recipharm is provided.

Process validation has been performed for the four finished product manufacturing sites.

Catalent: Process parameters, in-process controls, microbial controls, in-process holds and release tests indicate that the process is under control and consistently produces product that meets all predetermined quality attributes and in-process controls.

Rovi: Process parameters, in-process controls, microbial controls, in-process holds and release tests indicate that the process is under control and consistently produces product that meets all predetermined quality attributes and in-process controls.

Recipharm: Process parameters, in-process controls, microbial controls, in-process holds and release tests indicate that the process is under control and consistently produces product that meets all predetermined quality attributes and in-process controls.

Monza: The validation protocol and validation interim report have been provided. Validation shows consistent results for process parameters, in-process controls, microbial controls, in-process holds and release tests. Crimping parameters have also been qualified. Media fill and cleaning validation have been sufficiently performed.

Upon request from the CHMP, the final Time out of Refrigeration (TOR) and Cumulative Process Duration (CPD) supported by the PPQ exercise for each site up to the manufacture of the commercial labelled finished product were provided, except for Catalent and Recipharm which will be provided by September 2022 and October 2022, respectively (REC5).

The homogeneity of the diluted mRNA-1273.214 FP was evaluated with respect to osmolality, pH, and RNA concentration to qualify a mixing duration of 20 to 30 minutes. All of the data were within acceptance criteria and mRNA content shows no significant variation from top, middle, and bottom samples, confirming the suitability of the established process parameters.

The homogeneity of filled vials was evaluated by testing one vial from the beginning, middle, and end of the filling and visual inspection processes for RNA content, RNA ratio, mRNA purity, particle size and polydispersity, and RNA encapsulation. The results demonstrate consistent process performance.

Product specification

mRNA-1273 LNP-B

The following attributes have been included in the specification for mRNA-1273 LNP-B: appearance, mRNA identity by reverse transcription/Sanger sequencing, total RNA content by anion exchange chromatography, purity and product-related impurities by RP-HPLC, % RNA encapsulation by absorbance assay, mean particle size and polydispersity by DLS, lipid identity by UPLC-CAD (SM-102, cholesterol, DSPC, PEG2000-DMG), lipid content by UPLC-CAD (SM-102, cholesterol, DSPC, PEG2000- DMG), lipid impurities by UPLC-CAD (% individual impurities and sum of impurities), pH, osmolality, bacterial endotoxins (Ph. Eur. 2.6.14, kinetic chromogenic method) and bioburden (Ph. Eur. 2.6.12).

This section is similar to the registered information for the 0.1 mg/mL formulation, but has been updated with the following information (see additional scopes):

• Replacement of the RP-HPLC method for purity and related impurities with a new RP-IP-HPLC

• The new specification for Product-related Impurities

• To provide the new name for the Absorbance-based Assay used to assess the % RNA Encapsulation

mRNA-1273.529 LNP-B

The specifications proposed for the newly introduced mRNA-1273.529 LNP are identical to the specifications applied to the prototype mRNA-1273 LNP-B, and only the analytical method used for identity testing is newly developed to unequivocally identify the mRNA-1273.529 LNP-B variant sequence. A validation summary of the method has been presented.

As described above, a new RP-IP-HPLC test method is also being introduced for control of both mRNA-1273 LNP-B and mRNA-1273.529 LNP-B. The specifications were updated as a consequence to this change (see additional scopes).

Batch analysis data from all the manufacturing sites have been provided.

Finished product

The proposed finished product release specification for the bivalent Original/Omicron BA.1 vaccine is provided. It includes tests for appearance (visual), total RNA content by anion exchange chromatography, mRNA identity and mRNA ratio by RP-HPLC, purity and product-related impurities by RP-IP-HPLC, % RNA encapsulation by by absorbance assay, *in vitro* translation (methionine labelling), lipid identification by HPLC-CAD (SM-102, cholesterol, DSPC, PEG2000-DMG), lipid content by HPLC-CAD (SM-102, cholesterol, DSPC, PEG2000-DMG), lipid impurities by HPLC-CAD (% individual impurities and sum of impurities), particle size and polydispersity by DLS, pH , osmolality , particulate matter , container content (USP), bacterial endotoxin (*Ph. Eur.* 2.6.14, kinetic chromogenic method), sterility (*Ph. Eur.* 2.6.1) and container closure integrity (stability only).

The specification for the bivalent vaccine is based on the specification for the prototype formulation.

A test for identity and RNA ratio have been added to ensure that the intended content of each individual mRNA is present in the finished product. Since the total RNA content method measures total RNA and cannot distinguish RNA from different mRNA sequences, a measurement of the ratio of the two RNA components present in the finished is needed to ensure that each component is present at the intended level. Together, control of total RNA content and RNA ratio ensure that each finished product batch contains the intended dose of the individual RNA components. During the review the MAH was asked to clarify whether the RNA ratio method is specific to intact RNA or whether RNA fragments or adducts will also contribute to the result. The MAH clarified that RNA ratio determination is based on total RNA but argues that because of the high consistency of the purity of batches produced, only slight differences will occur. The explanation is only partially supported as average purity results do not rule out single batch combination with higher purity differences however, the issue will not be further pursued since the level of RNA fragments and RNA lipid adducts are controlled at release for each mRNA-LNP used in the manufacture of the mRNA-1273.214 DP.

mRNA-1273.214 FP is first digested with primer-guided RNase H, which targets a specific sequence within the open reading frame (ORF) of the RNAs present in the finished product. This digestion results in short RNA oligonucleotides, that are specific to each individual mRNA-1273 RNA component present in the finished product (14 nucleotides for CX-031302 mRNA and 21 nucleotides for CX-024414 mRNA comprising the mRNA-1273.214 finished product). As such, a bivalent finished product would result in two unique oligonucleotides after RNase H digestion. The resulting RNA oligos are separated by ion-pairing reversed phase chromatography and detected online by UV. A detailed validation summary has been presented. The method was shown to be specific, accurate, precise, and linear for the determination of RNA ratio.

As indicated in the imelasomeran active substance section, a new analytical procedure for determination of purity (RP-IP-HPLC) has been introduced within this variation application (see section 'additional scopes') to replace the existing RP-HPLC method. The validation characteristics evaluated were system suitability, specificity, linearity, accuracy; precision, range, robustness, determination of the detection and the quantitation limits and stability of standard and sample preparation solutions. Validation results are acceptable. A bridging study was performed to demonstrate comparable results with both RNA purity methods. Results showed higher purity values with the new method, specially at the level of the finished product. This change in method resulted in the revision of the assay specification limits for release and for end of shelf life to account for the higher results consistently produced by the new method as requested by CHMP (MO). Nonetheless, the MAH is recommended to reassess the need to adjust the purity specification limits at the level of elasomeran active substance and finished product intermediates (REC10).

During the review it was noticed that the in vitro translation test is not specific for the two different mRNAs. Since this is only a qualitative test that demonstrates the production of a protein of the expected size in vitro, it was raised that there is no proof of functionality of both mRNAs if the test is conducted at the finished product level. The in vitro translation test should be performed at the level of the mRNA-LNP intermediates as well, unless potency of both mRNA-LNPs in the final product can be demonstrated otherwise (Major Objection). In response, the MAH indicated that they have utilised in vitro relative protein expression (IVRPE) to quantitatively confirm the intended protein expression level of each monovalent LNP comprising the mRNA-1273.214 finished product. Two IVRPE assays are implemented as characterisation tests with optimised method parameters and product-specific reference standards to assess the protein expression of both mRNA-1273-B LNP and mRNA-1273.529 LNP-B, as part of analytical comparability. A strong correlation between protein expression as measured by IVRPE as well as in-vivo immunogenicity to total RNA content and purity has been shown. On the basis of this strong correlation, the protein expression of the monovalent LNP can be assured when RNA content and purity are well

controlled within their respective specification limits. The mRNA-1273.214 finished product manufacturing process consists of thaw and pooling of mRNA-1273 and mRNA-1273.529 LNPs, dilution, and mixing prior to sterile filtration and fill steps. Thus, protein expression is expected to remain consistent from monovalent LNPs to the bivalent finished product.

Spikevax is currently controlled using the In-Vitro Translation (IVT) assay as part of the finished product specification. The In-Vitro Relative Protein Expression (IVRPE) assay is not part of the routine Quality Control testing, but rather a characterisation assay as part of analytical comparability. The MAH has committed to work on the required implementation of the IVT assay at the mRNA-LNPs stage and submit the proposed change in Control Strategy by End of December 2022 (REC1). This is acceptable given the strong correlation between RNA content / purity and in vivo immunogenicity.

The impurity profile of mRNA-1273.214 finished product is the same as that of the mRNA-1273LNP/mRNA-1273.529 LNP-B. With the exception of potential extractables/leachables, no additional impurities are anticipated to formed or be introduced during mRNA-1273.214 finished product manufacturing. An evaluation of potential extractables and leachables from manufacturing components and container closure systems used in the mRNA-1273 FP manufacturing process at all manufacturing sites has been presented. Of the compounds characterised, none were identified as cohort of concern, cohort of very high concern, or potent mutagens.

Batch analysis data from 3 GMP batches of the bivalent finished product from each of the proposed finished product manufacturing sites (Catalent, Patheon, Rovi and Recipharm) have been presented together with their Certificates of Analysis (CoAs) as far as available. All results met the release specifications. Results are pending for purity and product-related impurities. The dossier should be updated, and the final CoA should be provided when the complete data are available, in early September 2022 (REC4). This is acceptable since the comparability exercise supports extrapolation of the mRNA-1273 FP data to mRNA-1273.214 in the meantime.

Stability of the product

mRNA-1273 LNP and mRNA-1273.529 LNP-B

An initial shelf life of 12 months is proposed for mRNA-1273 LNP and mRNA-1273.529 LNP material stored in the commercial container closure system, when stored at the recommended long-term storage condition of -60°C to -90°C.

Stability data from 30 mRNA-1273 LNP lots, 7 mRNA-1273 LNP-B lots and three additional stability modelling lots are available. Data have been collected for up to 18 months at -60ºC to -90 ºC and 12 months at 5±3ºC.

Three mRNA-1273.529 LNP registration stability batches have been established and stored at -60ºC to -90 ºC for up to 3 months and 5±3ºC for up to 2 months. Stability samples were stored in a scaled-down container closure system (50-mL Aramus bag). All results from samples stored at -60ºC to -90 ºC met the specification limits.

Samples were tested for appearance, pH, particle size, polydispersity, RNA content, % RNA encapsulation, purity (RE-HPLC), product related impurities (RP-HPLC), lipid content and lipid impurities, bacterial endotoxin, lipid identification, identity (Sanger sequencing), osmolality and bioburden.

The scaled down bag is representative of the storage container used for commercial manufacture with the same product contact surfaces. Based on the sample volume and size of the scaled-down container, the surface area:volume ratio is considered worst case in the stability studies.

Degradation rates for purity of mRNA-1273 LNP and mRNA-1273.529 LNP were estimated for two storage temperatures using the stability study results available.

These results support the proposed shelf life of 12 months for both mRNA-1273 LNP and mRNA-1273.529 LNP.

Finished product

The proposed shelf-life for the bivalent vaccine finished product is the same as for the prototype vaccine:

9 months at -50ºC to -15ºC.

After removal from the freezer, the unopened vaccine vial may be stored refrigerated at 2°C to 8°C, protected from light, for a maximum of 30 days. Within this period, up to 12 hours may be used for transportation at 2°C to 8°C (see SmPC section 6.4).

Chemical and physical stability has also been demonstrated for unopened vaccine vials when stored for 12 months at -50°C to -15°C provided that once thawed and stored at 2°C to 8°C, protected from light, the unopened vial will be used up within a maximum of 14 days (instead of 30 days, when stored at -50ºC to -15ºC for 9 months).

The proposed shelf-life is based on the shelf-life for the prototype vaccine and the stability model developed.

This is agreed since the mRNA-1273 associated variant vaccine sequences include only the changes relative to the CX- 024414 mRNA sequence required to incorporate the specific mutations of the variant S protein sequence. The RNA length is highly conserved between the prototype mRNA-1273 vaccine and mRNA1273 associated variant vaccines. As an example, the RNAs comprising mRNA-1273.214 finished product are within 9 nucleotides of each other relative to a total length of approximately 4000 nucleotides. It is concluded that the extensive data available in the stability program and shelf-life assessment for the prototype mRNA-1273 vaccine is directly applicable to mRNA-1273 associated variant vaccines. Due to the application of the same formulation, manufacturing process and the use of the same manufacturing sites as the prototype vaccine, extensive prior experience is leveraged for the bivalent finished product and comparability has been proven.

Stability from 3 PPQ batches manufactured at Patheon Monza, 1 PPQ batch manufactured at Catalent, Bloomington and one clinical batch from Moderna, TX stored for up to 3 months at -60°C to -60°C ± 5°C, for up to 3 months at-5°C ± 3°C and/or for up to 3 weeks at 25°C ± 5°C have been presented. Samples were tested for appearance, total RNA content, mRNA identity, purity, product-related impurities, %RNA encapsulation, in vitro translation, lipid identification, lipid content, lipid impurities, particle size, polydispersity, pH, particulate matter, container closure integrity test and bacterial endotoxin.

All results were within the specification limits and support the proposed shelf-life.

In conclusion, the proposed shelf-life for the bivalent vaccine is agreed: 9 months when stored at the recommended long-term storage condition of -50°C to -15 °C. After removal from the freezer, the unopened vaccine vial may be stored refrigerated at 2°C to 8°C, protected from light, for a maximum of 30 days. Within this period, up to 12 hours may be used for transportation at 2°C to 8°C.

Chemical and physical stability has also been demonstrated for unopened vaccine vials when stored for 12 months at -50°C to -15°C provided that once thawed and stored at 2°C to 8°C, protected from light, the unopened vial will be used up within a maximum of 14 days (instead of 30 days, when stored at -50ºC to -15ºC for 9 months).

Once thawed, the vaccine should not be re-frozen.

The unopened vaccine may be stored at 8°C to 25°C up to 24 hours after removal from refrigerated conditions.

This is in-line with the wording in section 6.3 in the SmPC.

The MAH is recommended to submit updated stability data when available (REC3).

GMO

Not applicable.

Additional scopes

Type II: To Change the manufacturing process of the biological/immunological active substance CX-031302 to introduce a new manufacturing kit line (kit 8) at (US) ModernaTX, Inc Norwood, MA USA facility for mRNA-1273 RNA.

The qualification has generated data at commercial scale to support and complement concurrent laboratory-scale studies. Process verification acceptance criteria were defined in the associated protocol and are provided in table below. For mRNA-1273 variant processes that use a manufacturing process and control strategy equivalent (equivalent except for mRNA sequence specific control elements) to the prototype mRNA-1273 process, one confirmatory process verification lot was performed to demonstrate process consistency per site per the requirements specified in the Process Validation Master Plan. The introduction of CX-031302 mRNA also included the qualification of the additional identical Train 8; enabling Train 8 for prototype mRNA-1273 and variants at Moderna Norwood. The MAH has successfully completed process verification of the CX-031302 mRNA manufacturing process as a means of demonstrating that the commercial-scale manufacturing process is capable of consistently delivering quality product. The PPQ verification has generated data at commercial scale to support and complement laboratory-scale studies.

Category	Acceptance Criteria
Consistency	PPQ verification batches meet specifications for all CQAs, CPPs, and CIPCs to demonstrate consistency and reproducibility. Non-consecutive batches, separated by failed batches whose failure can be attributed to a non-process related cause, are acceptable.
Robustness	Process parameters and IPCs that fail to meet the pre-established parameter ranges must be assessed and determined to not impact the consistent performance of the unit operation and the validity of the study.
Compliance	Any validation protocol exceptions must be described in the final report and determined not to impact the validity of the study.

The following table includes a summary of commercial sites and process trains validated for CX-031302 mRNA manufacture.

Site	Process Train	PPQ Verification Lot		Validation Status
		Moderna Lot Number	CMO Lot Number	
ModernaTX Norwood (75 L IVT)	8	4011422002	N/A	Qualified

Lonza Visp (75 L IVT)	5	4011822001	57001	Qualified

Abbreviations: CMO = contract manufacturing organisation; IVT = in vitro transcription; N/A = not applicable; PPQ = process performance qualification

The results of the CPP, PP, critical and non-critical IPC and release testing of the CX-031302 mRNA process verification batches were within the specifications and the prior defined acceptance criteria. Based on the outcome of the process verification exercise, the CX-031302 mRNA manufacturing process has been successfully verified at Moderna Norwood and Lonza Visp.

CHMP comment:
The process verification including 1 batch for the Moderna Norwood site produced in the new Kit 8 and 1 batch from Lonza Visp was successfully conducted. Therefore, the manufacture of CX-031302 at Moderna Norwood (including Kit 8) and Lonza Visp at 75 l IVT scale is considered acceptable.

Manufacturing process development:

Comparability studies:

All CIPC and IPC results met the acceptable ranges. Release results met both specification and comparability acceptance criteria and extended characterisation results met the comparability expected ranges established from development, clinical, Scale A, preliminary Scale B, and Scale B CX-024414 mRNA data. Therefore, the results from the Scale B (75 L IVT scale) PPQ lots of CX-031302 mRNA manufactured at ModernaTX (Norwood, MA) and Lonza (Visp, Switzerland) demonstrated that the pre-change and post-change manufacturing processes and quality attributes were comparable.

CHMP comment:
The comparability data clearly indicates that the quality of the CX-031302 mRNA is comparable to the quality of the parental CX-024414 mRNA, as all testing is within the comparability acceptance criteria.

Elucidation of Structure and Other Characteristics:

The structure, physicochemical properties of CX-031302 mRNA, were studied using a variety of techniques applicable to mRNAs. Unless otherwise indicated, data were generated from GMP lot 84112C0702. The data generated from these analyses confirm the physico- chemical structure of CX-031302 mRNA.

Process impurities:

Double Stranded RNA (dsRNA):

Double stranded RNA (dsRNA) can potentially be formed during in vitro transcription. dsRNA can be recognised by receptors in the innate immune system, leading to production of immune-stimulatory cytokines. The innate immune responses of CX-031302 mRNA were assessed by a double stranded RNA (dsRNA) ELISA.

Residual Protein:

Residual protein was tested by a sandwich pAb ELISA method.

CHMP comment:
The characterisation of the new mRNA CX-031302 is considered acceptable.

Impurities:

No new impurities as compared to parental mRNA.

Specification:

No change in specifications compared to CX-024414.

Analytical procedures:

The analytical methods used for release testing of CX-031302 and prototype CX-024414 are identical with the exception of the identity method, since this is the only method that is sequence-specific.

Validation of analytical procedures:

Confirmation of mRNA Sequence by RT-PCR and Sanger Sequencing has been validated and shown to be suitable for the purpose of determining the mRNA identity of CX-031302. The validation characteristic evaluated was specificity and intermediate precision.

Specificity:
To assess specificity, CX-031302 (mRNA-1273.529 RNA) was prepared and analysed once per SOP-1019. Testing was performed by one analyst and tested on the ABI 3500xL Genetic Analyzer.

SeqScape software was used to assemble and compare the sequencing data for each sample type to the corresponding reference sequence listed below. There must be two-fold coverage on each DNA strand, except near the ends of the RT-PCR product, where four-fold coverage was provided on a single DNA strand.

Additionally, each test article was also aligned against the incorrect reference sequence. The CX-031302 (mRNA-1273.529 RNA) test article was aligned to the CX-024414 (mRNA-1273 RNA) reference sequence.

mRNA	Reference Sequence	Expected Result
CX-031302 (mRNA-1273.529 RNA)	mRNA-1273.539 RNA ORF	Conforms
	mRNA-1273 RNA ORF	Does not Conform

Acceptance Criteria:

Sequence matches description of the region of the mRNA with a minimum coverage of two-fold on each DNA strand (forward and reverse), except near the ends of the RT-PCR product, where four-fold coverage is provided on a single DNA strand.

CX-031302 (mRNA-1273.529 RNA) test articles must not conform to the CX-024144 (mRNA-1273 RNA) ORF reference sequence.
Intermediate Precision:

To establish the precision within laboratory variations, such as different days, different analysts, different equipment, etc.

Experimental Design:

The specificity experiment was repeated by a second analyst, on a second day, and tested on a separate Genetic Analyzer instrument (ABI 3730xL). CX-031302 (mRNA-1273.529 RNA) was prepared and analysed per SOP-1019. The intermediate precision validation run passed system suitability, thus no discrepancies were generated.

Acceptance Criteria:

The test articles must meet the specificity criteria

The sequence generated on the ABI 3730xL for CX-031302 (mRNA-1273.529 RNA) matched the description of the mRNA coding region with a minimum of two-fold coverage of each DNA strand, except near the ends of the RT-PCR product, where four-fold coverage was provided on a single DNA strand.

CHMP comment:
The validation of the identity assay for CX-031302 is acceptable.

Batch analysis:

The release data of the two batches produced so far (4011422002 Moderna Norwood; 4011822001 (57001) Lonza Visp) is provided in the dossier.

Justification of specification:

Justification for specifications are the same as for CX-024414 mRNA.

Reference standard materials:

The reference material as described for CX-024414 will serve as the reference material for CX-031302.

Container closure system:

The container closure system is the same as used for CX-024414.

Stability:

Additional scope:

Type II: To extend the storage period of the biological active substance CX-024414 from 9 months to 36 months when stored at long-term storage condition of -60°C to -90°C, not in accordance with an approved stability protocol.

and

Type II: To change the storage conditions of the biological active substance CX-244141 from {-20°C ± 5°C/Ambient RH} to {-60°C to -90°C}, when stability studies have not been performed in accordance with a currently approved stability protocol.

Stability summary and conclusion:

The CX-031302 registration stability program was executed according to ICH Q1A (R2), Stability Testing of new Drug Substances and Products, and ICH Q5C, Stability Testing of Biotechnological/Biological Products. An initial shelf life of 36 months is proposed for CX-031302 mRNA material stored in the commercial container closure system, when stored at the recommended long-term storage condition of -60°C to -90°C.

The properties of CX-031302 mRNA with respect to the attributes that affect product potency have been systematically and thoroughly assessed. These attributes include fidelity of the RNA sequence including cap, tail, and open reading frame, and integrity of the RNA. Direct measurements of those attributes have been established and are included in the routine release panel for CX-031302 mRNA. The stability of CX-

031302 mRNA will be evaluated against a broad set of stability data and modelling encompassing both CX-024414 mRNA registration lots and additional variant RNAs associated with the mRNA-1273 program.

The product quality attribute expected to change most during the manufacturing and distribution of the product is mRNA purity, which represents the fraction of intact mRNA. The degradation of RNA in the product has been extensively studied by applying a sensitive chromatographic assay to assess the formation of RNA degradants. The principal route of degradation for RNA is hydrolytic chain scission to species that elute prior to the main peak (RNA fragments). mRNA purity correlates with protein levels measured in the in vitro relative protein expression assay. Direct measurement of RNA degradation utilising the RNA purity assay by RP-HPLC is precise, accurate, and the most stability-indicating measure of product activity.

The degradation rates can be determined from the purity analysis over different stability time points. Based on the stochastic nature of the degradation mechanism, there is a dependence on the rate of degradation on RNA size (length). Since CX-024414 and CX-031302 mRNAs have approximately the same overall length (~4,000 nt), a similar rate of degradation is expected across these different sequences.

Stability modelling results:

Degradation rates for RNA purity were estimated for 3 different storage temperatures using the stability study results available for CX-024414 registration and variant RNA lots as of May 10, 2022. The rates along with their 95% confidence intervals are summarised in Table 4. These rates are expressed as a percentage of initial purity lost in the next month, not as a decrease in purity percentage units. For example, a 10% loss per month for 70% purity results in 63% after 1 month, since 10% of 70% purity is 7 purity percentage units lost. The longest timepoint available for purity was 24 months at -60°C to -90°C. The statistical models are based on first-order kinetics. The degradation rate for RNA purity stored at -60°C to -90°C is not significantly different from zero. The modelling confirmed similar degradation rates for CX-024414 mRNA and variant RNA supporting the same shelf life for CX-024414 and CX-031302 mRNA. These results support a proposed shelf life of 36 months for CX-031302 mRNA when stored at -60°C to -90°C per ICH guidelines.

Temperature	Estimated Degradation Rate, % purity per month	Lower 95% CI for Degradation Rate	Upper 95% CI for Degradation Rate
-70°C	-0.0	-0.9	0.9
-20°C	-0.7	-0.8	-0.6
5°C	-10.2	-10.6	-9.8

Abbreviations: CI = confidence interval

CHMP comment:
The shelf-life claim of 36 month at -60°C to -90°C is not acceptable. So far, the data provided for the stability model only includes 1 batch (DH-06126, CX-027367) that was stored at -60°C to -90°C for 12 months. The MAH is asked to either provide more data or adapt the shelf life claim according to the data available. Furthermore, the MAH is asked to provide information on the mRNA CX-027367 characteristics to justify the comparability of this mRNA with CX-024414 and CX-031302.
It is understood that the CX-031302 mRNA will be stored only at -60°C to -90°C for long-term storage. Therefore, the MAH is asked to justify why the supporting CX-031302 PPQ lot is not placed at -60°C to -90°C but at -20 ± 5°C.

The MAH is asked to justify why the PPQ lot produced at Lonza Visp is not mentioned as supporting stability study (Table 1) and no stability protocols are included in the document.
The MAH is asked to provide the batch data of the additional CX-031302 batches included in the stability studies (4011422007 and 4011822002).

Post approval stability protocol and stability commitment:

No additional post-approval protocol is provided.
Stability data:

1 month stability data of the CX-031302 was provided.

CHMP comment:
In this section Table 2 indicates a batch (MTDS20002) that was stored for 24 months at -60°C to - 90°C however the data was not provided and needs to be submitted.

Type IA: To change an approved stability protocol of the active substance mRNA-1273 RNA to decrease from {-20°C ± 5°C/Ambient RH} to {-60°C to -90°C)}.

Some aspects of these variations are already discussed in conjunction with the introduction of mRNA-031302. However, also additional documents in the dossier of the original mRNA CX-024414 are affected.

In section 3.2.S.2.2 the storage of the AS is described as follows.

CX-024414 mRNA is stored at -60 to -90°C, after an optional interim storage temperature at -15 to -25°C, in containers as defined in Section 3.2.S.6 {CX-024414 – Lonza Visp}.

In section 3.2.S.6 however the document is still referring to storage at -15°C to -25°C.

The MAH proposed to change the post approval stability protocol from -20°C to ±5°C to -60 to -90°C. However, the 10 L, 20 L, and 60 L IVT scale, CX-024414 mRNA lots manufactured at the US sites are still stored at -15 to -25°C.

CHMP comment:
The MAH is asked to provide an updated section 3.2.S.6 that includes the new storage temperature of -60 to -90°C and adapt the suitability assessment of the container closure system.

The MAH needs to clearly define the length of the optional interim storage at -15 to -25°C indicated in section S.2.2, and the proposal needs to be justified with stability data and included in the shelf-life claim.

In the stability summary it is mentioned that the long-term storage moving on will be -60°C to -90°C for CX-024414, however in the present proposed document it is stated that 10 L, 20 L, and 60 L IVT scale, CX-024414 mRNA lots manufactured at the US sites are still stored at -15 to -25°C. This needs to be clarified and the section adapted accordingly (as well as all potentially affected section like S.2.2)

The post approval stability commitment is now only for storage at -60°C to -90°C. However, the 10 L, 20 L, and 60 L IVT scale, CX-024414 mRNA lots manufactured at the US sites are still stored at -15 to -25°C. Therefore, the MAH is asked to retain also the -15 to -25°C storage conditions in the post approval commitment.

Type IA:

To tighten the {Bioburden} in-process limits, applied during the manufacture of the active substance {CX-024414}, from {Report Results} to {≤ 20 CFU/10 mL} for Lonza AG Lonzastrasse 3930 Visp Switzerland for 75 L scale at Manufacturing Stages-Post-load Adjustment prior to Oligo dT1 Chromatography (IVT TFF Post Hold), dT TFF Harvest prior to

Filtration for Cap Reaction (dT TFF Post Hold), Post-Load Adjustment prior to Oligo dT2 Chromatography (Cap TFF Post Hold), Pre-final filtration stage.

To tighten the {Bacterial Endotoxins} in-process limits, applied during the manufacture of the active substance {CX-024414}, from {Report Results} to {≤ 10 EU/mL} for Lonza AG Lonzastrasse 3930 Visp Switzerland for 75 L at Manufacturing Stages -Post-load Adjustment prior to Oligo dT1 Chromatography (IVT TFF Post Hold), dT TFF Harvest prior to Filtration for Cap Reaction (dT TFF Post Hold), Post-Load Adjustment prior to Oligo dT2 Chromatography (Cap TFF Post Hold), Pre-final filtration.

To tighten the {Bioburden} in-process limits, applied during the manufacture of the active substance {CX-024414}, from {Report Results} to {≤ 20 CFU/10 mL} for ModernaTX, Inc One Moderna Way Norwood, MA 02062 USA for 60 L, 75 L scale at Manufacturing Stages - Post-load Adjustment prior to Oligo dT1 Chromatography (IVT TFF Post Hold), dT TFF Harvest prior to Filtration for Cap Reaction (dT TFF Post Hold), Post-Load Adjustment prior to Oligo dT2 Chromatography (Cap TFF Post Hold), Pre-final filtration stage

To tighten the {Bacterial Endotoxins} in-process limits, applied during the manufacture of the active substance {CX-024414}, from {Report Results } to { ≤ 10 EU/mL} for ModernaTX, Inc One Moderna Way Norwood, MA 02062 USA for 60 L, 75 L at Manufacturing Stages -Post-load Adjustment prior to Oligo dT1 Chromatography (IVT TFF Post Hold), dT TFF Harvest prior to Filtration for Cap Reaction (dT TFF Post Hold), Post-Load Adjustment prior to Oligo dT2 Chromatography (Cap TFF Post Hold), Pre-final filtration.

CHMP comment:
The tightening of the in-process controls for bioburden and bacterial endotoxin is considered acceptable.

Type IA: To add {Filter Integrity Test} as a new in-process test applied during the manufacture of the active substance {CX-024414}. The limit is set to Per site-specific acceptance criteria for 75 L IVT.

CHMP comment:
The addition of the in-process controls filter integrity test is considered acceptable

Type IB: Minor changes in the manufacturing process of the CX-024414 to the chromatography column in MC1 (already used at Norwood, US) at Lonza Visp as alternative to pre-packed chromatography column with diameter of 45.7 cm (Repligen). To use the chromatography column packed in-house used for the oligo dT chromatography process steps that is currently used at Moderna Norwood, is introduced at Lonza Visp, for the manufacturing of CX-024414.

The chromatography column Packing in MC1 is already approved and used for Norwood. Lonza uses an equivalent one and will start to use the chromatography column Packing in MC1 as alternative to the pre-packed chromatography column with diameter of 45.7 cm for the bivalent vaccine. The information is included in Table 6 in section S.2.2 Description of the Manufacturing Process and Process Controls {CX-024414 – Lonza Visp} for the 75 l scale.

CHMP comment:
The introduction of the chromatography column at Lonza is considered acceptable, as this column is already used for CX-024414 production at the Moderna Norwood site.

Type II: To change the manufacturing process of the biological/immunological active substance CX-031302 to introduce a new manufacturing kit line at (US) ModernaTX, Inc, MA, USA facility for mRNA-1273 RNA.

Changes are described and validation data provided. Batch analysis data are also provided.

CHMP comments
All issues were appropriately addressed.

Type II: To replace the current Purity and Product-related Impurities test procedure (RP-HPLC) with an improved RP-IP-HPLC test for the CX-024414 used in the manufacturing process of the active substance CX-024414 and consequentially, due to the replacement of the analytical method, to change the "Product related impurities" specification limit for the active substance (CX-024414) used in the manufacturing process of the active substance DS CX-024414 from < 30% pre-main peak to ≤ 30% RNA Fragments. There are editorial changes to remove the reference numbers of the methods from the Sections on CX-024414 specification that will be included in the end of the list.

It is intended to replace the current reverse-phase high-performance liquid chromatography (RP-HPLC) method by an improved reverse-phase ion pair high-performance liquid chromatography (RP-IP-HPLC) method to assess RNA purity in the mRNA-1273 RNAs CX- 024414 and CX-031302, mRNA-1273 LNP and mRNA-1273 Drug Product test samples.

Separation of the RNA fragments and main peak (intact full length mRNA) for the previous RP-HPLC method was performed using the tangent line function within Chromeleon software. The x-intercept of the tangent line marked the vertical splitting of the RNA fragment peak and the main peak. The tangent line method was employed to improve integration consistency for chromatograms.

The improved RP-IP HPLC method provides improved separation between RNA fragments and intact full-length mRNA. This level of separation improvement comes from the systemic chemistry of mobile phases, column, and HPLC chromatographic configurations. The splitting of RNA fragments from the main peak is more easily identified based on the observed valley or local minimum. The clear separation results in more precise integration. The improved resolution achieved provides more consistent and accurate classification of peak areas into fragments and main peak.

Method description:

3.2.S Drug Substance CX-024414 mRNA: Section 3.2.S.4.2 Analytical Procedures has been updated to include the method description of the RP-IP-HPLC in replacement of the RP-HPLC method.

In addition, current sections have been consolidated into one single document for all manufacturing sites (Lonza Visp, Moderna TX and Lonza Biologics), and by removing references to SOP numbers.

3.2.S Drug Substance mRNA-1273 LNP and mRNA-1273 LNP-B: Section 3.2.S.4.2 Analytical Procedures has been updated to include the method description of the RP-IP-HPLC in replacement of the RP-HPLC method. In addition, current sections have been consolidated into one single document for all manufacturing sites (Lonza Visp, Moderna TX and Lonza Biologics), and by removing references to SOP numbers.

3.2.P Drug Product mRNA-1273 Drug Product 0.20 mg/mL and mRNA-1273 Drug Product 0.10 mg/mL: Section 3.2.P.5.2 Analytical Procedures has been updated to include the method description of the RP-IP-HPLC in replacement of the RP-HPLC method. This section has also been updated by removing references

to SOP numbers, and to provide the new name for the Absorbance-based Assay used to assess the % RNA Encapsulation.

Method validation:

Method validation is presented in validation report QC-MVR-0025 and applies to mRNA-1273 RNAs (CX-024414 and CX-031302), mRNA-1273 LNP and mRNA-1273 Drug Product. Refer to QC-MVR-0025 for details of the initial and supplemental validation results. The validation characteristics evaluated were: system suitability, specificity, linearity, accuracy; precision, range, robustness, determination of the detection and the quantitation limits and stability of standard and sample preparation solutions.

Bridging study:
A bridging study has been performed in order to demonstrate comparable results with both RNA purity methods. The bridging report QC-OTH-0801 is provided in section 3.2.P.5.6 Justification of Specifications {mRNA-1273 Injection}. This bridging study report presents the mRNA-1273 analytical results generated using improved Reversed Phase Ion-Pair (RP-IP) HPLC method (SOP-1142) for the analysis of mRNA purity, as compared to the current method (SOP-0996).

Testing of the test articles listed were performed on both SOP-0996 and SOP-1142. Both methods were performed concurrently on the same day. The same vial of reference standard CX-024414, lot number DH-03180.1 was used to prepare standards for each method.

Reported values by each method are presented in the bridging report QC-OTH-0801.

Based on method validation and bridging study of both methods, revised acceptance criteria for mRNA Purity and Product-related Impurities is proposed.

CHMP comment:

RNA Purity is a release test for mRNA-1273 RNAs, mRNA-1273 LNP and mRNA-1273 Drug Product. A RP-IP-HPLC has been introduced as new method to substitute the current RP-HPLC test. The method has been fully validated using adequate validation parameters. The validation report has been provided. The relevant dossier sections have been updated accordingly to include method description and method validation (Sections 3.2.S.4.2 Analytical Procedures (for both mRNA and mRNA-LNP), 3.2.P.4.2 Analytical Procedures, 3.2.S.4.3 Validation of Analytical Procedures (for both mRNA and mRNA-LNP) and 3.2.P.4.3 Validation of Analytical Procedures).

A bridging study has been performed to compare results obtained with both methods using mRNA, mRNA-LNP and DP samples. This study demonstrated that values obtained with the new method are higher. This is especially the case for DP samples. With the new method, an improved separation between RNA fragments and full-length mRNA can be achieved. This results in a more precise integration and areas that have been (falsely) defined as impurities are now added to the main peak that represent the intact RNA. Despite this observation, the specification limits for RNA purity have not been adjusted. Specifications must be revised in order to maintain the current, clinically justified, RNA purity range (MO).

The MAH's proposal to remove the SOP numbers from the product specification was not considered acceptable, and he was requested to include them back.

3. Assessment of the responses to the request for supplementary information

3.1. Major objections

CX-031302:

1. The shelf life claim of 36 months at -60°C to -90°C is not acceptable. So far, the data provided for the stability model only includes 1 batch (DH-06126, CX-027367) that was stored at -60°C to -90°C for 12 months. The MAH is asked to either provide more data or adapt the shelf life claim according to the data available. Furthermore, the MAH is asked to provide information on the mRNA CX-027367 characteristics to justify the comparability of this mRNA with CX-024414 and CX-031302.

MAH's response: Justification for the shelf-life for the CX-031302 is supported by the cumulative stability data acquired for the CX-024414 and associated variants. The primary shelf-life-limiting attribute for mRNA drug substance is RNA purity. For different RNA sequences formulated and processed similarly, the purity loss rate depends mainly on sequence length. The length of CX-024414 and associated variants is very similar, with overall length approximately 4000 nucleotides, varying within a 20-nucleotide range. RNA purity degradation for CX-031302 is expected to be very similar to purity degradation rates for CX-024414 on this basis.

There is no significant loss of purity for storage at -70°C; the estimated loss rate for RNA Purity is 0.0% per month. One study with 7 time points to 24 months is available at -70°C. The estimated shelf life for CX-024414 following the standard ICH approach is greater than 60 (5 years) for storage at (-60 to -90 °C). A 36-month shelf life for CX-024414 mRNA and CX-031302 when stored at -70°C is proposed, limited to no more than 12 months beyond the longest time point tested (24 months).

The main size purity degradation pathways of RNAs are hydrolysis, transesterification, and oxidation. All these pathways require the presence of reactive species (acids, bases, or oxidising species) at the reaction site. At -70 °C the CX-031302 and CX-024414 are expected to be below the glass transition temperature (Tg). When stored below the Tg, the chain mobility of macromolecular species, such as RNA, is essentially zero, therefore significantly suppressing the transesterification degradation pathway. Similarly, at -70 °C, the mobility of the reactive species (required for hydrolysis or oxidation reactions) is significantly restricted, and therefore size purity degradation rates are expected to be reduced.

CX-027367 is the mRNA encoding the S protein sequence of SARS-CoV-2 B.1.351 (Beta) variant, incorporating the S-2P mutation. Relative to CX-024414, the spike protein encoded by CX-027367 includes the following mutations: L18F-D80A-D215G-L242-244del-R246I-K417N-E484KN501Y-D614G-A701V. The CX-023767 mRNA sequence was designed relative to the CX-024414 sequence, with changes introduced to match the B.1.351 variant-specific mutations. CX-027367 has a total length of 4092 nucleotides; the lengths of CX-024414 and CX-031302 are 4101 nucleotides, and 4092 nucleotides, respectively.

Assessment of the response: The MAH provided 24-month stability data for a batch of CX-0124414 stored at -60°C to -90°C and described the characteristics of the CX-027367 mRNA that is used in the supportive stability study with 12 month data at -60°C to -90°C available.

Issue solved.

Drug product:

2. The bridging study demonstrates that the new purity method consistently produces results that are higher compared to the current method. This is especially the case for the Drug Product (up to 6%), but also for mRNA and mRNA-LNPs. The purity specifications should be adjusted accordingly to reflect the current, clinically justified specifications.

MAH's response:
The stability and release acceptance criteria for RNA purity and fragments are based on the minimum purity required to ensure efficacy throughout shelf life.

Data on RNA fragments and RNA lipid adducts differences are provided

The MAH agrees to provisionally adjusting the mRNA purity release specification for drug product tested using the improved method (SOP-1142) to account for the observed offset between purity methods. Side-by-side testing of 20 GMP DP lots will be conducted to assess the magnitude of the offset between methods in real-world representative release laboratory testing. Please refer to the revised Section 3.2.P.5.1 Specification(s) {mRNA-1273.214 – 0.10 mg/mL} reflecting the updated release purity limit for mRNA-1273.214 DP. The provisionally revised Justification of Specification section will be updated accordingly and provided with the closing sequence for this procedure.

The adjustment will be reviewed and revised as supported by this updated dataset comparing results from SOP-0996 and SOP-1142 for GMP release testing. The MAH will review the release and End of Shelf Life (EOSL) specifications based on the results of these comparisons, additional stability data, to confirm the magnitude of the shift remains within the bounds that have been analysed to date. A revised justification of specification will be provided by the end of October 2022, leveraging additional comparative data between SOP-0996 and SOP-1142.
The specification limits applied to the mRNA-1273 prototype DP (including both 0.2mg/mL and 0.1mg/mL strengths, in vial and pre-filled syringe presentations), remain unchanged, as the currently approved analytical method (SOP-0996) is retained for the prototype product. This is reflected in the current version of the Specifications sections, provided with this response.

The MAH would like to clarify that the current specifications are based on safety and efficacy considerations. We acknowledge that the introduction of the improved purity assay for CX-031302 mRNA, mRNA-1273.529 LNP and mRNA-1273.214 DP has not followed a traditional approach and provide some additional context for introducing the new purity test for the bivalent components along with rationale for maintaining the current specifications. We also commit to providing additional comparative test results for the current and improved methods to confirm that the purity method change has no impact on patient safety or efficacy.

The MAH additionally confirmed to change the end of shelf life limits.

Assessment of the MAH's response: The MAH agreed that the release and end of shelf life specifications will be adapted according to the assay induced shift in purity results. The final revised documents were provided.

Conclusion: Issue solved.

3. The in vitro translation test that is performed as release test on the DP, is not able to distinguish between the two different mRNAs. Thus, there is no proof of functionality for both mRNAs if the test is done on the DP level. The in vitro translation test should be performed on the level of the mRNA-LNP intermediates as well unless functionality of both mRNA-LNPs in the final product can be demonstrated otherwise.

MAH's response The MAH has utilised in vitro relative protein expression (IVRPE) to quantitatively confirm the intended protein expression level of each LNP comprising the mRNA-1273.214 drug product.

The MAH provides study data to demonstrate correlation between IVRPE and in-vivo immunogenicity of drug product

Immunogenicity was assessed after one and two injections of mRNA-1273 in mice by measurement of serum SARS-CoV-2 S2P spike binding antibody titers in the qualified enzyme-linked immunosorbent assay (ELISA).

The MAH provides relationship data for in vivo immunogenicity and purity results. The MAH provides comparison datasets and correlation models to measure the relationship between titer and IVRPE

Assessment of MAH's response: The control of functionality of each mRNA entering the composition of the mRNA-1273.214 DP at the mRNA-LNP stage should be controlled.

Conclusion: Issue not solved.

MAH's 2nd response: The MAH acknowledges the CHMP's comments and commits to improve the control of functionality of each mRNA entering the composition of the mRNA-1273.214 DP at the mRNA-LNP stage.

Spikevax is currently controlled using the In-Vitro Translation (IVT) assay as part of the Drug Product (DP) specification. The In-Vitro Relative Protein Expression (IVRPE) assay is not part of the routine Quality Control testing, but rather a characterisation assay as part of analytical comparability. IVRPE will remain as part of analytical comparability at the mRNA-LNPs stage to support bivalent vaccine development with any process, scale, and site introduction. The MAH commits to work on the required implementation of the IVT assay at the mRNA-LNPs stage and submit the proposed change in Control Strategy by End of December 2022.

Assessment of MAH's 2nd response: The MAH commits to submit work on the required implementation of the IVT assay at the mRNA-LNPs stage and submit the proposed change in Control Strategy by End of December 2022.

Conclusion: Issue solved with recommendation (REC1).

3.2. Other concerns

Drug substance:

CX-031302:

3.2.S.1.2

4. The MAH is asked to clearly indicate which mutations of the B.1.1.529 variant were included in the CX-031302 mRNA. So far it is only stated which mutations the B.1.1.529 variant itself might contain.

MAH's response
The B.1.1.529 or Omicron variant emerged in South Africa and is currently circulating globally and was designated as a Variant of Concern on November 26, 2021. Specific additional details are provided.

Assessment of the response: The MAH provided the requested information. However, this information should be included in section S.1.2 and submitted.

Issue not solved.

2nd response of the MAH: The MAH provides with this response document a revised version of section 3.2.S.1.2 Structure {CX-031302} which integrates the elements previously provided in response to question Q2 (Round #3), related to mutations of the B.1.1.529 variant included in the CX-031302 mRNA.

Assessment of the 2nd response: The MAH provided the requested information in the respective document.

Issue solved.

5. The MAH is required to provide further clarification on specific aspects of the CX-031302 structure and alignment of the description/ colour coding with the figure.

Assessment of the response: The MAH commits to align the description and color-coding of CX-024414 at the next opportunity.

Issue solved.

6. It is understood that the CX-031302 mRNA will be stored only at -60°C to -90°C for long-term storage. Therefore, the MAH is asked to justify why the supporting CX-031302 PPQ lot is not placed at -60°C to -90°C but at -20 ± 5°C.

MAH's response: The MAH confirms that the proposed long term storage condition for the CX-031302 material is -60°C to -90°C. The supporting CX-031302 PPQ lot presented was stored at -20°C±5°C which is the storage conditions currently approved for the prototype CX-024414 and, as such, the condition applicable to CX material at the time of manufacturing.

An additional batch of CX-031302 has been placed on stability at –60°C to 90°C storage condition to support the proposed long term storage condition.

Assessment of the response: The MAH explained that the change in storage condition was made after the PPQ storage was started.

Issue solved.

7. The MAH is asked to justify why the PPQ lot produced at Lonza Visp is not mentioned as supporting stability study (Table 1) and no stability protocols are included in the document.

MAH's response: Section 3.2.S.7.1 Stability Summary and Conclusions {CX-031302} was updated and is provided with this response.

Assessment of the response: Table 1 still requires update.

Issue not solved.

2nd response of the MAH: Table 1 of section 3.2.S.7.1 Stability Summary and Conclusions {CX-031302}-EU-EMA describes stability study that was initiated to support the introduction of production process train 8 at Moderna TX, Inc (Norwood, MA, USA). Only batch 4011422002 from Moderna TX, Inc (Norwood, MA, USA) was executed in this context.

The stability studies conducted on Lonza Visp PPQ batch 4011822001 to support the introduction of the new CX-031302 material is described in section S.7.1, Table 3. Stability protocols for both batches are detailed in the 3.2.S.7.1 Stability Summary and Conclusions {CX-031302}-EU-EMA section: 4011422002 (Table 5 and Table 6) and 4011822001 (Table 12 and Table 13). The section is re-submitted.

Assessment of the 2nd response: The MAH explained why the batch was not included in Table1 and included it in Table 3. However, Table 3 is titled "CX-031302 mRNA -60°C to -90°C Stability Lots" and the PPQ batch 4011822001 is not even stored at -60°C to -90°C. Therefore, inclusion of the batch in Table 3 should be clarified.

Issue not solved.

3rd response of the MAH: The MAH renamed the table.

Assessment of the 3rd response:

Issue solved.

8. The MAH is asked to provide the batch data of the additional CX-031302 batches included in the stability studies (4011422007 and 4011822002).

MAH's response: Section 3.2.S.7.1 Stability Summary and Conclusions {CX-031302} was updated accordingly and is provided with this response.

Assessment of the response: The answer is not completely understood. The MAH was asked to provide the batch data of 4011422007 and 4011822002 in an updated section S.4.4.

Issue not solved.

2nd response of the MAH: Please find attached a revised version of section 3.2.S.4.4 Batch Analyses {CX-031302} including batches analysis data for 4011422007 and 4011822002.

Assessment of the 2nd response: The MAH included the batch data in the respective dossier section.

Issue solved.

3.2.S.7.3

9. In section S.7.3 Table 2 indicates a batch (MTDS20002) that was stored for 24 month at -60°C to - 90°C however the data was not provided and needs to be submitted.

MAH's response: The 24 months stability data for the batch MTDS20002 stored at -60°C to -90°C are provided with this response (refer to Section 3.2.S.7.3 Stability Data {CX-024414}, Table 54).

Assessment of the response: The MAH provided the requested information.

Issue solved.

CX-024414:

3.2.S.6

10. The MAH is asked to provide an updated section 3.2.S.6 that includes the new storage temperature of -60 to -90°C and adapt the suitability assessment of the container closure system.

MAH's response: The MAH provided the updated the Section 3.2.S.6. The container closure system suitability assessment was updated considering the new storage temperature of -90°C to -60°C.

Assessment of the response: The MAH provided an updated section.

Issue solved.

3.2.S.2.2/S.7

11. The MAH needs to clearly define the length of the optional interim storage at -15 to -25°C indicated in section S.2.2, and the proposal needs to be justified with stability data and included in the shelf life claim.

MAH's response.

The MAH provides further clarification on interim storage durations.

Assessment of the response
The MAH is asked to provide further updated sections.

Issue not solved

2nd response of the MAH: The MAH acknowledges the CHMP's request and provides following updated sections including an optional interim storage duration of up to 3 months (90 days) at -15°C to 25°C:

- 3.2.S.2.2 Description of Manu Process and Process Controls {CX-024414}-US

See section 3.2.S.2.2.3.10 – Storage

- 3.2.S.2.2 Description of Manufacturing Process and Process Controls {CX-024414}- Lonza Visp

See section 3.2.S.2.2.3.10 – Storage

- 3.2.S.7.1 Stability Summary and Conclusions {CX-031302}-EU-EMA

See first paragraph

- 3.2.S.7.1 Stability Summary and Conclusions {CX-024414} EU-EMA

See first paragraph

Assessment of the 2nd response: The MAH included the requested information in the dossier.

Issue solved.

3.2.S.7.1/2

12. In the stability summary it is mentioned that the long term storage moving on will be -60°C to -90°C for CX-024414, however in the present proposed document it is stated that 10 L, 20 L, and 60 L IVT scale, CX-024414 mRNA lots manufactured at the US sites are still stored at -15 to -25°C. This need to be clarified and the section adapted accordingly (as well as all potentially affected section like S.2.2)

MAH's response: The MAH would like to clarify that the batches currently included in the ongoing stability studies remain stored at the initial long term storage temperature of -25°C to -15°C. The MAH has not applied for this change for the material manufactured at the 10L, 20L and 60L IVT scales, since only the 75L IVT scale is currently used for routine commercial manufacturing.

In the event that a batch will need to be manufactured at any of the smaller scales, the storage conditions of -90°C to -60°C, if approved, will apply.

Assessment of the response: The MAH clarified that so far as only 75l IVT is manufactured right now. In case of foreseen production of smaller scales, a variation will be submitted.

Issue solved.

13. The post approval stability commitment is now only for storage at -60°C to -90°C. However, the 10 L, 20 L, and 60 L IVT scale, CX-024414 mRNA lots manufactured at the US sites are still stored at -15 to -25°C. Therefore, the MAH is asked to retain also the -15 to -25°C storage conditions in the post approval commitment.

MAH's response: See answer to question 12.

Assessment of the response: The answer to question 12 clarifies the storage conditions that will be applied to 10 L, 20 L and 60 L batches if produced again.

Issue solved.

Drug product intermediates:

14. The MAH should provide information on the IPCs for the drug product intermediate.

Summary of the MAH's response

The MAH provided information on the IPCs for the drug product intermediate and to reflect the current specifications in Module 3 of the dossier, manufacturing information

Assessment of the response

Module 3 has been updated with the required information.

The question is resolved.

<u>mRNA-1273 LNP</u>

15. It remains unclear whether 3.2.S.4.2 Analytical Procedures {mRNA-1273 LNP} has been updated as intended. A detailed comparison between the updated and the current version should be provided.

Summary of the MAH responses:

The MAH confirms that the latest submitted version of Section 3.2.S.4.2 Analytical Procedures {mRNA-1273 LNP} has been updated as intended and provides a detailed comparison (redline document) between the current version, that was submitted with the Line extension procedure X064, and the proposed updated version included in this procedure.

In the redline document (refer to document 3.2.S.4.2 Analytical Procedures {mRNA-1273 LNP} v10 redline), the text added with this variation appears in red, while the removed parts appear as strikethrough.

Section 3.2.S.4.2 Analytical Procedures {mRNA-1273 LNP} is also attached to this response, including the correction to reflect the SOP update in Table 1.

Assessment of the responses:

The question has been raised since it has been stated in the reviewer's guide that new name for the Absorbance-based Assay used to assess the % RNA Encapsulation has been introduced 3.2.S.4.2 Analytical Procedures {mRNA-1273 LNP}. However, the wording has not been consistently used for mRNA-1273 LNP and mRNA-1273.529. The MAH is asked to review the submitted documents. In the case that besides the name change any other changes will be implemented a detailed comparison between the updated and the current version as submitted with the response to the 1st CMC LoQ should be provided.

Summary of the MAH responses to outstanding issue:

Please find with the submission a revised version of section 3.2.S.4.2 Analytical Procedures {mRNA-1273 LNP - ROW}, submitted with the responses to the 1st round of CMC questions dated 03 August 2022. The analytical method name was aligned to "absorbance-based assay", as described initially in the reviewer's guide, and as presented for the mRNA-1273.529 LNP material. The MAH confirms that there is no change to the method itself, and the same method is used for the analysis of both mRNA-1273 LNP and mRNA-1273.529 LNP materials.

Assessment of responses to outstanding issue:

The dossier has been updated as requested.

The question is resolved.

16. Apparently, incorrect method IDs are provided for purity testing in Section 3.2.S.4.2 (Table 2) and 3.2.S.4.3 (Tables 1-3). The dossier should be updated accordingly.

Summary of the MAH responses to outstanding issue:

Section 3.2.S.4.2 was updated as part of response to the 1st round of CMC questions to include the references to the analytical method SOPs (or method IDs), as requested by the Agency

Please refer to the Section 3.2.S.4.2, Table 1, submitted with the responses to the 1st LOQs for the correct version of the Section (Note: Table 2 of Section 3.2.S.4.2 relates to instruments, equipment and reagents for the identity test).

An updated Section 3.2.S.4.3 Validation of Analytical Procedures {mRNA-1273 LNP - ROW} is provided here to reflect the correct reference to method IDs (Table 1), method transfer report (Table 2) and method validation report (Table 3) for the purity test.

Assessment of responses to outstanding issue:

The MAH provided the updated documents.

The question is resolved.

<u>mRNA-1273.529 LNP</u>

17. Section 3.2.S.2.4 refers to the respective section for 1273 LNP-B, and according to the document the manufacturing process and controls are identical. However, during manufacture of the PPQ batches apparently a reduced set of controls has been applied. It is unclear which parameters and controls apply – a clarification is requested, and the dossier should be updated accordingly, if necessary.

Summary of the MAH responses to outstanding issue:

The following microbial control strategy elements are currently applicable for the manufacture of the mRNA-1273 LNP-B:

- Post-use sanitisation of the mixing skid
- Pre-filtration bioburden and endotoxin testing of the mixed product
- Clarification (0.2 µm terminal filter) of the mixed product & filter integrity testing

The pre-use sanitisation controls previously listed in 3.2.S.2.4 {mRNA-1273 LNP}-US [Seq 0247] and Section 3.2.S.2.4 {mRNA-1273 LNP}-Lonza Visp [Seq 0342] are now moved under site cleaning

validation requirements. Post use cleaning is always performed, while pre-use cleaning is performed depending on the established clean hold time and actual durations between batches.

The microbial controls for water and CX-024414 presented in current Section 3.2.S.2.4 {mRNA-1273 LNP}-US [Seq 0247] and Section 3.2.S.2.4 {mRNA-1273 LNP}-Lonza Visp [Seq 0342] were determined to be redundant to the description of control of quality of water described in the site-specific Sections 3.2.A.1 and the specifications for CX-024414 mRNA in Section 3.2.S.4.1 {CX-024414} [Seq 0254]; they were consequently removed from the recently submitted Section 3.2.S.2.4 {mRNA-1273 LNP}-Rovi Granada [Seq 0237].

Description of microbial testing performed after post-use sanitisation of the skid testing was also determined to be an element of cleaning procedure and cleaning validation rather than process-specific microbial control, and therefore beyond the scope of the Section 3.2.S.2.4, and was removed from Section 3.2.S.2.4 in recent introductions.

These redundant details will be removed from the current Section 3.2.S.2.4 {mRNA-1273 LNP}-US [Seq 0247] and Section 3.2.S.2.4 {mRNA-1273 LNP}-Lonza Visp [Seq 0342] to align the content of the 3.2.S.2.4 sections as an editorial update at the next opportunity.

Assessment of responses to outstanding issue:

The MAH clarified that several controls were moved to site cleaning requirements as these are not considered process-specific specific. The approach is deemed acceptable. The MAH will remove the redundant information in the dossier section with the next opportunity.

The question is resolved.

18. Section 3.2.S.5 incorrectly refers to CX-024414 (instead of CX-031302) and should be corrected accordingly.

Summary of the MAH responses to outstanding issue:

The MAH clarifies that the reference is correct with the following rationale. RNA reference material is used as a system suitability standard for several release tests and is also used as a reference standard for measurement of total RNA content. With the development of mRNA-1273 variant mRNA such as CX-031302, and related variant mRNA-1273 LNP and DP materials, CX-024414 RNA reference material will be used to support testing of mRNA-1273 variant RNA, LNP, and DP materials. As a system suitability standard, this material is used to assess the system suitability of the analytical testing, for example the consistency of retention time or peak area response in an HPLC-UV method. In this application of the reference material, it is justified to use a qualified reference material with a different mRNA sequence from the test sample to assess system suitability, i.e., the use of prototype CX-024414 mRNA reference material to support the testing of variant CX-031302 mRNA or mRNA-1273.529 LNP.

Similarly, the prototype reference material can be used to quantitate total RNA content for variant LNP and DP test samples. Since the mRNA-1273 RNAs all have similar lengths, they have very similar molar extinction coefficients. For example, the calculated sequence-corrected coefficient for CX-031302 mRNA (33.99 µg/mL) and CX-024414 mRNA (34.01 µg/mL) are within 0.06% of each other. Thus, the use of a single RNA standard is suitable for total RNA content measurement across different variant mRNA-1273 materials.

The MAH agrees that in cases where an attribute is measured by comparison of a test sample against a comparator, a qualified reference standard should be used. Most of the analytical test methods used for testing release and stability of CX-031302 do not require this type of comparison to report results and therefore do not utilise product-specific reference standards.

- For Total RNA content testing, the reference material is used as the calibrator against which samples are compared to obtain the total RNA content values; a sequence-specific reference standard is not required for this assay.

- Sanger sequencing test methods are used to confirm identity for both mRNA-1273 RNA and LNP. These test methods can experimentally measure the nucleotide sequence of specific regions of the RNA. Test results are compared against the theoretical mRNA sequence to confirm identity of the test sample. Since the data reporting is using a theoretical mRNA sequence and not compared against the reference material sequence, a product-specific reference standard is not utilised for these test methods. In this case, the mRNA reference material is used as a positive control for the test method and serves as a system suitability standard.

- Assays such as %PolyA tailed variants, use a relative measurement of a specific peak of interest (such as a PolyA tail peak) relative to a total peak area within the same test sample to report results. The measurement of purity and product-related impurities by ion pairing reversed-phase chromatography uses a similar approach, where the purity and impurity peaks are measured in relation to the total peak area within a sample chromatogram. In both cases, a product-specific reference standard is not needed to measure the attributes of interest. To assess the system performance parameters such as peak area or retention time consistency within the run as system suitability, any standard that chromatographs similarly to the analyte of interest can be used, and a product-specific reference standard is not necessarily needed. In the case of the RP IP HPLC purity method, a single mRNA standard (CX-024414) is used to assess system suitability.

- For in vitro translation (IVT) for protein expression testing, after the mRNA test sample is translated into protein and labelled, it is separated on a gel and then compared against a protein molecular weight ladder standard to determine size. Its size is not compared against that of the mRNA reference material tested on the same gel. In this case, the mRNA reference material is used as a positive control for the test method and serves as a system suitability standard.

Assessment of responses to outstanding issue:

The MAH justified the use of CX-024414 as RNA reference material.

The question is resolved.

19. The process qualification summary document in section S.2.5 refers only to the Moderna Norwood and Lonza Visp mRNA-1273.529 LNP-B PPQ batches, but not to the PPQ batch manufactured at Rovi Granada. This should be clarified. In addition, the document title should be changed from "3.2.S.2.5 Process Validation and/or Evaluation {mRNA-1273 LNPs-B}" to "3.2.S.2.5 Process Validation and/or Evaluation {mRNA-1273.529 LNP-B}" and references in the summary document to other sections should be reviewed and revised as appropriate.

Summary of the MAH responses:

The MAH acknowledges the comment and provides a new version of the Section 3.2.S.2.5 Process Validation and/or Evaluation {mRNA-1273.529 LNP-B}, updated with the data from Rovi Granada and revised nomenclature for mRNA-1237.529 LNP-B material.

Assessment of the responses:

Module 3 has been updated as requested.

The question is resolved.

20. A formal comparability protocol is presented in 3.2.S.2.6 (of note, the protocol seems to be duplicated). The comparability acceptance criteria for purity/mRNA-related impurities seem to be

based on the current RP-HPLC method (Table 11, 13, and 14). However, due to the off-set/no determination of IG2, results obtained by the proposed new RP-IP-HPLC method are not directly comparable to results obtained by the current method. The comparability criteria should be updated accordingly.

Summary of the MAH responses to outstanding issue:

The formal comparability protocol lists impurity ranges that include impurity groups associated with the current RP-HPLC test method (SOP-0996) reporting as the method continues to be used for release testing of CX-024414 mRNA and mRNA-1273 LNP-B. With global approval of the purity method change to SOP-1142, the protocol will be updated to completely align with SOP-1142 impurity reporting.

Specification limits for purity and impurities associated with both purity methods (SOP-0996 and SOP-1142) are identical. When establishing the comparability criteria for purity and impurities by SOP-0996, tolerance interval analysis of a dataset of representative clinical and commercial lots distributed beyond the lower purity specification limit and upper impurity specification limits.

Thus, the established comparability criteria are closely aligned with the lower purity specification limit and upper impurity specification limits for SOP-0996. With the same specification limits being applied for SOP-1142, the currently established comparability criteria can still be applied to data generated using SOP-1142 to demonstrate comparability.

Assessment of responses to outstanding issue:

The MAH approach to maintain the comparability ranges until global approval of the purity method change is acceptable. However, the MAH´s position that the comparability criteria based on data originating from method SOP-0996 can still be applied to results obtained with method SOP-1142 is not endorsed. For future comparability studies, the comparability criteria for mRNA purity/impurities should be updated accordingly in line with the specification limits for mRNA purity/impurity.

The question is resolved with recommendation (REC8).

21. The loss rates/CI reported in Tables 5 and 7 of Section 3.2.S.7.1 slightly differ – a clarification is requested. In addition, the stability programmes differ between Moderna, Rovi, and Lonza – a justification is requested.

Summary of the MAH responses to outstanding issue:

Please find attached a revised version of section 3.2.S.7.1 Stability Summary and Conclusions mRNA-1273.529 LNP-B EU. For clarity, the title of Table 5 has been corrected to clarify it covers both -70°C and 5°C storage temperatures. The results presented have been updated with the latest stability modelling results and are now in line with Table 7. Table 7 provided the correct degradation rates for mRNA-1273 LNP at -70°C and 5°C as established based on the latest data available in May 2022. The complete subsection 3.2.S.7.1.1 "Stability Modelling Results" has been revised to integrate the latest statistical analysis performed in May 2022 (data from a previous analysis dated December 2021 remained).

Different stability protocols have been established for the registration batches from each site, on the basis of the knowledge cumulated at the time each protocol was established. The stability protocols being designed for the post approval stability studies and stability commitments will result from the same extensive stability data evaluation; stability protocols are being progressively harmonised across sites in terms of testing frequency and testing panel.

The cumulated knowledge of product degradation profile allowed the MAH to reduce the testing frequency for some of the quality attributes in the stability studies. The shelf life limiting attribute is mandatory at

each time point, instead PH, RNA content, % RNA encapsulation, lipids content and lipid impurities testing frequency has been reduced. The protocol in Table 11 is the new standard harmonised protocol, revised based on the extensive stability data available and will be implemented going forward for each new mRNA-1273 LNP-B stability studies. The protocols in Table-9 and Table-10 represent pre harmonisation stability programs for mRNA-1273 LNP-B stability studies.

Assessment of responses to outstanding issue:

The MAH provided the corrected documents and provided a clarification for the different stability programs.

The question is resolved.

22. The MAH states that mRNA purity is the stability limiting attribute; however, for two development lots Lot DH-06191.1 and DH-06191.2) after 12 months storage at -60°C to -90°C, OOS results are obtained for %encapsulation. This observation should be further discussed by the MAH.

Summary of the MAH responses to outstanding issue:

As shown in section 3.2.S.7.3 Stability Data {mRNA-1273 LNP – ROW}, % encapsulation is stable over an extended time (at least 18 months demonstrated for several lots) when mRNA-1273 LNP is stored at -70°C.

The lower than expected %encapsulation values for development lots DH-06191.1 and DH-06191.2 were observed after a freezer unit where the materials were chambered, was re-located to a new laboratory. Testing of original retains of this material stored in a different freezer unit at -70°C for > 12 months resulted in %encapsulation > 90% for both lots. Thus, the stability vials likely had undergone an unintended destabilisation (for example, an uncontrolled thaw and refreeze over extended duration) during the freezer re-location and the material was compromised. This arm of the stability study will be halted with no further data generated.

Assessment of responses to outstanding issue:

The MAH provided the justification for the OOS for % encapsulation which was due to the re-location of the freezer unit the samples were stored in.

The question is resolved.

23. Section 3.2.S.7.3 for mRNA-1273.529 LNP should be updated. The heading of this section changes from page 5 on. 'mRNA-1273 LNP' should be replaced in text and tables by 'mRNA-1273.529 LNP' where applicable.

Summary of the MAH responses:

The MAH is providing an updated Section 3.2.S.7.3 for mRNA-1273.529 LNP, including the correction of the headings.

Assessment of the responses:

Module 3 has been updated as requested.

The question is resolved.

24. A summary of the deviations observed in the stability program for mRNA-1273 LNP, mRNA-1273.529 LNP or other mRNA-1273 LNP Stability Modelling Lots should be provided.

Summary of the MAH responses:

Please find attached to this response document a listing of all major deviations and OOS results observed in the stability program for mRNA-1273 LNP, mRNA-1273.529 LNP and other Stability Modelling Lots. No major deviation potentially impacting the Stability Model Exercise was identified.

Assessment of the responses:

Documentation has been provided as requested.

The question is resolved.

Drug product:

mRNA 1273 DP injection

25. Section 3.2.P.1 lists only the composition of 0.1 mg/mL, 3.2 mL product; the description of the composition of the 0.2 mg/mL, 6.4 mL product is missing and should be added.

Summary of the MAH responses to outstanding issue:

Section 3.2.P.1 Description and Composition of the Drug Product {mRNA-1273.214 DP - 0.10 mg/mL} [Seq 0337] was issued upon EMA recommendation to only submit information specific to the bivalent mRNA-1273.214 DP product version. It covers the description of the 0.10 mg/mL presentation composition for 3.2 mL and 6.3 mL fill volumes.

The bivalent mRNA-1273.214 DP product version will not be manufactured at the 0.20 mg/mL strength. As a consequence, the composition of the monovalent specific presentations described as part of the Spikevax platform 3.2.P.1 Description and Composition of the Drug Product {mRNA-1273 DP - ROW} [Seq 0334], was not reported in the new mRNA-1273.214 specific section version.

Assessment of responses to outstanding issue:

The MAH clarified that the bivalent vaccine will only be produced at 0.10 mg/ml. However, the question relates to module *DP mRNA-1273, injection* that describes the current monovalent mRNA-1273 DP. The 0.2 mg/mL strength should be described in this module. An update with the closing sequence is acceptable.

The question is resolved with recommendation (REC9).

<u>Manufacturing process development</u>

26. Some release test results (purity and product-related impurities) are pending for all DP manufacturing sites and should be provided.

Summary of MAH's response:
The purity and product-related impurities results collected during PPQ on the Unlabeled mRNA-1273.214 DP intermediate are presented in section 3.2.P.2.3 Manufacturing Process Development {mRNA-1273.214} for each manufacturing site:

- Catalent – As part of interim characterisation testing in Table 27.
- Recipharm – As part of interim characterisation testing in Table 31.
- Rovi – As part of interim characterisation testing in Table 35.

- Patheon - As part of interim characterisation testing in Table 39.

Similarly, the submitted section 3.2.P.5.4 Batch Analyses {mRNA-1273.214} only presented the purity and product-related impurities data collected on the Unlabeled DP intermediate. Purity being the shelf-life indicating parameter, it is tested during routine operation after thawing and labelling / packaging activities. (These activities were not yet completed at time of submission). The final CoA is issued after purity testing of the labelled DP (LDP), supporting release of the Labelled mRNA-1273.214 DP batches.

The MAH provided information on commercial label and pack activities. Section 3.2.P.5.4 Batch Analyses {mRNA-1273.214} will be updated with final release results from all sites in Early September 2022.

Assessment of MAH's response:

The MAH has provided final CoAs for Catalent, Rovi and two Monza batches. Thus, the final CoA for the Recipharma PPQ lots and 1 Monza lot are missing. The MAH states that final CoAs will be available by end of August 2022 and section 3.2.P.5.4 Batch Analyses {mRNA-1273.214} will be updated in early September 2022.

Conclusion: Issue solved. Remaining CoA and updated section 3.2.P.5.4 Batch Analyses {mRNA-1273.214} will be provided in early September 2022 (REC4).

27. Final results for the CPP TOR/CPD should be provided for all DP manufacturing sites.

Summary of MAH's response:

The final Time out of Refrigeration (TOR) and Cumulative Process Duration (CPD) supported by the PPQ exercise for each site up to the manufacture of the commercial labelled mRNA- 1273.214 DP batches (LDP) are provided. For the Catalent and Recipharm sites, the final PPQ report covering final TOR and CPD up to the LDP stage are not yet available (Refer to Item 12).

Assessment of MAH's response:

TOR/CPD results have been provided for Rovi and Monza batches. The MAH commits to providing the TOR/CPD results from Catalent and Recipharm by September 2022 and October 2022, respectively.

Conclusion: Issue solved. Outstanding TOR/CPD results will be provided in September/October 2022 (REC5).

28. The comparability reports are missing and should be provided.

Summary of MAH's response

The overall mRNA-1273.214 comparability report DS-IND-0143 covering all four sites (Catalent, Recipharm, Rovi, Patheon Monza) is provided with this response. Please note that the report also includes the results obtained for the site Patheon Greenville, that is not registered in EU (as not relevant for EU market supply), and therefore not presented in Section 3.2.P.2.3.

Assessment of MAH's response:

The comparability report DS-IND-0143 has been provided.

Conclusion: Issue solved.

<u>Manufacture:</u>

29. Pooling of the two mRNA-LNPs is not adequately described under 3.2.P.3.3.2.1 LNP Pooling (e.g. 1:1 pooling by mass is not mentioned etc).

Summary of the MAH responses to outstanding issue:

Please find attached revised versions of the following P.3.3 sections with a revised description of the mRNA-LNPs pooling step:

- 3.2.P.3.3 Description of Manufacturing Process and Process Controls {Catalent - mRNA-1273.214} - See 3.2.P.3.3.1.1 LNP Pooling

- 3.2.P.3.3 Description of Manufacturing Process and Process Controls {Rovi -mRNA-1273.214} - See 3.2.P.3.3.2.1 LNP Pooling

The same description of the LNP pooling step than provided for the Patheon Monza and Recipharm sites have been incorporated.

The Critical In-Process Control (CIPC) for weight ratio was already described in the IPC tables (Table 2 for Catalent and Rovi; Table 3; Table 2 for Recipharm and Patheon Monza).

Assessment of responses to outstanding issue:

The MAH provided more detailed descriptions of the pooling step.

The question is resolved.

30. It is unclear why filtration pressure has been removed from the table of clarification process parameters (Table 9, 3.2.P.3.3 Description of Manufacturing Process and Process Controls {Recipharm – mRNA-1273.214}), although the legend indicates that this parameter is still controlled. The table should be revised accordingly. The same is true for Monza site.

Summary of MAH's response:
The MAH would like to highlight that all IPCs are now described together in Table 3 of Sections 3.2.P.3.3 {mRNA-1273.214 - Recipharm} and 3.2.P.3.3 {mRNA-1273.214 - TFS Monza}. It is confirmed that filtration pressure is still listed as an IPC for the clarification filtration step for both sites.

Assessment of MAH's response:
The MAH has clarified that filtration pressure during the clarification step is controlled as IPC at Recipharm and Monza. This measure was listed both as process parameter and IPC before. Now clarification filtration pressure is consistently defined as IPC for all sites and has been removed from the lists of process parameters.

Conclusion: Issue solved.

31. Flowrate, filtration pressure and duration of filtration have been removed from the list of sterile filtration process parameters (Table 10 of Section 3.2.P.3.3 Description of Manufacturing Process and Process Controls {Recipharm – mRNA-1273.214}), as compared to the 0.2 mg/ml process, although the text indicates that parameters are maintained. The table should be revised accordingly. The same parameters should also be included in the respective table for Monza site. Also for Monza, the description in the text indicates that filtration duration and filtration pressure are controlled.

Summary of MAH's response:
The MAH would like to highlight that all IPCs are now described together in Table 3 of Sections 3.2.P.3.3 {mRNA-1273.214 - Recipharm} and 3.2.P.3.3 {mRNA-1273.214 – TFS Monza}.

With regards to Recipharm, the following evolution can be noted and compared to the 0.20

mg/mL product version:

- Filtration pressure limit for Sterile Filtration remains a CIPC and is listed in Table 3 of Section 3.2.P.3.3. The limit is unchanged.
- The corresponding parameter is also listed in Table 3 of Section 3.2.P.3.3 for TFS Monza.
- Allowable duration of filtration is governed by sterile filtration validation as well as duration of site media fill, whichever is shorter. The duration is set at maximum for Recipharm and the parameter has been added in Table 10 of Section 3.2.P.3.3.
- The corresponding parameter (maximum 36 h for each filtration line) has also been added to Table 10 of Section 3.2.P.3.3 for TFS Monza. Actual durations for this process step are summarised below.
- The filtration flux parameter for sterile filtration (Table 10 of Section 3.2.P.3.3) has been removed since it is not relevant for sites performing filtration with on-line filling. The filtration is governed by the (vial) fill rate and is not a directly controlled process parameter. As part of process fit, the MAH verifies that at the maximum fill rate on the line, the flow-rate is below the stated flux limit.

The MAH provided information on the flow rate used on various manufacturing lines.

Assessment of MAH's response:
The MAH has clarified that Filtration pressure during Sterile Filtration is controlled as CIPC. The filtration process duration has been added to the list of process parameters for Monza and Recipharm. It is acceptable that the filtration flux has been removed as process parameter for sites performing filtration with on-line filling.

Conclusion: Issue solved.

32. In the process validation report for Monza, a high number of process parameters and critical process parameters is listed for the different manufacturing steps. This is not consistent with the PP given in section 3.2.P.3.3. and the CPP given in section 3.2.P.3.4. This should be clarified.

Summary of MAH's response
The MAH has implemented a consistent set of CPPs and CIPCs (presented in Section 3.2.P.3.4) across all manufacturing sites. Individual manufacturing sites have different definitions for CPPs as governed by their specific strategies to achieve control over operational and business risks. Thus, at the site level, additional controls may be implemented, where relevant, for operational needs based on site specific SOPs and control systems.

TFS Monza operationally defines multiple CPPs in each manufacturing step to ensure successful execution and control. All parameters that are controlled are CPPs. The MAH uses a stricter definition for CPPs – failure of which has risk impact on batch quality. The combination of IPCs (including CIPCs) with defined PPs and CPPs ensures that the batch quality is maintained. These have been consistently defined and used by the MAH across global manufacturing sites. Adapting these to each site would result in highly inconsistent control strategy, since as stated above, each site has their own definitions as well as terminology. In all cases, parameters with potential to impact to batch quality are controlled as CPPs or CIPCs and have resulted in consistent drug product quality over more than a 1000 drug product batches at 7 fill-finish manufacturing sites across the globe.

Assessment of MAH's response:
The MAH explains that in addition to the CPPs and CIPCs that are consistently defined for all DP manufacturing sites, each site has its specific control strategy that might include further controls. It is further clarified that the definition of a CPP is different between the MAH and Monza manufacturing site. It is considered confusing that in the process validation report different CPPs and PPs are defined than in the associated dossier parts. In addition, the definition of a CPP should generally be the same for all sites however, this can be accepted.

Conclusion: Issue solved.

33. To avoid misunderstanding, table 3 in section 3.2.P.3.3 Description of Manufacturing Process and Process Controls {Rovi – mRNA-1273.214} should be updated to include all process parameters for this step, not only the changed parameters. It is expected that buffer charge, buffer transfer rate and process temperature are maintained as process parameters for the dilution step.

Summary of MAH's response:
The Section 3.2.P.3.3 Description of Manufacturing Process and Process Controls {Rovi – mRNA-1273.214} has been revised accordingly and is provided with this submission.

Assessment of MAH's response:
Table 3 in section 3.2.P.3.3 Description of Manufacturing Process and Process Controls {Rovi – mRNA-1273.214} has been revised as requested.

Conclusion: Issue solved.

34. All process parameters for the DP dilution/mixing steps at Catalent should be given in section 3.2.P.3.3. Only buffer charge is currently indicated as (critical) process parameter here. It is expected that the process parameters used in the monovalent vaccine (mixing speed, mixing duration, process temperature, buffer transfer rate) at this steps will be maintained.

Summary of MAH's response
The Section 3.2.P.3.3 Description of Manufacturing Process and Process Controls {Catalent – mRNA-1273.214} has been revised accordingly and is provided with this submission.

Assessment of MAH's response:
Section 3.2.P.3.3 Description of Manufacturing Process and Process Controls {Catalent – mRNA-1273.214} has been revised as requested.

Conclusion: Issue solved.

35. In section P.3.3.it is stated for the optional interim storage step that the thaw duration is qualified during process qualification. The thaw durations should be updated in the dossier for all sites. In addition, the maximum interims storage time should be indicated in the process description for all sites.

Summary of the MAH's response
The vial thaw durations have been qualified and were summarised. The corresponding process description are provided for all sites in the corresponding 3.2.P.3.3 sections.

Conclusion.
The CHMP requests an update to relevant dossier sections to further define vial thaw durations. Issue not solved.

MAH's 2nd response:
Please find with this response document the revised CTD sections:

- 3.2.P.3.3 Description of Manufacturing Process and Process Controls {mRNA-1273 DP -

0.10 mg/mL-Rovi}

- 3.2.P.3.3 Description of Manuf Process and Process Controls {mRNA-1273 DP-

0.10mg/mL-Catalent}

As requested, the thaw duration times are detailed in Table 12 and 13, respectively, and the currently assigned maximum duration of 6 months interim storage is detailed in section 3.2.P.3.3.2.12 "Vial Interim Storage".

Assessment of the MAH's 2nd response: The MAH defined the maximum duration of interim hold time and provided the updated documents.

Conclusion: Issue solved.

Process validation:

36. Three consecutive PPQ lots have been manufactured at Catalent vial line 3 according to the modified process. The MAH should clarify whether other lines are also intended to manufacture the bivalent vaccine.

Summary of MAH's response
The MAH confirms that qualification data will be provided to support Catalent Line 1 and Flexible filler line before manufacturing of the bivalent vaccine on these lines.

Assessment of MAH's response:
The MAH has confirmed that qualification data will be provided to support Catalent Line 1 and Flexible filler line before manufacturing of the bivalent vaccine on these lines.

Conclusion: Issue solved.

37. In section 3.2.P.3.5 Process Validation and/or Evaluation {Rovi-Variant}, Table 7 shows not "Total Reject Rate (%)" as indicated, but the absolute number of rejected vials. This should be corrected.

Summary of MAH's response
The MAH confirms that Table 7 in Section 3.2.P.3.5 {mRNA-1273.214 DP – Rovi SSRR} present the actual count of vials rejected and not the % as stated in the column header. The updated Table is provided below. The corrected Section is provided with this submission.

Assessment of MAH's response:
The table has been corrected.

Conclusion: Issue solved.

38. The final PPQ reports for all DP manufacturing sites should be provided when available.

Summary of MAH's response:
The final PPQ reports covering complete manufacturing operations up to the Labelled Drug Product /LDP) step at each site are made available.

Assessment of MAH's response:
PPQ reports have been provided for Rovi and Monza. The final PPQ reports for Catalent and Recipharm will be provide in September and October 2022.

Conclusion: Issue solved. Outstanding PPQ reports will be provided in September and October 2022 (REC6).

39. In contrast to the other DP manufacturing sites, at Recipharm only one lot has been included in a dilution mixing study that demonstrates similar pH, osmolality and mRNA content at the bottom, middle and top of the vessel after specific mixing times. Since the mixing procedure has been changed at Recipharm, control of homogeneity including mRNA-LNP ratio would have been expected for all three PPQ lots. Although homogeneity is further shown by analysing the ratio of the two different mRNA-LNPs during begin, middle and end of filling and inspection for all PPQ batches, further proof of homogeneity after mixing should be provided.

Summary of MAH's response:
In the mRNA-1273.214 DP process validation, no mixing was intended on the pooled LNPs prior to dilution step for mRNA-1273.214 process. Mixing was performed only after dilution, using same mixing speed and mixing duration as for the mRNA-1273 process already validated for the 0.20 mg/mL product (Section 3.2.P.3.3).

In Recipharm, Monts, pooling, dilution and mixing of mRNA-1273.214 DP solution can be performed in one of the four equivalent (1000-L) vessels. During this PPQ campaign, a mixing homogeneity study was performed with sampling from top, middle and bottom of vessel during compounding of PPQ batch 223033 using a specified vessel to confirm and demonstrate the mixing homogeneity which was previously validated. This was limited to one batch to restrict open-vessel interventions required for sampling which adds significant bioburden risk to the compounded solution.

Furthermore, homogeneity of the diluted solution after mixing is supported using the RNA ratio results from the PPQ characterisation sample "23-PPQ" and RNA content results from PPQ characterisation sample "22-PPQ".

These results will be included in the final PPQ report.

The additional results summarised above, in combination with the earlier conducted mixing homogeneity study, confirm the ability to achieve a homogeneous bulk after dilution using the parameters listed in Table 8, Section 3.2.P.3.3 {mRNA-1273.214 - Recipharm}.

Assessment of MAH's response:
The MAH is of the opinion that including only 1 batch in the mixing study at Recipharm was sufficient because the aim was to confirm and demonstrate the mixing homogeneity which was previously validated. This is not fully agreed since the mixing procedure has been changed and the LNP pooling mixing step has been omitted. However, the MAH provides further data to demonstrate homogeneity after the dilution step. This data supports homogeneity however, samples have not been taken from different places of the mixing vessel. Nevertheless, taken into account the data from samples taken at begin, middle and end of filling, this is accepted.

Conclusion: Issue solved.

40. During process validation at Monza, an OOS result for filling homogeneity was observed. This deviation was caused by a non-correct installation of the filter housing. In the report, it is stated that other parameters were in the expected range, only mRNA content at different filling stages was affected. Since homogeneity is not routinely analysed, the MAH should clarify how it can be excluded that non-correct filter installation leads to an unnoticed inhomogeneity of mRNA content during filling.

Summary of MAH's response
The MAH wants to clarify that the filling homogeneity results were not OOS against release acceptance criterion for RNA content. During process validation at TFS, Monza, a bulk homogeneity study was performed on the final bulk solution in order to demonstrate homogeneity of the dispersion throughout the compounding tank. Samples were collected from top, middle and bottom of the compounding tank after mixing and demonstrated adequacy of the mixing speed and duration.

However, during demonstration of Filling Homogeneity on batch MV10001/1, results out of the CMO internal equivalency critierion were obtained for the total RNA content for the End samples. An investigation was initiated and concluded that these results could be linked to incorrect assembly of the filters in the sterile filtration line. The MAH provided information on the RNA content and the CAPAs defined to prevent non correct installations of machinery.

The operational result of the incorrect assembly was an increase of the pressure on the sterilisation filter. The incorrectly assembled filter caused reversal of flow (outlet to inlet) in the filter. This led to a rapid increase in filtration pressure. However, it must be noted that the controls installed to detect the increase in pressure functioned as intended and the filtration pressure alarm limit (1.3 bar) was not exceeded. The (differential) filtration pressure is monitored continuously during the manufacturing operations. Pressure transmitters, connected to the SCADA system, are installed on the stainless-steel line upstream and downstream of each sterilising filter with alarms. An excursion beyond the filtration pressure acceptance criteria would lead to a stop of the operations and a filter change-out.

Thus, the CAPAs already implemented along with the active pressure monitoring system significantly reduces the risk for this failure mode for inhomogeneity or for it to go undetected during routine operations.

Assessment of MAH's response:
The implemented CAPAs to reduce the risk of incorrect filter installation are deemed sufficient.

Conclusion: Issue solved.

Control of drug product:

41. The MAH should clarify whether the RNA ratio method is specific to intact RNA or whether RNA fragments or adducts will also contribute to the result. In the latter case, it should be justified how an equal amount of functional RNA can be ensured when mRNA-LNPs with different purities are used for formulation of the DP.

Summary of the MAH responses to outstanding issue:

The MAH clarifies that the RNA ratio is determined based on total RNA, including intact mRNA, RNA fragments and RNA lipid adducts, this is consistent with total RNA content determination. The level of RNA fragments and RNA lipid adducts are controlled at release for each mRNA-LNP used in the manufacture of the mRNA-1273.214 DP.

The proportion of intact RNA for the mRNA-LNPs components might be slightly different depending on their individual purities at release, but the rate of purity loss is expected to be similar for each component. The ratio of functional RNAs in the DP is not guaranteed to be identical for all components, but will be limited to a relatively narrow range by the in-process controls on Weight of LNPs pooled, the LNP incoming release purity, the similarity of purity loss rates per component, as well as the specifications on (Total) RNA Ratio in the final product.

Additionally, the MAH has performed the following assessment of the mRNA release purity from 728 mRNA-1273 LNP commercial batches across three manufacturing sites (Moderna Norwood, MA, Lonza

Portsmouth, NH, and Lonza Visp, Switzerland) to determine the process capability of each site as well as understand site-to-site differences. A statistical summary is provided in Table 1. The results demonstrate a high degree of consistency for purity across the manufacturing history, further supporting the conclusion above.

Assessment of responses to outstanding issue:

The MAH clarified that RNA ratio determination is based on total RNA but argues that because of the high consistency of the purity of batches produced only slight differences will occur.

The question is resolved.

42. The dossier should be updated with final DP batch analysis results and the final CoA for the PPQ lots from the four DP manufacturing sites should be provided when the complete data is available.

Summary of MAH's response
Already addressed in response to Q27.

The question is resolved.

43. In section 3.2.P.5.5 Characterisation of Impurities {mRNA 1273.214-Patheon (Monza)}, the MAH refers to "Section 3.2.P.3.5 {mRNA 1273.214 – Patheon (Monza)} – Ext-0820)" regarding sterile filter leachables. However, leachables are not addressed in this section/report. The MAH should provide the relevant information for sterile filter leachables including a risk assessment for the unknown esters.

Summary of the MAH responses to outstanding issue:

The MAH notes that the filter extractables and their toxicology assessment was provided in Section 3.2.P.5.5 – Patheon (Monza). However, EXT-0820 was incorrectly referred to for filter leachables. An updated section 3.2.P.5.5 Characterisation of Impurities {mRNA-1273.214-Patheon (Monza)} is provided without the incorrect cross-reference raising confusion (See revised section 3.2.P.5.5.1).

As noted in the P.5.5 section, a formal Toxicology assessment of unknown esters is not possible when the structure as well as concentration is not known. These structures (= extractables) were detected in an extraction study under worst-case conditions. The amounts of any such substances appearing as leachables is expected to be lower in practice.

The Sartorius Sartopore 2 XLG filters are commonly used for manufacture of parenteral products. In light of their usage for large batch sizes and for a product dosed in small volumes over a limited number of doses, the filters are considered suitable for use.

Assessment of responses to outstanding issue:

The MAH stated that the cross-reference was removed. However, it is still included in section 3.2.P.5.5.1.3.1.2 in the provided document. Therefore, the MAH is asked again to provide the report of the leachable study conducted by Sartorius Validation Services. The explanation concerning the unknown esters is acceptable.

The question is not resolved.

Summary of the MAH 2nd responses to outstanding issue: The MAH acknowledges the comment. The report is provided in attachment to this response.

Assessment of 2nd responses to outstanding issue: The MAH provided the requested document. However, the section 3.2.P.5.5 should be resubmitted with the correct cross-reference to this report in section 3.2.P.5.5.1.3.1.2 with the closing sequence.

The question resolved with recommendation (REC7).

44. The programme for annual requalification of PRM/WRM lot D009222001 should be provided and Section 3.2.P.6 updated accordingly

Summary of the MAH responses to outstanding issue:

The primary/working reference material (PRM/WRM) lot D00922001 will be requalified annually as per internal SOP. Section 3.2.P.6 Reference Standards {mRNA 1273.214} has been updated accordingly; see section 3.2.P.6, Page 2, last paragraph.

Assessment of responses to outstanding issue:

The MAH provided the updated document.

The question is resolved.

Stability:

45. As stated in the CHMP scientific advice from July 2022, the reduction to only one single stability verification batch will only be acceptable when enough data from several batches is available that demonstrates that number and sequence of RNAs has no influence on stability. In section 3.2.P.8.1 Stability Summary and Conclusion {mRNA-1273.214}, the MAH states that "Stability studies representing similarly formulated lots of mRNA-1273, mRNA-1273.211 and mRNA 1273.351 are available for comparison" and indeed stability data is provided for such lots in 3.2.P.8.3. However, no RNA degradation slopes are presented for these lots, so it is unclear how this data should justify comparable stability behaviour of the variant vaccines. In addition, there is no information provided for the variants: strain, RNA length, concentration, etc. is unknown. The MAH should provide more information on the variant vaccine DP lots used for demonstrating comparable stability and present the RNA degradation slopes in comparison to the slopes calculated for the prototype vaccine.

Summary of the MAH responses to outstanding issue:

Please find attached the updated stability report DPAD-00880 version 5.0 which integrates the latest stability data available for the calculation of the RNA degradation rates. The similarity of RNA degradation slopes was assessed in this updated comprehensive evaluation using all stability study results available for Drug Product as of May 25, 2022. 81 development and GMP lots were studied at six different storage temperatures, although not all lots were studied at all six temperatures.

Assessment of responses to outstanding issue:

The MAH provided an updated stability report including RNA degradation slopes. However, no further data on the variants used as supportive data was provided.

The question is not resolved.

Summary of the MAH 2nd responses to outstanding issue: Supportive data from stability studies for development and clinical lots mRNA-1273.214 are described in Sections 3.2.P.8.1 and 3.2.P.8.3; development and clinical stability data from additional mRNA-1273 variant vaccines are also provided, including: mRNA-1273.351 (Beta), mRNA-1273.617.2 (Delta), mRNA-1273.529 (Omicron BA.1), mRNA-1273.211 (Prototype + Beta), and mRNA-1273.213 (Beta + Delta). These bracket mRNA-1273 RNA sequence lengths between 4092 and 4101 nucleotides and concentrations between 0.1 and 0.5 mg/mL.

Assessment of 2nd responses to outstanding issue: The MAH provided the requested information.

The question is resolved.

46. It was stated in the CHMP scientific advice from July 2022 that "the MAH's proposal to assign the shelf-life claims from the prototype mRNA 1273 vaccine to mRNA-1273 associated variant vaccines might be acceptable provided that accelerated data is available that confirms comparable stability behaviour." For one PPQ lot from Catalent, stability data at 25°C is available. In addition, for a developmental mRNA-1273.214 Drug Product Lot (DHM-82829) stability data at 5°C and 25°C is provided. However, these results have not been used for calculating RNA degradation slopes, so no conclusion on comparability is possible. The MAH should demonstrate that the RNA degradation rates at accelerated temperature for the mRNA-1273.214 Drug Product lots are comparable to the prototype degradation rates to support the proposed shelf life.

Summary of the MAH responses to outstanding issue:

Available mRNA-1273.214 Drug Product Lot (DHM-82829) stability data at 5°C and 25°C was included in a revised version of the comprehensive stability evaluation using all stability study results available for Drug Product as of May 25, 2022. Please find attached the updated stability report DPAD-00880 version 5.0 which integrates the latest stability data available for the calculation of the RNA degradation rates.

Inclusion of the 25°C accelerated stability data for Lot 6017222001 of mRNA-1273.214 produced at the Catalent site will be added to the comprehensive RNA degradation rate evaluation at the next update of DPAD-00880.

Assessment of responses to outstanding issue:

The MAH provided the updated stability report that included the developmental mRNA-1273.214 Drug Product Lot including calculated RNA degradation slopes.

The question is resolved.

47. In section 3.2.P.8.1.4.2 *Variant Stability Study Results*, the links to the Tables are misleading and should be corrected.

Summary of the MAH responses to outstanding issue:

Please find attached a reformatted version of section 3.2.P.8.1 Stability Summary and Conclusion {mRNA 1273.214}. The links in Table 11, section 3.2.P.8.1.4.2, which were designed to link tables located in section 3.2.P.8.3 have been removed to avoid confusion.

Assessment of responses to outstanding issue:

The MAH provided an updated document

The question is resolved.

48. The stability model uses RNA purity as a measure of DP stability. The method for measuring RNA purity has been changed and it has been noted that the results for DP samples are about 5% higher when the new method is used (see assessment of the introduction of the new method for determination of RNA impurity). Therefore, calculation of degradation slopes should be based only on values obtained from one of the methods, not from mixed results. The MAH should confirm that this is the case.

Summary of the MAH responses to outstanding issue:

The purity method employed to obtain each result used in stability modelling is tracked and accounted for in the calculation of degradation slopes. Purity results for most stability studies have been obtained with only the original method (SOP-0996) or only the new method (SOP-1142), and these degradation slopes are based only on values obtained from one of the methods. However, there are a limited number of stability studies with early time points obtained using the original purity method, and later time points obtained using the new method.

In order to evaluate all available data, instead of omitting results obtained with mixed methods, two approaches are used to account for the different purity methods:

- First, degradation rates (slopes) obtained with the two methods are compared using an Analysis of Covariance (ANCOVA) statistical model to assess for poolability of slopes across purity methods.

- Second, when results from the two methods are included in a unified overall model, additional terms are added to the ANCOVA model to assess the potential difference in initial purity due to method and the potential difference in purity slope due to method.

This modelling approach permits an overall assessment using all available data, rather than excluding some results because of the method improvement introduced during certain stability studies.

Assessment of responses to outstanding issue:

The MAH explained that additional terms to the statistical model were introduced to account for the different purity methods used.

The question is resolved.

General issues:

49. Removal of reference to SOP numbers in the specifications is not acceptable. These references should be re-established for all components of Spikevax.

Summary of the MAH responses:

The MAH provides in attachment the corrected Specifications sections including the reference to SOP numbers.

Assessment of the responses:

Module 3 has been updated as requested.

The question is resolved.

50. Updated stability data should be provided for all components of Spikevax.

Summary of the MAH responses:

The MAH is providing in attachment to this response the updated stability data for CX-031302, mRNA-1273.529 LNP-B and mRNA-1273.214 Drug Product. The MAH commits to update the stability sections for all the remaining components of Spikevax by October, 2022.

Assessment of the responses:

CX-031302

The MAH provided 3-month data at -20°C and 3-month data of the accelerated stability study at 5°C.

mRNA-1273.529 LNP-B

The following additional data have been provided.

Lot 5011122003: Data for three months for, stored between -60°C to -90°C (previously no data). All results are within the specifications.

Lot 5011422001: Stored at 5°C ± 3°C for two months (previously 1 month). Result for purity is OOS (68% vs NLT 70%)

Lot 5011422001: Stored at -80°C ± 10°C for 3 months (previously no data). All results are within the specifications.

Outstanding Issue:

Batch 5011522001 manufactured at Rovi has been removed from the updated section 3.2.S.7.3 Stability Data {mRNA-1273.529 LNPs} without any notice what is not acceptable. Table 1 in 3.2.S.7.3 Stability Data {mRNA-1273.529 LNPs} should be revised and data from batch 5011522001 for long term and accelerated storage should be provided. According to the stability protocol for this batch testing under accelerated conditions is forseen after 1 and two months and some data have to be available.

Summary of the MAH responses to outstanding issue:

mRNA-1273.529 LNP-B

Data from Rovi Granada was inadvertently removed upon request from another agency. The MAH apologises for the version mistake and provides an updated version including the stability data collected on Rovi Batch 5011522001.

Assessment of responses to outstanding issue:

Stability data provided for the mRNA CX-031302 is acceptable for the time being.

Stability data for Rovi Batch 5011522001 have been provided for long term and accelerated conditions. Result for purity is OOS (67% vs NLT 70%) under accelerated conditions. All other results are within the specifications. The MAH commits to update the stability sections for all the remaining components of Spikevax by October, 2022 (REC). Therefore, the currently available data are acceptable.

However, for the Rovi mRNA-1273.529 LNP stability batch 5011522001 the results for encapsulation are presented as <94% (less than; specification limit NLT 85%). Numerical values should be provided unless otherwise justified. This also applies to other stability batches where the results are reported as >94% (more than). 3.2.S.7.3 Stability Data {mRNA-1273.529 LNPs} should be revised accordingly.

MAH Response to additional outstanding issue:

The MAH acknowledges the comment, confirms that the correct result is >94% for encapsulation, and provides with this response the corrected section 3.2.S.7.3 Stability Data {mRNA-1273.529 LNPs} with

this submission. The MAH would like to specify that the 94% corresponds to the upper quantitation limit of the validated method.

Assessment of responses to outstanding issue:

The stability data for encapsulation have been corrected. It has been clarified that 94% corresponds to the upper quantification limit of the encapsulation method.

Issue solved with recommendation (REC3).

Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance imelasomeran, bivalent Original/Omicron BA.1 finished product and data to support the additional scopes have been presented in a satisfactory manner. The results of tests carried out, indicate consistency and uniformity of important product quality characteristics and these in turn lead to the conclusion that the product is expected to have satisfactory and uniform performance in clinical use.

Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

3.3. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. The MAH should submit work on the required implementation of the IVT assay.
2. The MAH should review the suitability of IPCs.
3. The MAH should update the stability sections for all the components of Spikevax.
4. The MAH should provide the remaining CoA from specified sites and update section 3.2.P.5.4 Batch Analyses {mRNA-1273.214}.
5. The MAH should provide the TOR/CPD results from specified sites.
6. The MAH should provide the final PPQ reports for specified sites.
7. The MAH should resubmit the section 3.2.P.5.5 with the correct cross-reference to this report in section 3.2.P.5.5.1.3.1.2 with the closing sequence.
8. The MAH should update the comparability criteria in the comparability protocol for mRNA purity for future comparability exercises.
9. The MAH should update the DP composition section of the dossier and submit this in the closing sequence.
10. The MAH should reassess the need to adjust the purity specification limits at the level of active substance and intermediates finished product.

4. Non-clinical aspects

4.1. Pharmacology

4.1.1. Brief summary

mRNA-1273 encodes the S protein of the Wuhan-Hu-1 isolate of SARS-CoV-2, whereas mRNA-1273.529 encodes the S protein of the SARS-CoV-2 BA.1 variant (Omicron). In addition, both vaccines include two proline mutations to stabilise the S protein into the pre-fusion conformation (S2P). Non-clinical bivalent mRNA-1273.214 was a 1:1 bench side mix of mRNA-1273 and mRNA-1273.529.

All vaccines were formulated into a mixture of four lipids: SM-102, cholesterol, DSPC and PEG2000-DMG.

The MAH conducted four non-GLP compliant primary pharmacodynamics studies to demonstrate the immunogenicity and efficacy of mRNA-1273, mRNA-1273.529 and mRNA-1273.214. Three of these studies were conducted in mice and one study were conducted in non-human primates (NHPs). In all studies, the vaccines were administered intramuscularly (IM), which is the intended administration route in human.

The preclinical mRNA-1273 and mRNA-1273.529 drug products used in these studies were mRNA formulations prepared with the same method as the Good Manufacturing Practice mRNA-1273 and mRNA-1273.529 clinical drug products.

4.1.2. Primary pharmacodynamics

Study MOD-5019: Evaluation of Immunogenicity and Antigen-Reactive B Cell Responses of Omicron-Matched mRNA Vaccine Boosters in Mice

Study design:

The immunogenicity and antigen-reactive B cell responses of mRNA-1273, mRNA-1273.529 and mRNA-1273.214 were analysed. Eight female BALB/c mice per group were administered intramuscularly with three or four mRNA vaccine doses.

Animals of the 3-dose regimen (group 1-4) were administered 0.25 µg mRNA-1273 on Day 1 and Day 22. At Day 50, these mice were boosted with 0.25 µg of mRNA-1273, mRNA-1273.529, or mRNA-1273.214. Animals who received the 4-dose regimen (group 5-8) were administered 0.25 µg mRNA-1273 on Day 1 and Day 22. At Day 50 and Day 78, these mice were boosted with 0.25 µg of mRNA-1273 or mRNA-1273.529. Mice boosted with mRNA-1273.214 were not included in the 4-dose regimen part of this study.

Animals of Groups 1 (3 dose-regimen) and 5 (4 dose regimen) received phosphate-buffered saline (PBS) control article on the same dosing schedule as the active groups.

Table 1: Study Design for Study MOD-5019

Study Report Group (n = 8/group)	Primary Series (Dose 1 and 2)			Booster 1 (Dose 3)			Booster 2 (Dose 4)			Readouts
	Treatment (IM)	Dose Level (µg)	Dose Schedule	Treatment (IM)	Dose Level (µg)	Dose Schedule	Treatment (IM)	Dose Level (µg)	Dose Schedule	
Three Doses Administered										All groups: Blood (Days 21, 36, 49): Antibody responses (ELISA, PSVN)
1	PBS Control	0	Day 1, 22	PBS	0	Day 50	NA			Groups 1-4 (3 doses): Sacrifice (Day 64): Serum: Antibody responses (ELISA, PSVN assay) Spleen and LN: Antigen-specific B cell response
2	mRNA-1273	0.25		mRNA-1273	0.25					
3	mRNA-1273	0.25		mRNA-1273.529	0.25					
4	mRNA-1273	0.25		mRNA-1273.214*	0.25					
Four Doses Administered										
5	PBS Control	0	Day 1, 22	PBS	0	Day 50	PBS	0	Day 78	Groups 5-8 (4 doses): Sacrifice (Day 93): Serum: Antibody responses (ELISA, PSVN assay)
6	mRNA-1273	0.25		mRNA-1273.529	0.25		mRNA-1273.529	0.25		
7	mRNA-1273	0.25		mRNA-1273	0.25		mRNA-1273	0.25		
8	mRNA-1273	0.25		mRNA-1273	0.25		mRNA-1273.529	0.25		

Abbreviations: IM = intramuscular; LN = lymph node; mRNA = messenger RNA; NA = not applicable; PBS = phosphate-buffered saline; PSVN = pseudovirus neutralization.
* mRNA-1273.214 was a 1:1 bench side mix of separately formulated mRNA-1273 and mRNA-1273.529.

To analyse the vaccine immunogenicity, blood was collected from all animals on Day 21 (before Dose 2), Day 36 (2 weeks after Dose 2), and Day 49 (before Dose 3). Blood was also collected from animals of the 3-dose regimen on Day 64 (2 weeks after Dose 3) and from animals of the 4-dose regimen on Day 93 (2 weeks after Dose 4).

Serum samples were analysed for stabilised Wuhan-Hu-1 spike protein-specific (S-2P) or BA.1 spike protein-specific (S-2P.529) IgG antibody responses via an enzyme-linked immunosorbent assay (ELISA).

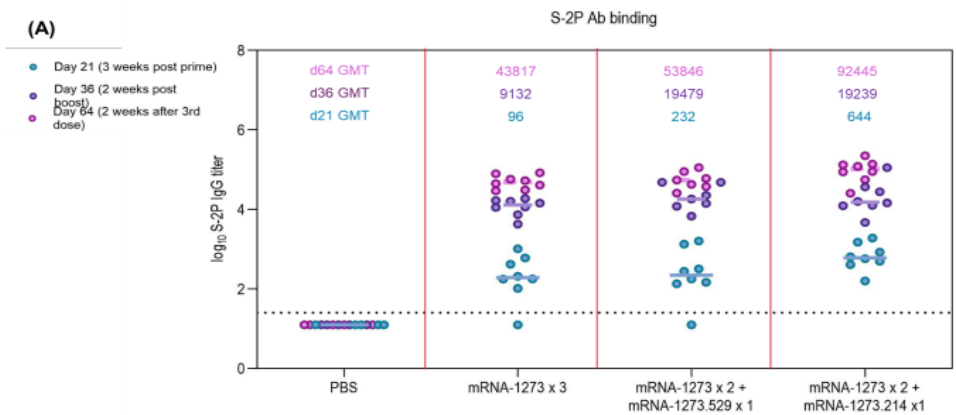
Neutralising antibody responses against Wuhan-Hu-1 spike-protein (D614G), BA.1 spike protein and BA.2 spike protein were analysed by a vesicular stomatitis virus (VSV)-based pseudovirus neutralisation (PSVN) assay.

Mice who received the 3-dose regimen were euthanised on Day 64, and mice who received the 4-dose regimen were euthanised on Day 93. Spleen and lymph nodes of the study animals were harvested for antigen-reactive B cell analysis.

Results of the 3-dose regime:

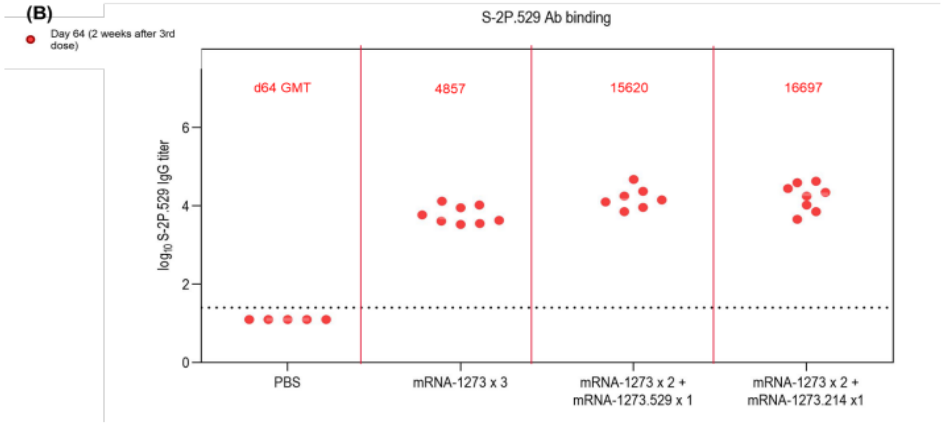
Figure 1: Binding Antibody Responses in BALB/c Mice After Dose 3

Figure 1: Binding Antibody Responses in BALB/c Mice After Dose 3



One dose of mRNA-1273 induced low Spike protein (SP)-specific IgG antibody titres in most mice (96 - 644 GMT). The SP-specific IgG antibody titres increased after a second dose of mRNA-1273 and SP-specific antibody responses could be detected in all mRNA-1273 vaccinated animals (9132 - 19479 GMT).

2 weeks after a booster dose of mRNA-1273 (group 2), the SP-specific IgG antibody titre increased even more (43817 GMT). Minimal higher GMT of SP-specific IgG antibodies were detected 2 weeks after a booster dose of mRNA-1273.529 (group 3, 53846 GMT) and mRNA-1273.214 (group 4, 92445 GMT). However, it has to be considered that the SP-specific IgG antibody titres after the primary vaccine series with mRNA-1273 were already higher in group 3 and 4 than in group 2 mice.

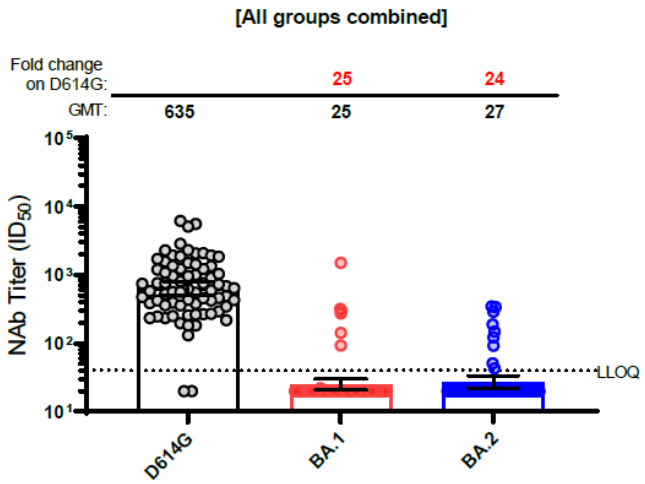


Abbreviations: Ab = antibody; GMT = geometric mean titer; IgG = immunoglobulin G; PBS = phosphate-buffered saline; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; S-2P.529 = Omicron-specific S-2P.

In a S-2P.529-specific ELISA, Omicron spike protein (S-2P 529)-specific IgG antibody responses did not differ remarkably in animals boosted with mRNA-1273 (4857 GMT), mRNA-1273.529 (15620 GMT) or mRNA-1273.214 (16697 GMT). The different boosters induced an S2P.529-specific IgG antibody titre slightly low than for Wuhan Spike-specific IgG antibodies. Similar to the Wuhan Spike protein titres, the GMT were higher in mice boosted with one of the both BA.1 specific vaccine candidates compared to mice, which were boosted with mRNA-1273. Also for these results, the already higher Wuhan SP IgG antibody titre in group 3 and 4 mice might have an impact on the Omicron-specific IgG antibody titre.

Figure 2: Neutralizing Antibody Responses in BALB/c Mike Before Dose 3

Figure 2: Neutralizing Antibody Responses in BALB/c Mice Before Dose 3

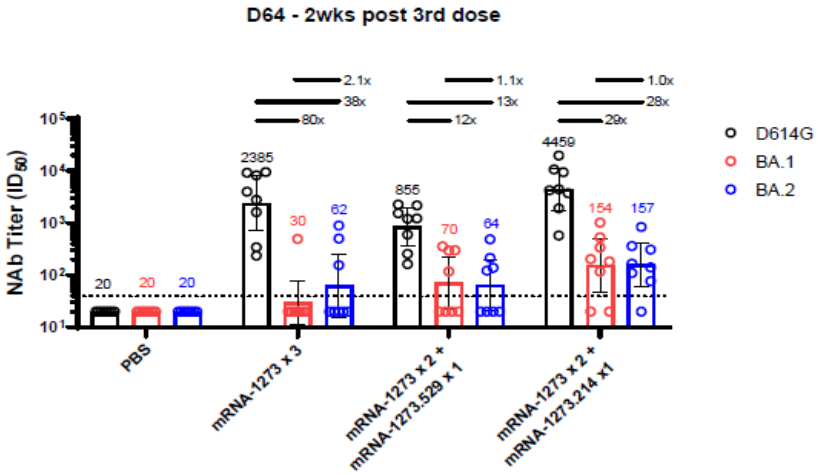


Abbreviations: GMT = geometric mean titer; ID50 = infectious dose 50; LLOQ = lower limit of quantification; NAb = neutralizing antibody.
Note: Mice received 0.25 µg mRNA-1273 on Day 1 and Day 22 (primary series; Dose 1 and 2).

On Day 49 after two mRNA-1273 doses, all animals showed S-2P Wuhan-Hu-1-specific neutralising antibody titre at an infection dose (ID50) of 635 GMT. At this time point, before the booster vaccination, the BA.1 and BA.2 specific neutralising antibodies were below the quantification limit (LLOQ).

Figure 3: Neutralizing Antibody Responses in BALB/c Mike Before Dose 3

Figure 3: Neutralizing Antibody Responses in BALB/c Mice After Dose 3



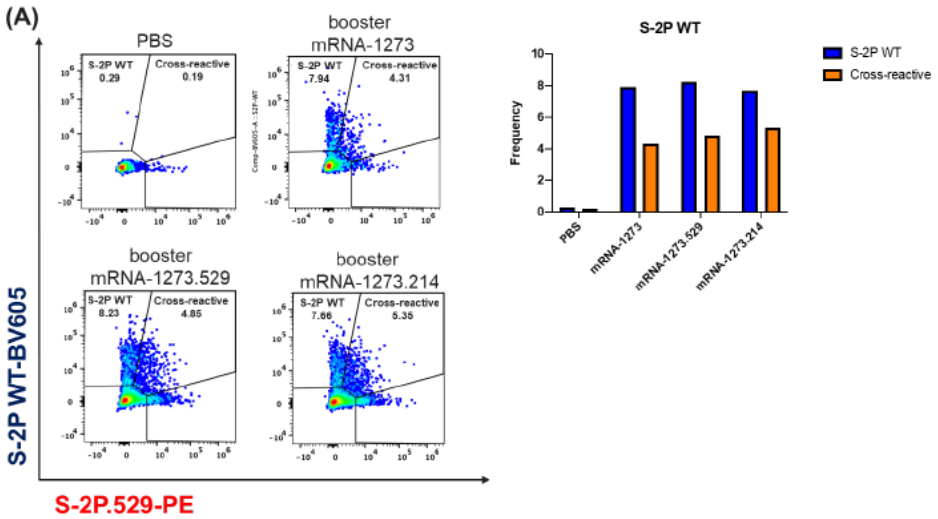
Abbreviations: GMT = geometric mean titer; ID50 = infectious dose 50; NAb = neutralizing antibody.
Note: The dotted line represents the lower limit of quantification.

After a booster (3rd) dose with mRNA-1273, mice showed an increased Wuhan S-2P specific neutralising antibody titre (2385 GMT) but only very low neutralising antibody titres against BA.1 (30 GMT) and BA.2 (62 GMT), which were below and shortly above the LLOQ, respectively. Animals boosted with mRNA-1273.529 showed increased Wuhan S-2P specific neutralising antibody titre (855 GMT) compared to mice of the primary vaccine regime (2x mRNA-1273), but the titre was slightly lower than for the mRNA-1273 boosted mice. Furthermore, the neutralising antibody titre against BA.1 was only slightly higher (70 GMT) and against BA.2 was similar (64 GMT) as compared to mRNA-1273 boosted animals. Animals boosted with mRNA-1273.214 showed increased Wuhan S-2P neutralising antibody (4459 GMT), which were higher than in mRNA-1273-boosted mice, and higher titres of neutralising antibodies against BA.1 (154 GMT) and BA.2 (157 GMT).

Overall, the neutralising antibody titres against BA.1 and BA.2 were still very low in Omicron-specific vaccine boosted mice.

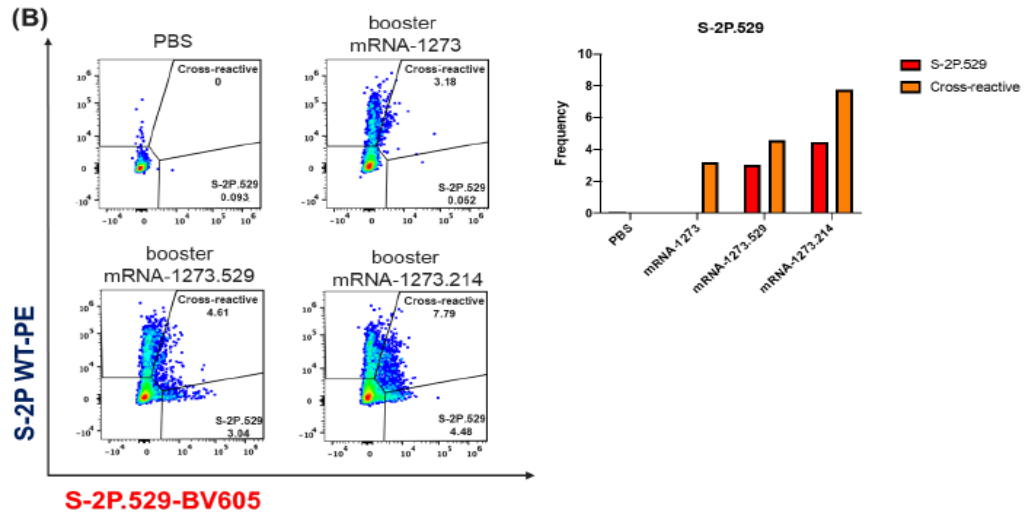
Figure 4: Frequencies of S-2P and S-P.529 and Cross-Reactive (WT+.529+) B Cells in Iliac Lymph Nodes in Mice 2 Weeks After Boost (Dose 3) With mRNA-1273 or BA.1-Matched mRNA Vaccines

Figure 4: Frequencies of S-2P and S-2P.529 and Cross-Reactive (WT+ .529+) B Cells in Iliac Lymph Nodes in Mice 2 Weeks After Boost (Dose 3) With mRNA-1273 or BA.1-Matched mRNA Vaccines



2 weeks post-dose on Day 64, spike protein antigen-specific B cell responses were observed in iliac lymph nodes of mice boosted with mRNA-1273, mRNA-1273.529 or mRNA-1273.214. For this study, pooled lymph nodes were analysed. The frequencies of antigen-reactive B cells are expressed as a percentage of all IgD- and IgM- class-switched B cells.

Similar high frequencies of Wuhan-Hu-1 S-2P D6146G-specific B cells were observed in all three booster groups. Similar, but lower frequencies of cross-reactive B cells were also observed in all three booster groups.



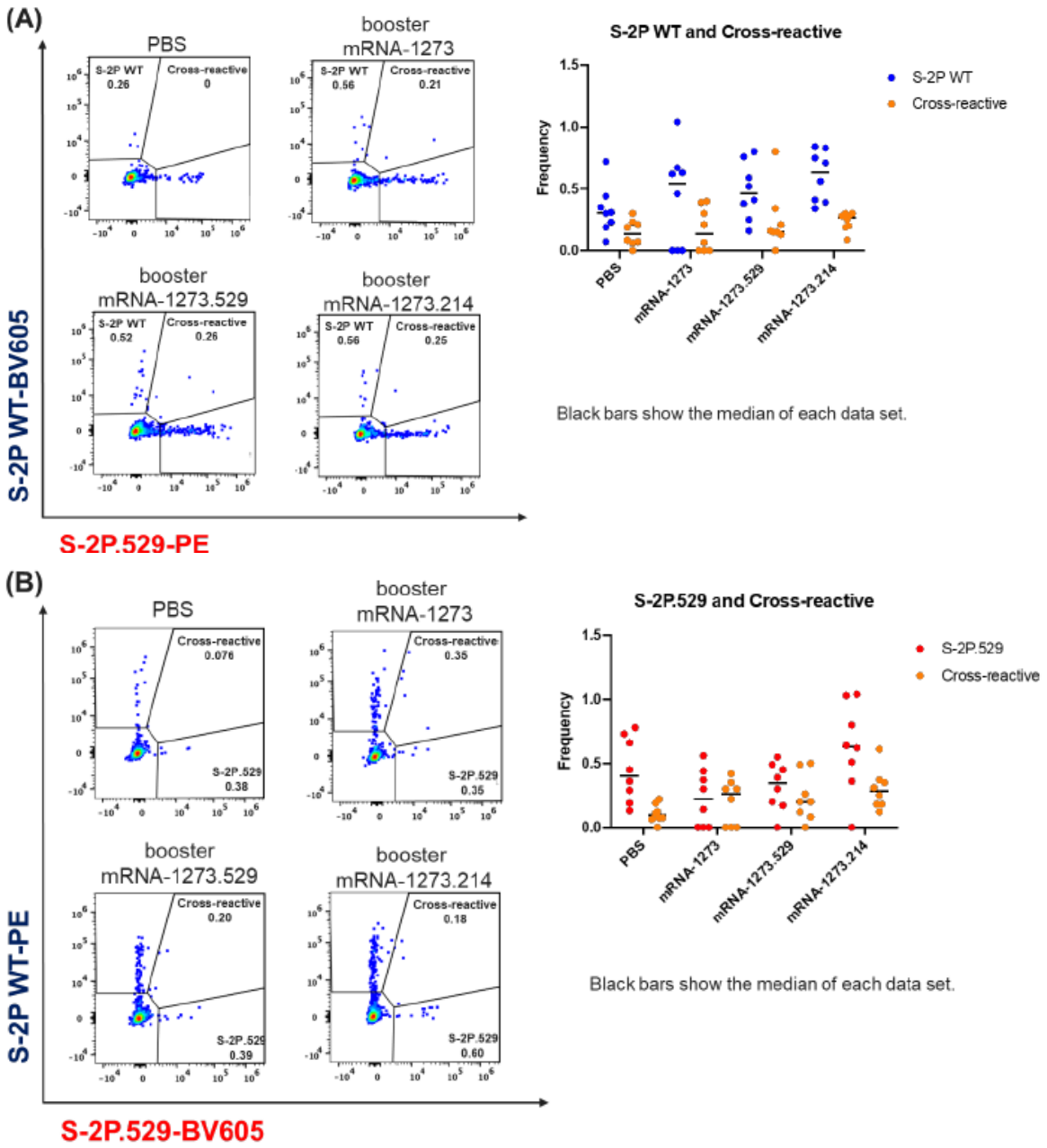
Abbreviations: mRNA = messenger RNA; PBS = phosphate-buffered saline; PE = phycoerythrin; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; S-2P.529 = Omicron-specific S-2P; WT = wild type.

Omicron-matched S-2P.529-specific B cell frequencies were detected in iliac lymph nodes of mice boosted with mRNA-1273.529 or mRNA-1273.214 (3.04% and 4.48%, respectively) but were not observed in mice boosted with mRNA-1273 (0.052%). It has to be noted, that the detected S-2P.529-specific B-cell frequencies were significantly lower than the Wuhan-specific B-cell frequencies in mRNA-1273.529 and

mRNA-1273.214 boosted mice. Furthermore, high frequencies of cross-reactive B cells were observed in all 3 booster groups. Especially, mRNA-1273.214 boosted mice showed significant high cross-reactive B-cells (mRNA-1273: 3.18%, mRNA-1273.529: 4.61%, mRNA-1273.214: 7.79%).

Figure 5: Frequencies of S-2P and S-P.529 and Cross-Reactive (WT+.529+) B Cells in Spleen in Mice 2 Weeks After Boost (Dose 3) With mRNA-1273 or BA.1-Matched mRNA Vaccines

Figure 5: Frequencies of S-2P and S-2P.529 and Cross-Reactive (WT+ .529+) B Cells in Spleen in Mice 2 Weeks After Boost (Dose 3) With mRNA-1273 or BA.1-Matched mRNA Vaccines



Abbreviations: mRNA = messenger RNA; PBS = phosphate-buffered saline; PE = phycoerythrin; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; S-2P.529 = Omicron-specific S-2P; WT = wild type.

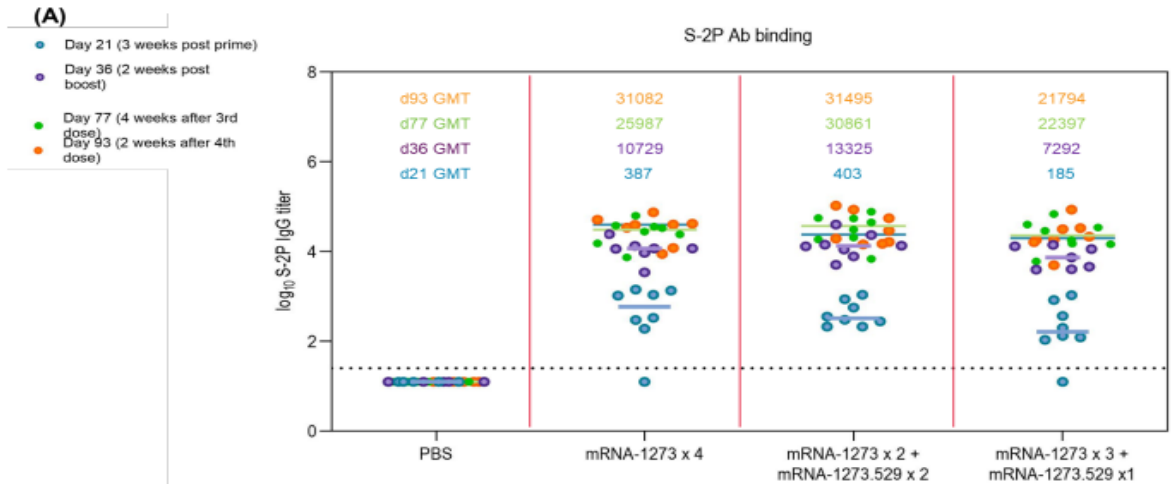
Spleens were processed by individual mouse and isolated cells were probed with recombinant Wuhan S-2P and Omicron S-2P.529 proteins. The frequencies of antigen-reactive B cells are expressed as a percentage of all IgD- and IgM-class-switched B cells.

In the spleen, minimal Wuhan and Omicron-matched S2P antigen-specific B cell responses were observed across all groups (<1%). In addition, cross-reactive B-cells frequencies were also very low across all groups (<0.5%). These results indicate that antigen-reactive B cells were not in the systemic circulation. The MAH explains that this result is likely due to the low dose of mRNA vaccine.

Results of the four dose regime:

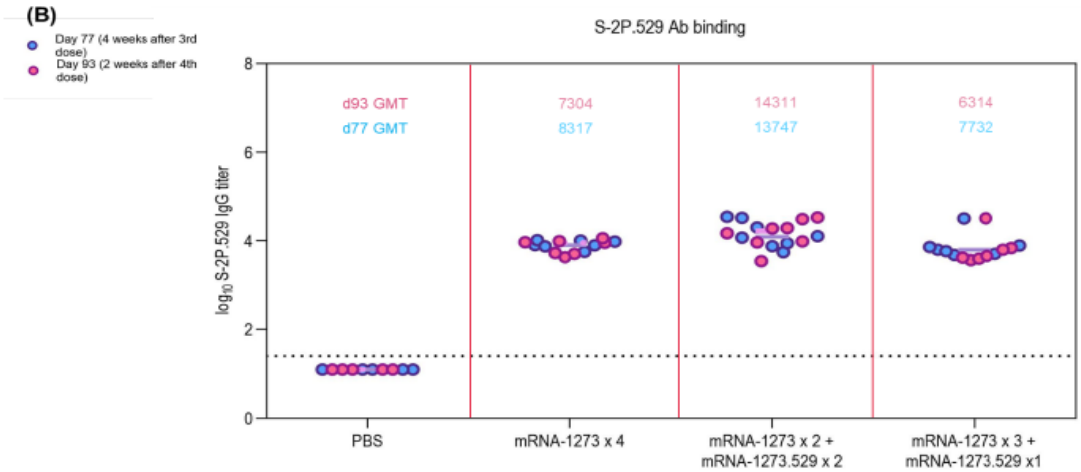
Figure 6: Binding Antibody Responses in BALB/c Mice After Dose 4

Figure 6: Binding Antibody Responses in BALB/c Mice After Dose 4



High IgG binding antibody titres against Wuhan S-2P and Omicron S-2P.529 proteins were detected after a 2-dose primary series with mRNA-1273 and 1x and 2x boosting with mRNA-1273 or mRNA-1273.529 compared to PBS control.

4 weeks after the first booster (Day 77), mRNA-1273 or mRNA-1273.529 induced an increased Wuhan S2P-specific IgG antibody response compared to GMTs after the 2nd vaccine dose (Day 36). Overall, the GMTs of all vaccine groups were similar. Furthermore, a notable increase in Wuhan S-2P IgG GMTs were not noted after the 4th dose (Day 93) across all booster groups, which might be due to the high levels of pre-existing immunity in these mice at this time point according to the MAH.



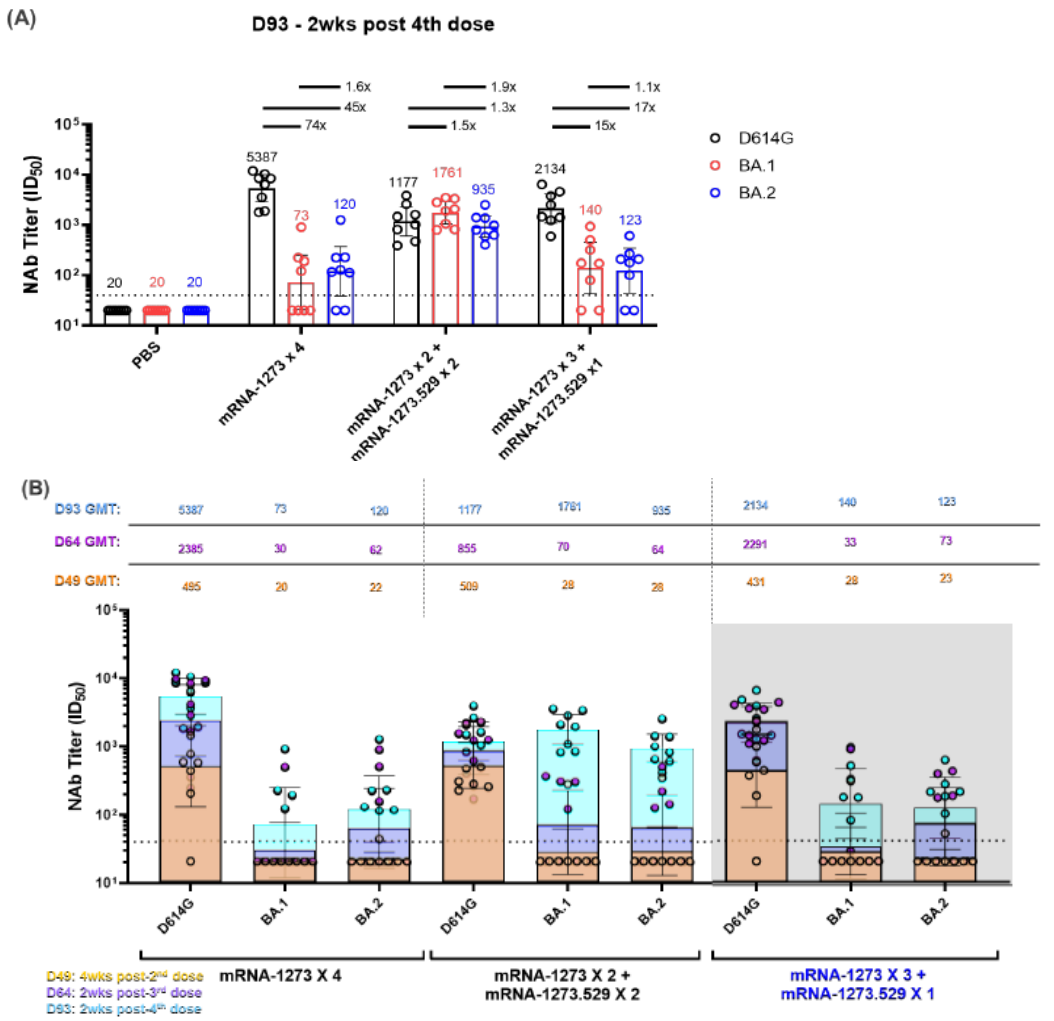
Abbreviations: Ab = antibody; GMT = geometric mean titer; IgG = immunoglobulin G; PBS = phosphate-buffered saline; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; S-2P.529 = Omicron-specific S-2P.

Similar results were observed for Omicron S2P.529-specific IgG binding antibody titre. The GMT for Omicron S2P.529-specific IgG binding antibody were slightly lower than the GMT for Wuhan S2P-specific IgG binding antibody across all groups. In general, a 4th vaccine dose did not increase the GMT compared to a 3rd vaccine dose. However, it has to be noted that the Omicron S2P-specific GMT was higher in mice boosted with 2x mRNA-1273.529 (14311 GMT) than in mice boosted with 2x mRNA-1273 (7304 GMT) and 1x mRNA-1273 + 1x mRNA-1273.529 (6314 GMT). Nevertheless, it should be also considered that the GMT after the primary vaccine series was higher in group 6 animals (2x mRNA-1273.529) than in group 7 (2x mRNA-1273) and 8 animals (1x mRNA-1273 + 1x mRNA-1273).

mRNA-1273.214 as a booster was not analysed in this 4-dose vaccine regime study.

Figure 7: Neutralizing Antibody Responses in BALB/c Mice After Dose 4

Figure 7: Neutralizing Antibody Responses in BALB/c Mice After Dose 4



Abbreviations: D = day; GMT = geometric mean titer; ID_{50} = infectious dose 50; NAb = neutralizing antibody.
Notes: The dotted line represents the lower limit of quantification.
(A) Neutralizing antibody titers on Day 93 (2 weeks post Dose 4)
(B) Neutralizing antibody titers on Days 49 (2 weeks post Dose 2), Day 64 (2 weeks post Dose 3) and Day 93 (2 weeks post Dose 4) overlayed.

2 weeks after the 4th dose, the D614G neutralising antibody response was increased in 4x mRNA-1273 (5387 GMT) vaccinated mice compared to 2x or 3x mRNA-1273 vaccinated mice. In addition, the BA.1 and BA.2 neutralising antibody responses were also slightly increased (73 and 120 GMT) compared to the

2x and 3x dose neutralising antibody titres. Nevertheless, the BA.1 and BA.2 neutralising antibody titres in 4x mRNA-1273 vaccinated mice were still very low.

In mice boosted 2x with mRNA-1273.529, the D614G neutralising antibody response (1177 GMT) was only minimal increased compared to the neutralising titres post-2nd and post-3rd dose, and was slightly lower than for 4x mRNA-1273 vaccinated mice. In contrast, BA.1 and BA.2 neutralising antibody titres were significantly increased after the 2nd mRNA-1273.529 booster dose (1761 GMT and 935 GMT) and reached comparable high titres than for D614G in this group. The MAH suggested that the Omicron-specific memory B cells measured after the first booster dose of mRNA-1273.529 responded to the second booster dose of mRNA-1273.529, resulting in a significant increase in neutralising antibodies against BA.1 and BA.2.

In mice boosted 1x with mRNA-1273.529, the D614G neutralising antibody response (2134 GMT) was similar high than post-3x mRNA-1273. BA.1 and BA.2 neutralising antibody titres were increased after the 4th dose with mRNA-1273.529 boost (140 GMT and 123 GMT) and showed similar high levels. Nevertheless, the Omicron-specific neutralising antibody titres in this group were still low and significantly smaller than in the mice boosted 2x with mRNA-1273.529.

In the study report, the MAH mentioned that the antigen-reactive B cell responses on Day 93 (after 4 doses) were similar to those on Day 64 (after 3 doses). Thus, Day 93 results were not submitted with this study data.

The MAH concluded that a single Omicron-matched booster (Dose 3) might be not enough to increase significantly Omicron-specific neutralisation antibody titres. However, it is driving the production of Omicron antigen-reactive B cells, which would be available to respond rapidly to subsequent vaccination with mRNA-1273.529. This was observed by the significantly increase in BA.1 and BA.2 neutralisation antibody titres after a second booster dose with mRNA-1273.529.

CHMP comment

3-dose regime:

One booster of Omicron-specific SARS-CoV-2 vaccines, monovalent mRNA-1273.529 and bivalent mRNA-1273.214, induced similar high Wuhan 1 and BA.1 SP-specific IgG antibody titres. Mice vaccinated with 2x mRNA-1273 and boosted 1x with mRNA-1273.214 showed the highest neutralising antibody titres against Wuhan-1, BA.1 and BA.2 SARS-CoV-2 variants compared to booster with mRNA-1273.529 or mRNA-1273. However, the neutralising antibody titres against BA.1 and BA.2 were relative low after 1 booster dose, even for Omicron specific vaccines. mRNA-1273.529 and mRNA-1273.214 induced antigen-reactive B cells to S-2P.529 in the draining lymph nodes, while boosting with mRNA-1273 did not. All 3 vaccines show antigen-reactive B-cells to Wuhan S-2P and cross-reactive B-cells in lymph nodes. However, minimal antigen-specific B cell responses (< 1%) were observed across all groups in spleen.

4-dose regime:

The Wuhan-1 and BA.1 SP-specific IgG antibody titre showed similar levels after the 4th vaccine dose than after the 3rd dose in all vaccine groups. Neutralising antibody titres against BA.1 and BA.2 were significantly increased after 2x booster doses of mRNA-1273.529. 1 booster dose of mRNA-1273.529 showed only little increase of neutralising antibody titre compared to 4x mRNA-1273. In addition, neutralising antibody titre against Wuhan-1 SP did not remarkably changed after 4th dose and was similar than after 3rd dose. The B-cell response after 4th dose data were not shown. The MAH mentioned that data are similar than after 3rd dose

In general, the Omicron-specific IgG binding antibody titre was slightly lower than the Wuhan-specific titre in all vaccine groups. Furthermore, one booster dose with a Omicron-matched vaccine (mRNA-1273.529 or mRNA-1273.214) induce high IgG titres against the Wuhan and Omicron spike protein, but they were not significant higher compared to the parent vaccine mRNA-1273. Furthermore, the Omicron-specific neutralising titres were relative low in the Omicron-matched vaccine boostered mice. However, a 2nd booster dose with mRNA-1273.529 induced high neutralising antibodies against BA.1 and BA.2. These data suggest, that similar results are expected for a 2nd booster dose of mRNA-1273.214. However, this vaccine variant was not tested for a 4-dose regime. In addition, one booster with an Omicron-matched vaccine induced memory B-cells in lymph nodes against Wuhan and Omicron, as well as cross-reactive B-cells.

Study MOD-5156: Evaluation of Immunogenicity of Omicron-Matched mRNA Vaccines as Primary Series in Mice

Study design

The immunogenicity of mRNA-1273 and the Omicron-matched monovalent mRNA-1273.529 and bivalent mRNA-1273.214 vaccines was analysed after 2-dose primary dose regimen. 6- to 8-week-old female BALB/c mice, 8 animals per group, received two intramuscular injections of 1 µg mRNA vaccines as a primary series approximately 3 weeks apart. PBS was used as a control.

Table 2: Study Design for Study MOD-5156

Table 2: Study Design for Study MOD-5156

Study Report Group (n = 8/group)	Primary Series (Dose 1 and 2)			Readouts
	Treatment (IM)	Dose Level (µg)	Dose Schedule	
1	PBS Control	0	Day 1, 22	**Serum (Day 21)** Antibody responses (ELISA) **Serum (Day 36):** Antibody responses (ELISA, PSVN assay)
2	mRNA-1273	1		
3	mRNA-1273.529	1		
6	mRNA-1273.214	1		

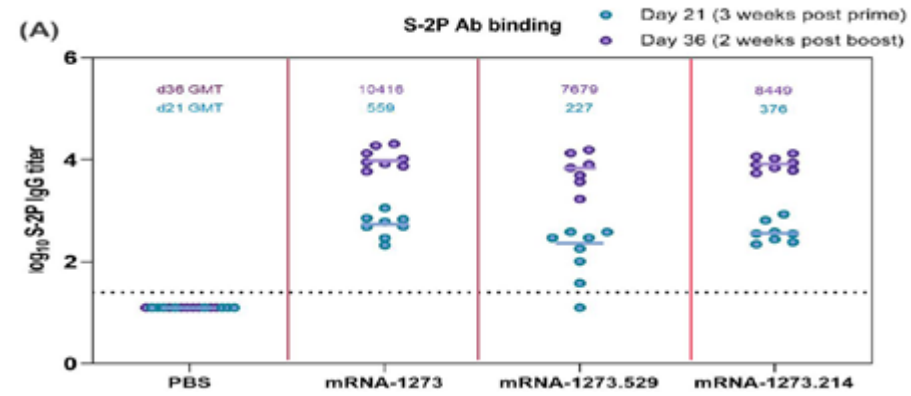
Abbreviations: ELISA = enzyme-linked immunosorbent assay; IM = intramuscular; mRNA = messenger RNA; NA = not applicable; PBS = phosphate-buffered saline; PSVN = pseudovirus neutralization.

[a] mRNA-1273.214 was a 1:1 bench side mix of separately formulated mRNA-1273 and mRNA-1273.529.

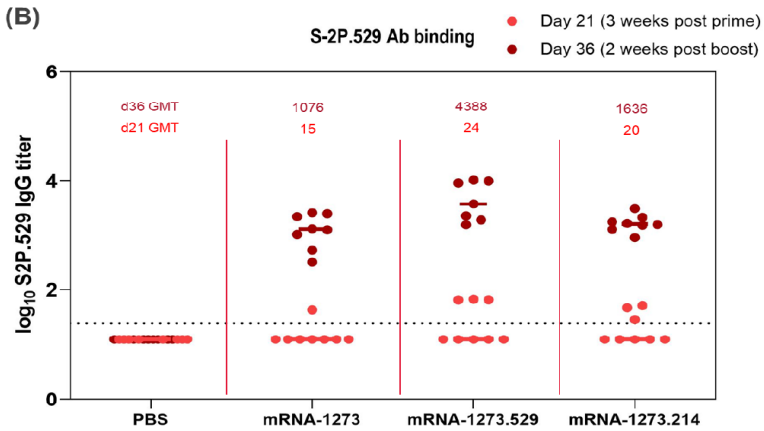
Serum samples were collected from all animals before the 2nd Dose (Day 21) and 2 weeks after the 2nd Dose (Day 36). Spike protein-specific IgG antibody titres were analysed by ELISA and neutralisation antibody responses were studied by VSV-based PSVN assay.

Results

Figure 8: Binding Antibody Responses in BALB/c Mice After Primary Series



Similar mean Wuhan-1 S2P-specific IgG antibody titres were measured for all three mRNA vaccines, 3 weeks after the 1st vaccine dose. However, two animals of the mRNA-1277.529 group showed only low S2P-specific IgG titre at the quantification limit. A 2nd dose of one of these three vaccines enhanced the IgG antibody response and all three vaccines reached similar high titres (7679 – 10416 GMT).

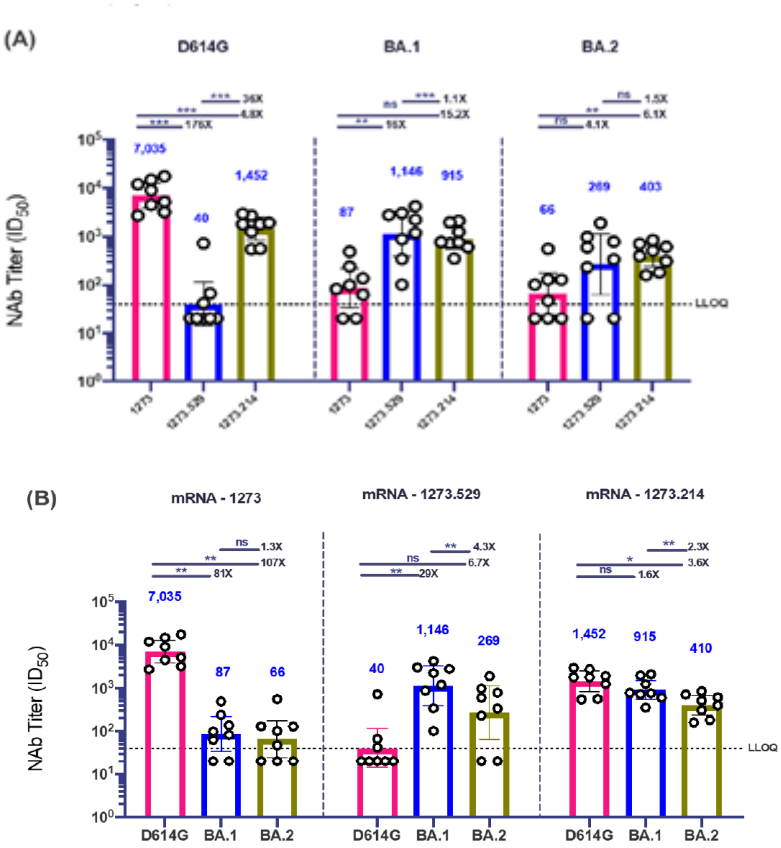


Abbreviations: Ab = antibody; GMT = geometric mean titer; IgG = immunoglobulin G; mRNA = messenger RNA; PBS = phosphate-buffered saline; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; S-2P.529 = Omicron-specific S-2P.

After the 1st vaccine dose, Omicron-matched S2P.529-specific IgG antibody titre was under the quantification level in almost all animals in all groups. A 2nd vaccine dose increased remarkably the S2P.529-specific IgG antibody responses in all vaccinated animals (1076 – 4388 GMT). The mRNA-1273.529 vaccinated mice showed a slightly higher titre (4388 GMT) than the mice of the other two groups.

Overall, the S2P.529-specific IgG antibody titres were slightly lower than S2P-specific IgG antibody titres after the 2nd vaccine dose in all groups.

Figure 9: Neutralizing Antibody Responses in BALB/c Mice After Primary Series (Day 36)



Abbreviations: ID_{50} = infectious dose 50; LLOQ = lower limit of quantification; mRNA = messenger RNA; NAb = neutralizing antibody; ns = not significant.

Note: Statistical analysis performed was nonparametric t-test; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.

2 weeks after the second dose, the GMT of serum neutralising antibody responses against Wuhan D614G were significantly higher in mRNA-1273 vaccinated mice (7035 GMT) compared with mRNA-1273.529 (40 GMT) or mRNA-1273.214 (1452) vaccinated mice. Nevertheless, mRNA-1273.214 showed robust neutralisation antibody response against Wuhan D614G.

As expected, serum neutralising antibody responses against BA.1 or BA.2 were higher in mice vaccinated mRNA-1273.529 (1146 and 269 GMT) or mRNA-1273.214 (915 and 410 GMT) compared with mRNA-1273 (87 and 66 GMT). Overall, mRNA-1273.214 provided the widest neutralisation coverage across the evaluated SARS-CoV-2 variants.

CHMP comment

Two primary doses of mRNA-1273, mRNA-1273.529 or mRNA-1273.214 induce robust Wuhan-1 and Omicron SP-specific IgG antibody titres in mice. However, the IgG antibody titres against Omicron SP were lower than Wuhan-1 SP-specific IgG titre for all three vaccines.

As expected, two doses of mRNA-1273 vaccine induced high neutralising antibody titre against Wuhan-1 spike protein, but only very low BA.1- and BA.2-specific titres. mRNA-1273.529 vaccine showed robust titre for BA.1 and BA.2 but only low for Wuhan. mRNA-1273.214 vaccine showed high titre for Wuhan, BA.1 and BA.2.

N-terminal domain- and RBD-specific antibody titres were not analysed in this study.

Together with the data of study MOD-5019 (Omicron-matched booster), these data show that one dose of mRNA-1273.529 or mRNA-1273.214 might be not sufficient to induce robust neutralising antibody

responses against Omicron variants BA.1 and BA.2. Nevertheless, immunogenicity data obtained from mice cannot be translated directly to human.

Study WASHU-01-MOD-5020:

The immunogenicity and protection against viral challenge was evaluated for different dose concentrations of mRNA-1273 and BA.1-matched booster vaccines.

The test articles in this study were 0.1 µg, 0.25 µg, 1 µg, or 5 µg of mRNA-1273, and 0.1 µg or 1 µg mRNA-1273.529 administered as a 2-dose primary series regimen (with or without a boost of mRNA-1273, mRNA-1273.529, control mRNA, or PBS). A non-translating mRNA (0.1 µg, 0.25 µg, or 5 µg control mRNA [UNFIX-01]) or PBS was used as a negative control.

WASHU-K18MOD7AB (cohort 1) and WASHU-K18MOD3/4 (cohort 2) studies

Study design

In cohort 1, seven weeks old K18-hACE2 female mice were injected with 2 intramuscular doses of 0.1 µg or 5 µg mRNA-1273 or non-translating mRNA-control 3 weeks apart. Blood was collected on Day 42 to measure the IgG antibody responses against Wuhan-1 and BA.1-speific Spike and RBD by ELISA and neutralising antibodies were measured by FRNT. Five weeks after the second dose, mice were challenged intranasally with 10^4 FFU of Wuhan-1 D614G or BA.1 SARS-CoV-2 variants. 6 days post-infection, mice were sacrificed and nasal wash, nasal turbinates and lung samples were harvested for virological, immunological, and pathological analysis.

In cohort 2, the effects of an mRNA-1273 booster dose on antibody responses and protection against BA.1 was evaluated in 7-week old K18-hACE2 female mice. The mice received 2 IM injections of 0.25 µg or 5 µg mRNA-1273 or mRNA-control 3 weeks apart (primary series). 17 to 19 weeks after the second dose, mice were boosted with 1 µg mRNA-1273 or mRNA-control. Blood samples were collected before the boost dose and at 4 weeks post-boost dose (before challenge), and samples were analysed for serum neutralising antibodies by FRNT. Four weeks after the boost dose, mice were challenged intranasally with 10^4 FFU of BA.1. 6 days post-infection, mice were euthanised and tissues (nasal wash, nasal turbinates, and lung) were harvested for virological analysis.

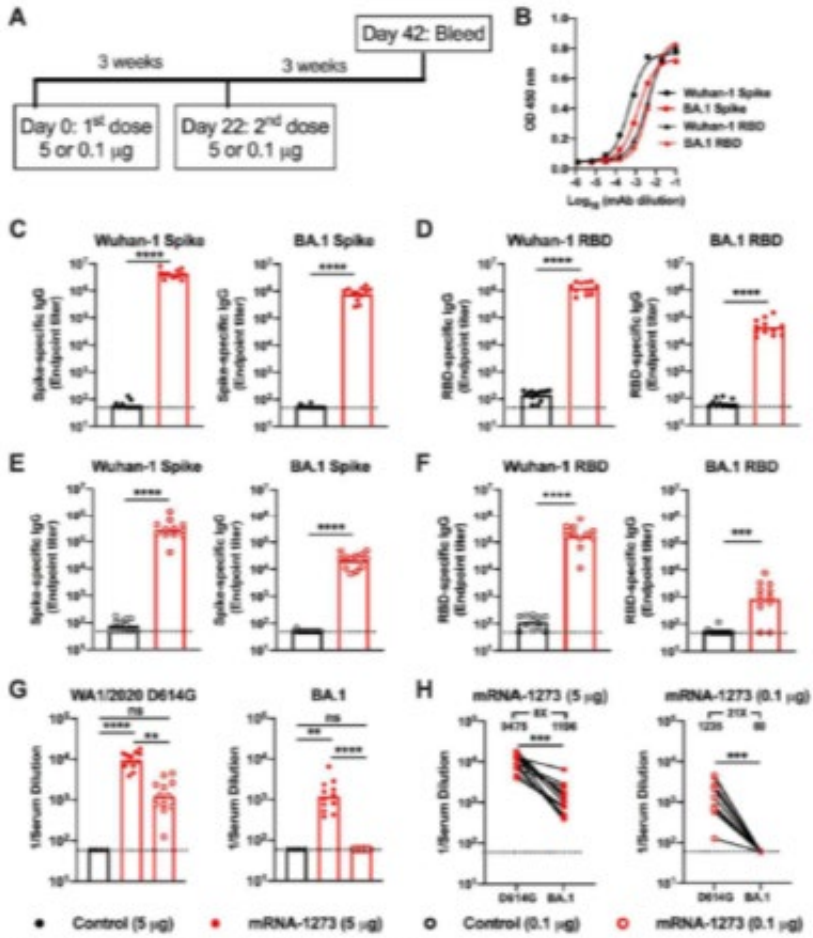
Table 3: Treatment Regimen for Study WASHU-K18MOD7AB and WASHU-K18MOD3/4: K18-hACEe2 Mice

		Primary Series			Boost			Challenge		
Cohort	Mouse Strain (Female)	Treatment	Dose Level (µg)	Dose Schedule	Treatment	Dose Level (µg)	Dose Schedule	Variant (10^4 FFU)	Time Point	Collection Time Points
1	K18-hACE2	mRNA control	0.1	Day 0, Day 21	N/A			WA1/2020 D614G or BA.1	Day 56/57	**Serum (Day 42):** Antibody responses (ELISA, FRNT) **Sacrifice (Day 62/63)** Body weight Viral burden (qRT-PCR) Cytokine/chemokine analysis Histopathology
			5							
		mRNA-1273	0.1							
			5							
2	K18-hACE2	mRNA control	0.25	Day 0, Day 21	mRNA control	1	Day 134/156	BA.1	Day 162/184	**Serum (Day 133/155 pre-boost; Day 160/182 post-boost):** Antibody response (FRNT) **Sacrifice (Day 168/190):** Viral burden (qRT-PCR)
			5			1				
		mRNA-1273	0.25		mRNA-1273	1				
			5			1				

Abbreviations: ELISA = enzyme-linked immunosorbent assay; FFU = focus-forming units; FRNT = focus reduction neutralization; mRNA = messenger RNA; N/A = not applicable; qRT-PCR = quantitative reverse transcription polymerase chain reaction.

Results

Figure 10: Antibody Responses of mRNA Vaccines in K18-hACE2 Mice



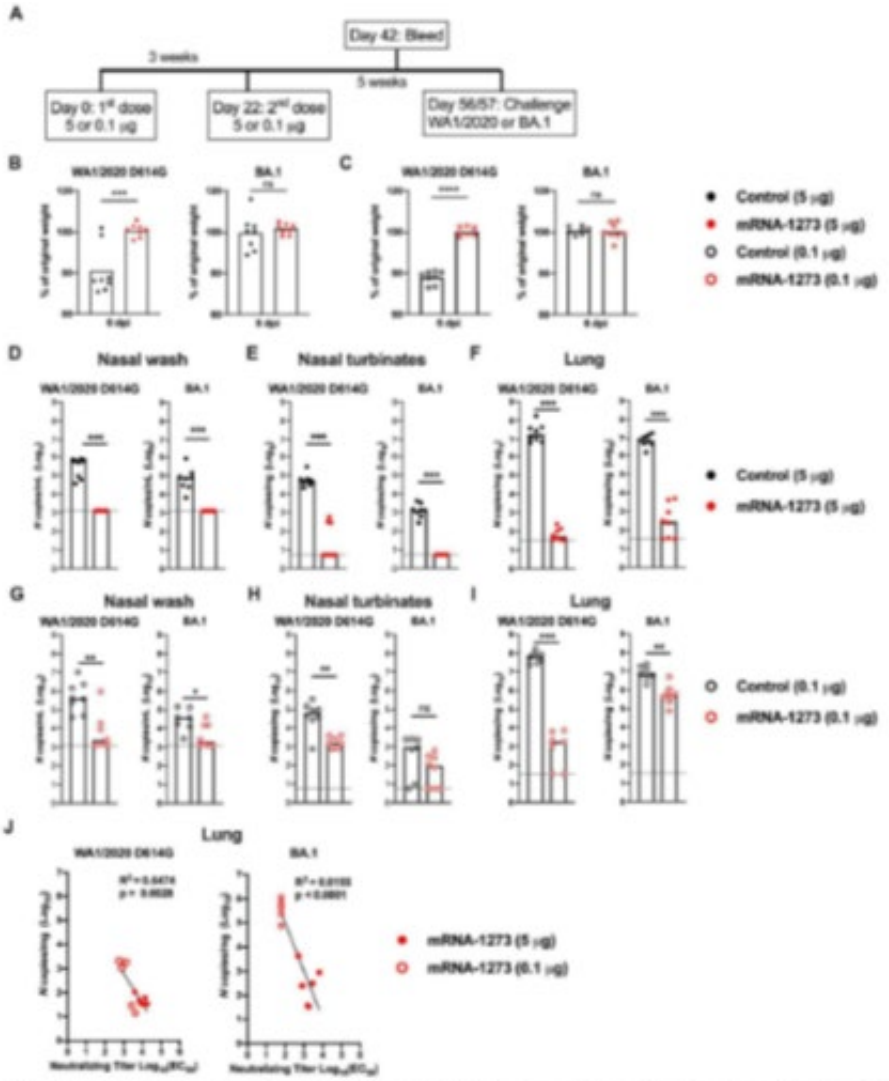
Abbreviations: ANOVA = analysis of variance; IgG = immunoglobulin G; mAb = monoclonal antibody; mRNA = messenger RNA; ns = not significant; OD = optical density; RBD = receptor-binding domain.

Notes: Statistical analyses performed were as follows: Mann-Whitney test (**C-F**); 1-way ANOVA with Dunn's post-test (**G**); and Wilcoxon signed-rank test (**H**); ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Source: Ying et al 2022b, Figure 1.

In cohort 1 K18-hACE2 mice, two doses of mRNA-1273 vaccine induced high Wuhan-1 and BA.1 Spike- and RBD-specific binding IgG antibody responses. mRNA-1273 showed dose-dependent increase of serum IgG antibody titres. In the 0.1 µg mRNA-1273 group, the IgG titres were about 10-fold lower than those in the 5 µg mRNA-1273 group. In both mRNA-1273 dose groups, the Wuhan-1 S- and RBD-specific IgG titres were higher than those observed against BA.1. In addition, serum neutralising antibody responses against both Wuhan-1 D614G and BA.1 could be detected after two doses of 5 µg mRNA-1273. For mice that received 0.1 µg of mRNA-1273, only moderate high neutralising antibody titres against Wuhan-1 D614G were observed. Neutralising antibody titre against BA.1 were very low and similar to control mRNA mice. Overall, the serum neutralising activity against BA.1 was lower in both mRNA-1273 dose groups when compared with Wuhan-1 D614G-specific neutralising antibody titres.

Figure 11: Protection Against SARS-CoV-2 Infection After mRNA Vaccination in K18-hACE2 Mice



Abbreviations: dpi = days post-infection; EC_{50} = half maximal effective concentration; mRNA = messenger RNA; ns = not significant; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Notes: Statistical analyses were performed as follows: unpaired t test (**B, C**); Mann-Whitney test (**D-I**); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

Source: Yinu et al 2022b, Figure 2.

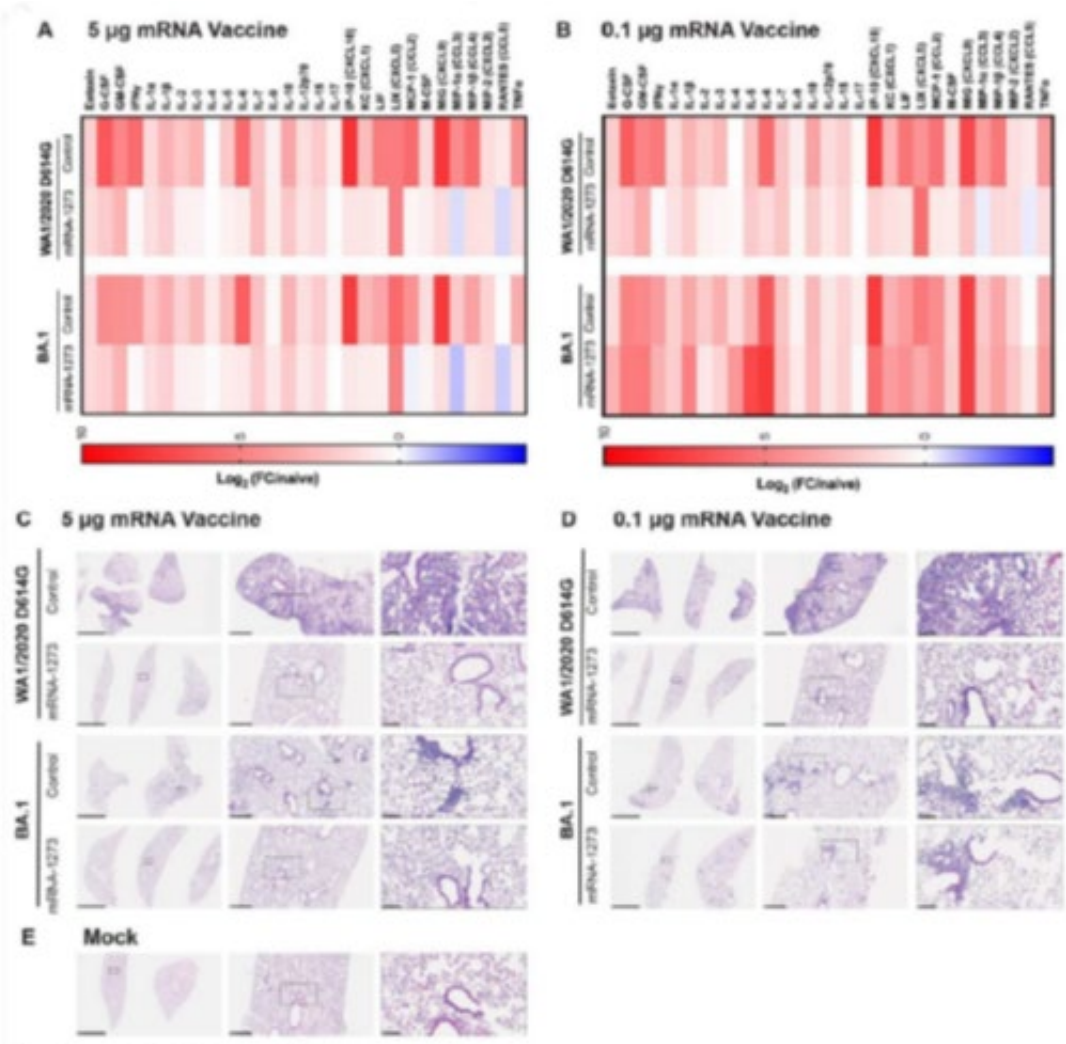
Five weeks after the completion of the primary vaccination series with 0.1 µg or 5 µg mRNA-1273, mice were challenged with 10^4 FFU Wuhan-1 D614G or BA.1. 6 days post infection, no weight loss was observed in 0.1 µg or 5 µg mRNA-1273 group mice. However, also the BA.1 challenged control mice did not reduced their body weight. Overall, 6 days post-infection, the control mice showed also lower viral load after BA.1 challenge than after Wuhan-1 D614G challenge, indicating a lower infection character of BA.1 in mice. The nasal washes, nasal turbinates and lung of 5 µg mRNA-1273-vaccinated mice showed significantly reduced viral load of Wuhan-1 D614G and BA.1. The 0.1 µg mRNA-1273 group showed also reduced viral load, but the viral load reduction compared to the negative control was less.

After the Wuhan-1 D614G- or BA.1-challenge, the viral load in the nasal wash was about 10^3 copies/mL in both mRNA-1273 dose groups. The viral load in the nasal turbinates was even lower in the 5 µg mRNA-1273 group at about 10^1 copies/mL after Wuhan-1 D614G- and BA.1-challenge. In the 0.1 µg mRNA-1273 vaccinated mice, the Wuhan-1 D614G- and BA.1 viral load in nasal turbinates was at 10^3 and 10^2 copies/mL, respectively. The viral load in lung of 5 µg mRNA-1273 vaccinated mice was at $10^{1.5}$

copies/mL after Wuhan-1 D614G challenge and at 10^2 copies/mL after BA.1 challenge. In 0.1 µg group mice, the viral load reduction in lung was only slightly lower than in the control mice and showed viral loads at 10^3 copies/mL after Wuhan-1 D614G challenge and 10^6 copies/mL after BA.1 challenge.

Serum neutralising antibody titres were inversely correlated with the viral load in the lung for BA.1, where the burden of infection generally decreased as the neutralising antibody titres increased. Thus, the highest levels of infection were observed in the BA.1-challenged mice with low neutralising antibody titres after vaccination with 0.1 µg mRNA-1273, and the lowest levels of infection were observed in the BA.1-challenged mice with high neutralising antibody titres after vaccination with 5 µg mRNA-1273. For Wuhan-1 D614G, similar results were shown, but without a clear correlation between vaccine dose, neutralisation antibody and level of infection.

Figure 12: mRNA Vaccine Protection Against Disease in K18-hACE2 Transgenic Mice



Abbreviations: FC = fold-change; mRNA = messenger RNA.
Source: Ying et al 2022b, Figure 3.

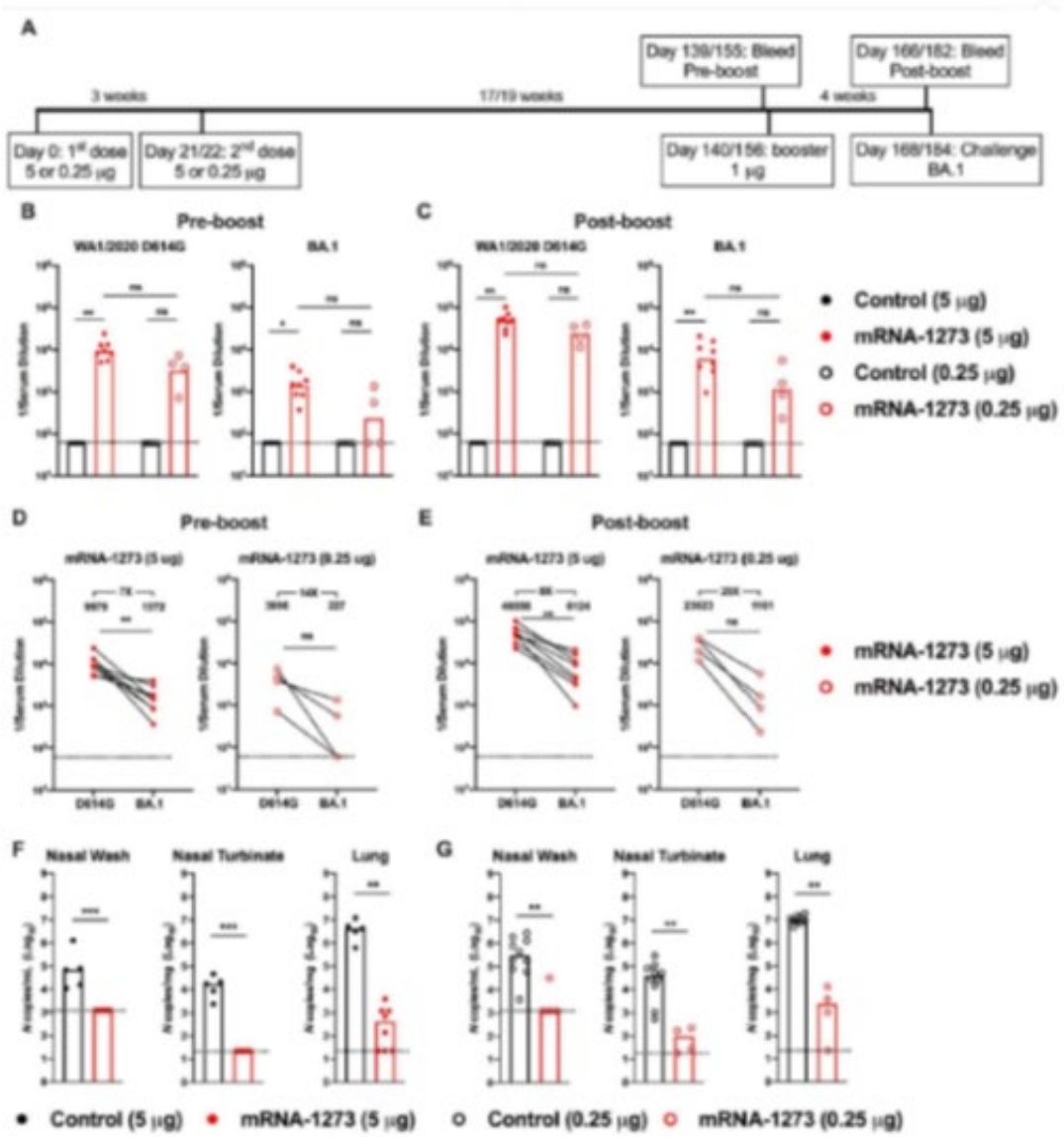
6 days after challenge with 10^4 FFU Wuhan-1 D614G or BA.1, cytokine and chemokine responses in lung homogenates were evaluated in mice, which received a 2-dose primary series of mRNA-1273 or mRNA negative control. In the control group, Wuhan-1 D614G or BA.1 infection resulted in an increased inflammatory response, as indicated by increased expression of several pro-inflammatory cytokines and chemokines in lung, including G-CSF, GM-CSF, IFN-γ, IL-1β, IL-6, CXCL1, CXCL5, CXCL9, CXCL10, CCL2, CCL4 and TNF-α.

In the 5 µg mRNA-1273 group, the inflammatory response was reduced compared to controls for both Wuhan-1 D614G or BA.1 infection. In addition, mice that received 0.1 µg mRNA-1273 showed low levels of cytokines and chemokines after Wuhan-1 D614G challenge. However, no protection was observed in these mice challenged with BA.1, where levels of pro-inflammatory cytokines and chemokines in the lung were similar to those observed in the mRNA control group.

In mice immunised with mRNA negative control and challenged with Wuhan-1 D614G, histological analysis of lung sections showed immune cell infiltration, alveolar space consolidation, vascular congestion and interstitial oedema, indicating severe pneumonia. However, mRNA negative control mice showed less focal airspace consolidation and immune cell infiltration in lung after BA.1 challenge.

Mice immunised with 0.1 µg or 5 µg mRNA-1273 did not develop lung pathology after challenge with Wuhan-1 D614G and findings were consistent with SARS-CoV-2 uninfected mock animals. After challenge with BA.1, 5 µg mRNA-1273 protected against mild pathological changes associated with infection. Protection against BA.1 was not observed in mice that received 0.1 µg mRNA-1273, where lung pathology findings were similar to those observed in the mRNA control group, with patchy immune cell infiltration, airway space thickening, and mild alveolar congestion.

Figure 13: A Booster Dose of mRNA-1273 Enhances Neutralizing Antibody Responses and Confers Protection in K18-hACE2 Mice



Abbreviations: ANOVA = analysis of variance; mRNA = messenger RNA; N = nucleocapsid; ns = not significant.

Notes: Statistical analyses were performed as follows: 1-way ANOVA with Kruskal-Wallis post-test (**B, C**). Wilcoxon signed-rank test (**D, E**); Mann-Whitney test (**F,G**); ** $p < 0.01$; *** $p < 0.001$.

Source: Ying et al 2022b, Figure 4.

17 to 19 weeks after a 2-dose primary series of 0.25 µg or 5 µg mRNA vaccines immunisation in cohort 2 mice, blood was collected before animals were boosted with 1 µg mRNA-1273 or a control.

Consistent with the results from cohort 1, pre-boost neutralising antibody titres against Wuhan-1 D614G and BA.1 were significant lower in mice immunised with 0.25 µg mRNA-1273 when compared with mice immunised with 5 µg mRNA-1273. In addition, the serum neutralising antibody activity was lower against BA.1 compared to Wuhan-1 D614G SARS-CoV-2 variant in both mRNA-1273 dose groups. This difference was even statistically significant in the 5 µg mRNA-1273 dose group. However, 2 of 4 mice vaccinated with 0.25 µg mRNA-1273 showed very low neutralising activity against BA.1, at the lower detection limit.

One month after boosting with 1 µg mRNA-1273, serum neutralising titres increased against both Wuhan-1 D614G and BA.1. However, neutralising antibody titres against BA.1 were lower compared with those

observed against Wuhan-1 D614G. Overall, the neutralising antibody titre increased dose-dependent and were higher in the 5 µg dose group compared to the 0.25 µg dose group.

Four weeks after boosting with 1 µg mRNA-1273 or control, mice were challenged with BA.1 and viral load was measured in the upper and lower respiratory tract. In mice vaccinated with 0.25 µg or 5 µg mRNA-1273 in primary series, and boosted with mRNA-1273, viral load was significantly lower than in the mRNA control groups. In the 5 µg mRNA-1273 group, very low BA.1 viral RNA was detected in the nasal wash (mean 10^3 copies/mL) or nasal turbinates (mean $10^{1.5}$ copies/mL, 5 of 8 mice), and low levels of BA.1 were observed in the lung (mean $10^{2.5}$ copies/mL).

In the 0.25 µg mRNA-1273 group, low levels of BA.1 were observed in the nasal wash (1 of 4 mice), nasal turbinates (3 of 4 mice), and lung (4 of 4 mice). Overall, evaluation of viral load 6 days post-infection showed that boosting with 1 µg mRNA-1273 improves neutralising antibody response and reduces BA.1 viral load in the upper and lower respiratory tract.

CHMP comment

K18-hACE2 mice:

Cohort 1:

In line with previous data, 2 doses of mRNA-1273 induce dose-dependent high IgG antibody titres against Wuhan and BA.1 spike protein and RBD. In addition, only two high doses of mRNA-1273 induces high neutralising antibody response. As expected, the neutralising antibody titre against BA.1 was lower than against Wuhan-1 Spike protein.

Furthermore, 2 doses of mRNA-1273 reduce dose-dependently D614G and BA.1 infection in nasal wash, nasal turbinates and lung. In general, BA.1 challenge caused lower viral load than D614G infection in vaccinated and control mice. Thus, BA.1 is less infectious than Wuhan-1 SARS-CoV-2 in mice. For analysis of viral load in the different tissues, different limits of quantification were set. However, the analysis was performed by the same q-RT-PCR method for all tissue samples. The MAH this with the different amount of sample for each tissue. After Wuhan and BA.1 challenge, pro-inflammatory cytokines and chemokine levels were not increased after high dose mRNA-1273 vaccination compared to control. In addition, the low dose of mRNA-1273 shows similar low levels of proinflammatory markers after Wuhan infection, but high inflammatory markers after BA.1 infection. Moreover, histopathology of lung tissue from vaccinated mice showed reduced inflammation in both dose groups.

Cohort 2:

In mice pre-boost and post-boost with mRNA-1273, a dose-dependent increase of neutralising antibody titre for BA.1 and Wuhan-1 was observed. However, neutralising antibody titres were lower for BA.1 than for Wuhan-1. In general, the titres were higher after booster (3rd dose) compared to pre-boost levels. After challenge with BA.1 SARS-CoV-2, a dose-dependent reduction of viral load was observed in nasal wash, nasal turbinate and lung. However, the higher dose (5 µg mRNA-1273) protects fully in the upper respiratory tract and reduced significant the viral load in the lung. The lower vaccine dose showed only partially protection against BA.1 in the upper and lower respiratory tract.

Overall, three doses of 5 µg mRNA-1273 seem to induce a robust immunogenicity and protection against Wuhan-1 and BA.1 SARS-CoV-2 infection in young K18-hACE2 mice. However, this study has some limitations. The group sizes were very small, especially for cohort 2. Also, an Omicron-matched vaccine was not analysed.

MOD-5020 study (Cohort 3)

Study design

6- to 8-week old female BALB/c mice received 0.1 µg or 1 µg of mRNA-1273, mRNA-1273.529 or PBS approximately 3 weeks apart. Blood was collected on Day 21 (before 2nd dose) and at Day 36 (2 week after 2nd dose), and samples were collected to measure IgG antibody responses against Spike protein-specific for Wuhan-1 and BA.1 and RBD-specific for Wuhan-1, BA.1, B.1.351 and B.1.617.2 by ELISA and neutralising antibodies by VSV-based PSVN assay. For the neutralisation antibody assay, only the 1 µg dose groups were evaluated. To evaluate the response to T-cells to peptide pools, spleens were collected from 1 µg dose groups on Day 36. T-cell responses were measured in splenocytes, restimulated with two separate peptide pools that together encompass the peptide library of the Wuhan-1 S protein. Antigen-specific CD4+ T-cell cytokine responses (IFN-γ, IL-2, TNF-α, IL-4, IL-5, and IL-13) and CD8+ T-cell cytokine responses (CD107a, IFN-γ, IL-2, and TNF-α) were assessed using intracellular cytokine staining.

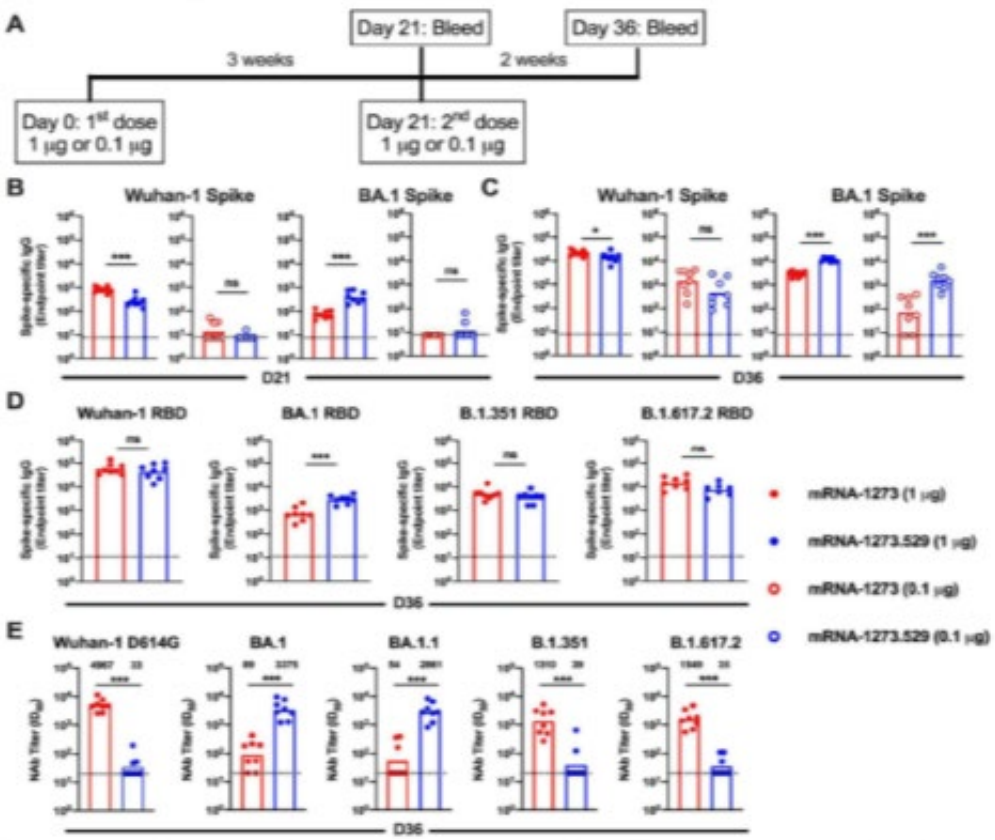
Table 4: Treatment Regimen for Study MOD-5020: BALB/c Mice

Cohort	Mouse Strain (Female)	Primary Series			Collection Time Points
		Treatment	Dose Level (µg)	Dose Schedule	
3	BALB/c	PBS Control	0.1	Day 1, Day 22	**Serum (Day 21, Day 36):** Antibody responses (ELISA, PSVN) **Spleen (Day 36):** T-cell response (ICS)
			1		
		mRNA-1273	0.1		
			1		
		mRNA-1273.529	0.1		
			1		

Abbreviations: ELISA = enzyme-linked immunosorbent assay; ICS = intracellular cytokine staining; mRNA = messenger RNA; PBS = phosphate-buffered saline; PSVN = pseudovirus neutralization.

Results

Figure 14: Antibody Responses in BALB/c Mice After Immunization with mRNA-1273 and mRNA-1273.529 Vaccines



Abbreviations: D = day; ID_{50} = 50% infectious dose; IgG = immunoglobulin G; mRNA = messenger RNA; NAb = neutralizing antibody; ns = not significant; RBD = receptor-binding domain.

Notes: Statistical analyses were performed as follows: Mann-Whitney test (**B-E**; ns; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

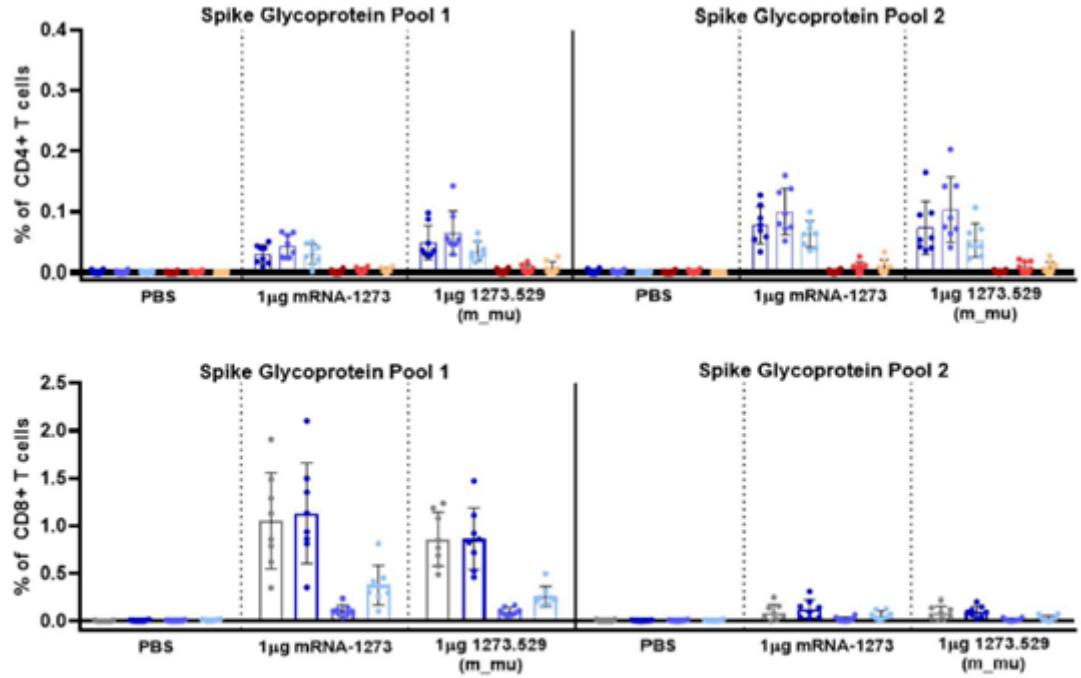
Source: Ying et al 2022b, Figure 5.

After the first dose (Day 21) in mice that received 1 µg of mRNA-1273, Wuhan-1 S-specific IgG antibody titres were higher than those against BA.1 spike protein. In contrast, Wuhan-1 and BA.1 Spike-specific IgG antibody titres were similar after vaccination with 1 µg of mRNA-1273.529. At 0.1 µg of either mRNA-1273 or mRNA-1273.529 vaccine, IgG antibody titres against both Wuhan-1 and BA.1 Spike protein were very low, near the LOD. 2 weeks after the second dose (Day 36), the IgG antibody titres increased against Wuhan-1 and BA.1 Spike protein in all groups. 1 µg mRNA-1273 produced higher IgG antibody titres against Wuhan-1 S compared to BA.1 S, and 1 µg mRNA-1273.529 produced similar high IgG antibody titres against Wuhan-1 and BA.1 S. It has to be noted, that the BA.1 S-specific IgG antibody titre was higher in mRNA-1273.529 vaccinated mice than in mRNA-1273 vaccinated mice. 2 doses of 0.1 µg mRNA-1273.529 resulted in higher IgG antibody titres against BA.1 Spike compared to Wuhan-1. In addition, 2 doses of 1 µg mRNA-1273 or mRNA-1273.529 produced similar IgG titres against Wuhan-1 RBD, B.1.351 RBD, and B.1.617.2 RBD. However, BA.1 RBD-specific titres were slightly higher in mice vaccinated with 1 µg mRNA-1273.529 compared to those vaccinated with 1 µg mRNA-1273.

Serum neutralising antibody responses were observed against Wuhan-1 D614G after a 2-dose primary series of 1 µg mRNA-1273, and slightly lower neutralising antibody titres observed against B.1.351 or B.1.617.2. In contrast, the serum neutralising activity was significantly reduced against the BA.1 and BA.1.1 compared with Wuhan-1 D614G in these animals. Mice immunised with 2 doses of 1 µg mRNA-1273.529 showed high neutralisation antibody titres against BA.1 and BA.1.1, but only low titres against

Wuhan-1 D614G, B.1.351, and B.1.617.2, with only few animals with detectable neutralising antibody titres. Overall, these data suggest that a primary series with mRNA-1273.529, a BA.1 matched vaccine, induces robust neutralising activity against BA.1 and BA.1.1, but not against other previous SARS-Co V-2 variants.

Figure 15: SARS-CoV-2 Spike Glycoprotein-specific T-cell Responses in BALB/c Mouse Splenocytes at Day 36



Abbreviations: CD = cluster of differentiation; mRNA = messenger RNA; m_mu = BA.1 Omicron spike; PBS = phosphate-buffered saline; S = spike; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.
Note: Splenocytes from mice dosed with a prime/boost regimen of 1 µg of mRNA-1273 or mRNA-1273.529 were stimulated with a peptide library spanning the entire sequence of the Wuhan-1 S glycoprotein at stimulation concentration of 1 µg/peptide/mL.

2 weeks after the second dose, CD4+ T-cell and CD8+ T-cell cytokine production to Wuhan-1 S glycoprotein were similar in mice dosed with 1 µg mRNA-1273 and mRNA-1273.529. In general, very low CD4+ T-cell levels were measured. Low levels of IFN-γ, IL-2 and TNF-α could be detected, but almost no IL-4, IL-5 and IL-13. Thus, these data indicate a slight shift to a Th1-skewed CD4+ T-cell response. In addition, moderate levels of CD107a and IFN-γ producing CD8+ T-cells were detected in pool 1 spike proteins from both vaccinated mice. In contrast, almost no CD8+ T-cell cytokines were measured.

CHMP comment

Dose-dependent increase of BA.1 and Wuhan-1 Spike-specific IgG antibody response after 1st and 2nd dose of mRNA-1273 and mRNA-1273.529. After the 2nd vaccine dose, the Spike-specific IgG antibody titres were even increased, similar to previous studies. After the 2nd dose, mRNA-1273.529 showed similar high Wuhan-1, but slightly higher BA.1 Spike-specific IgG titres compared to mRNA-1273. In addition, 2 doses of 1 µg mRNA-1273 or mRNA-1273.529 induce robust RBD-specific IgG responses against Wuhan-1, BA.1 (Omicron), B.1.351 (Beta) and B.1.617.2 (Delta). The antibody titres were similar high in both vaccine groups, except for Omicron where mRNA-1273.529 induced a slightly higher antibody response.

The neutralising antibody titres differed remarkably between mRNA-1273 and mRNA-1273.529 vaccinated mice. 2 doses of 1 µg mRNA-1273 induced robust neutralising antibody titres only against

Wuhan-1, Beta and Delta variants. In contrast, mRNA-1273.529 induces robust neutralising antibody titres only against the two Omicron variants BA.1 and BA.1.1.

In general, the Wuhan S-specific T-cell response was similar in mRNA-1273 and mRNA-1273.529 vaccinated mice. Low CD4+ T-cell response was observed in both groups. In addition, moderate CD107a and IFN-γ CD8+ T-cell responses were detected. However, the T-cell response against Omicron S-protein or another VoC was not analysed.

WASHU-129MOD5/6 study (cohort 4):

Study design

7-week old female 129S2 mice received 0.25 µg or 5 µg of noncoding mRNA (negative control) or mRNA-1273 on Day 0 and Day 21. 10 to 11 weeks after the second dose, mice were boosted homologous or heterologous with 1 µg of mRNA control, mRNA-1273, or mRNA-1273.529. Blood was collected before the booster dose and at 3 to 4 weeks post-boost dose to assess neutralising antibodies against Wuhan-1 D614G and BA.1 by FRNT. 3 or 4 days after the post-boost blood collection, mice were challenged with 10^5 FFU of Wuhan-1 N501Y/D614G or BA.1 intranasally.

Table 5: Treatment Regimen for WASHU-129MOD5/6: 129S2 Mice

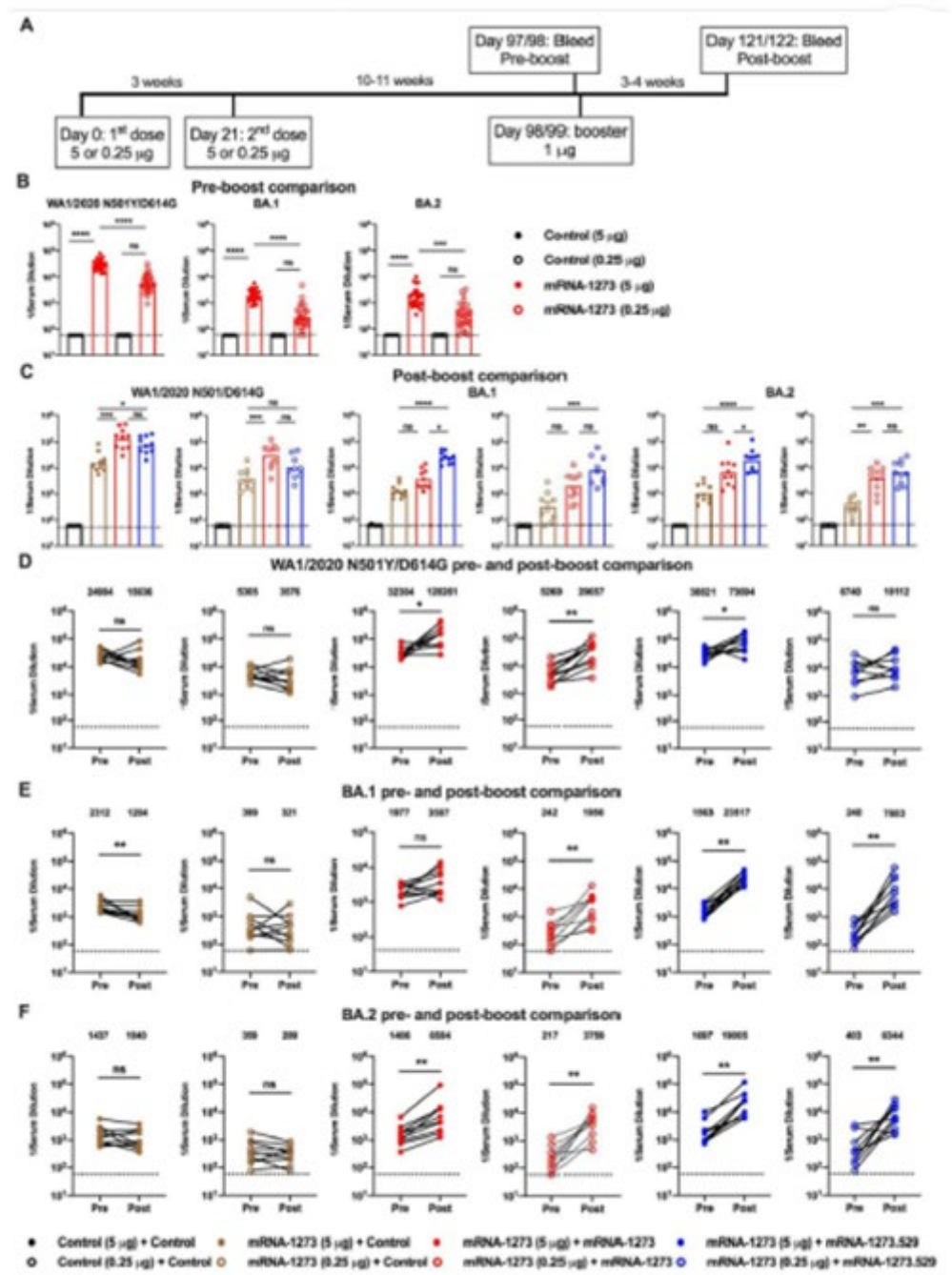
		Primary Series			Boost			Challenge		
Cohort	Mouse Strain (Female)	Treatment	Dose Level (µg)	Dose Schedule	Treatment	Dose Level (µg)	Dose Schedule	Variant (10^5 FFU)	Time Point	Collection Time Points
4	129S2	mRNA Control	5	Day 0, Day 21	mRNA Control	1	Day 98, Day 99	WA1/2020 N501Y/D614G or BA.1	Day 124/126	**Serum (Day 97/98 pre-boost; Day 121/122 post-boost):** Antibody response (FRNT) **Sacrifice (Day 127/129):** Viral burden (qRT-PCR) Cytokine/chemokine analysis
			0.25		mRNA Control	1				
		mRNA-1273	5		mRNA Control	1				
					mRNA-1273	1				
					mRNA-1273.529	1				
			0.25		mRNA Control	1				
					mRNA-1273	1				
					mRNA-1273.529	1				

Abbreviations: FFU = focus-forming units; FRNT = focus reduction neutralization; mRNA = messenger RNA; qRT-PCR = quantitative reverse transcription polymerase chain reaction.

The Wuhan-1 N501Y/D614G variant was used because the substitution of N501Y enables engagement of murine angiotensin-converting enzyme 2 and productive infection of conventional strains of laboratory mice. Mice were euthanised 3 days post-infection, and tissues of nasal wash, nasal turbinates, and lung were harvested for virological, cytokine, and chemokine analysis.

Results

Figure 16: Booster Doses of mRNA-1273 or mRNA-1273.529 Enhance Neutralizing Antibody Responses in 129S2 Mice



Abbreviations: ANOVA = analysis of variance; mRNA = messenger RNA; ns = not significant.

Notes: Statistical analyses were performed as follows: 1-way ANOVA with Dunn's post-test (**B, C**); Wilcoxon signed-ra test (**D, E**); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

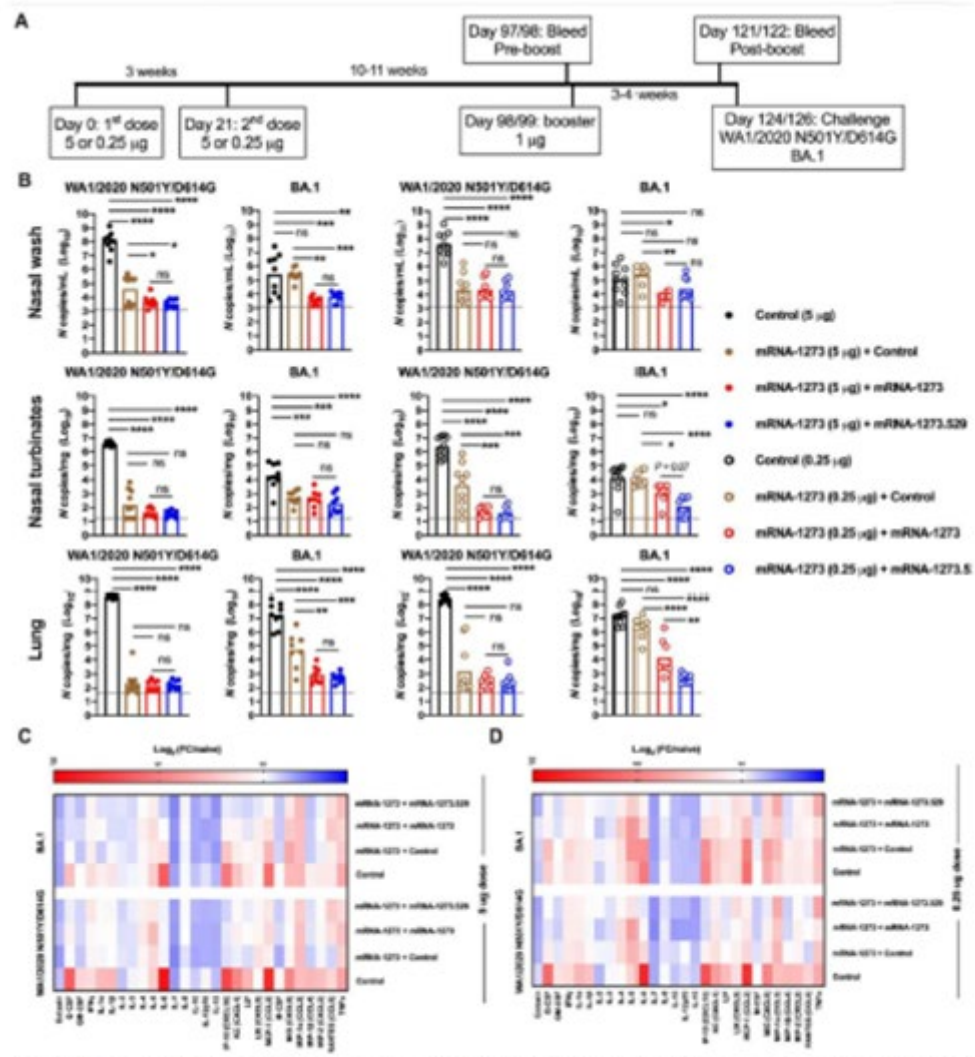
Source: Ying et al 2022b, Figure 6.

Two doses of 0.25 µg or 5 µg mRNA-1273 induced dose-dependent increase of neutralising antibody titres against different SARS-CoV 2 variants. mRNA-1273 induced strong neutralising antibody response against Wuhan-1 N501Y/D614G, but significantly lower neutralising antibody titres against BA.1 and BA.2.

One month after boosting with 1 µg mRNA-1273 or mRNA-1273.529, neutralising antibody titres against Wuhan-1 N501Y/D614G were overall increased compared to 2-dose group. The mRNA-1273 booster

induced slightly higher neutralising antibody response against Wuhan-1 N501Y/D614G compared to mRNA-1273.529 booster. The BA.2-specific neutralising antibody titre increased slightly higher in mRNA-1273.529 than in mRNA-1273 boosted mice. However, boosting with mRNA-1273.529 induced higher neutralising antibody titres against BA.1, but not in mRNA-1273-boosted mice.

Figure 17: Booster Doses of mRNA-1273 or mRNA-1273.529 Enhance Protection Against BA.1 Infection in 129S2 Mice



Abbreviations: ANOVA = analysis of variance; FC = fold-change; mRNA = messenger RNA; N = nucleocapsid; ns = no significant.

Notes: **A and B:** 1-way ANOVA with Tukey's post-test; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).
C and D: FC was calculated relative to naïve mice, and Log_2 values are plotted (2 experiments, n = 8 per grou naïve, n = 4).

Source: Ying et al 2022b, Figure 7.

3 days after infection with 10^5 FFU Wuhan-1 N501Y/D614G or BA.1, the viral BA.1 RNA levels in the upper and lower respiratory tracts in mice vaccinated with the control mRNA were significantly lower than those observed after Wuhan-1 N501Y/D614G infection, indicating a lower pathogenicity of BA.1 in mice. In mice primed with mRNA-1273 and boosted with mRNA-1273 or mRNA-1273.529, the Wuhan-1 N501Y/D614G and BA.1 viral load was significantly reduced in nasal wash, nasal turbinates and lungs compared to mRNA control, in a dose-dependent manner. In contrast to the high dose (5 µg), mice primed with the low dose (0.25 µg) of mRNA-1273 and boosted with mRNA-1273 showed less protection against BA.1 in the lung and nasal turbinates compared to 0.25 µg mRNA-1273.529 boosted mice.

Interestingly, mice primed with 5 µg mRNA-1273 but without booster showed comparable protection against Wuhan-1 N501Y/D614G than mice that got boosted with mRNA-1273 or mRNA-1273.529.

After Wuhan-1 N501Y/D614G challenge, mice primed with mRNA-1273 and boosted with either mRNA-1273 or mRNA-1273.529 had lower levels of pro-inflammatory cytokines and chemokines compared with mice that received 3 doses of control mRNA. Also, vaccinated mice without additional booster showed reduced cytokine and chemokine levels.

After BA.1 challenge, mice immunised with 5 µg mRNA-1273 and boosted with mRNA-1273 or mRNA-1273.529 or without additional booster showed reduced levels of chemokines and cytokines compared to the negative control mice. Furthermore, the BA.1-induced inflammatory response in mice primed with 0.25 µg mRNA-1273 without booster was similar to the response in negative control mice. In addition, lower levels of pro-inflammatory cytokines and chemokines were observed in mice primed with 0.25 µg mRNA and boosted with mRNA-1273.529 compared to those boosted with mRNA-1273.

CHMP comment

An increase of Wuhan- and Omicron-specific neutralising antibody titre was observed in mice when boosted with mRNA-1273 or mRNA-1273.529 (3rd dose). As expected, the BA.1 and BA.2 neutralising antibody titres were slightly higher in mRNA-1273.529 booster mice, and the Wuhan-specific neutralising antibody titre were slightly higher in mRNA-1273.

After challenge with Wuhan or BA.1 SARS-CoV-2, pro-inflammatory markers and viral loads in the upper and lower respiratory tract were reduced in all vaccinated mice. In addition, the mice vaccinated with control mRNA showed lower viral load and slightly lower levels of pro-inflammatory marker after BA.1 infection than after Wuhan-1 infection. Thus, the Omicron variant seems to be less infectious in mice than the Wuhan variant. Similar results were seen in humans.

Overall, mRNA-1273 and mRNA-1273.529 booster show similar good protection against Wuhan-1 and BA.1 infection. Also, the levels of pro-inflammatory markers were lower in boosted mice than in non-boosted or control mice. 3 days after challenge with the Wuhan variant, the viral loads in nasal wash, nasal turbinates and lung tissue were slightly above the lower detection limit in both booster groups. However, the viral load in nasal turbinates and lung tissue were slightly higher in both booster groups after BA.1 infection. The viral load in nasal wash after BA.1 infection was as low as after the Wuhan infection.

The dose concentration of the primary vaccination series seems to be important for a robust neutralising antibody titre, reduced viral load and decreased levels of pro-inflammatory markers. Mice, who were primed with a high vaccine dose, show higher neutralising antibody titre and better protection against SARS-CoV-2 variants. This effect was even observed after the booster vaccination. The booster vaccination was always 1 µg mRNA.

Study VRC-20-857: mRNA-1273 Primary Series and mRNA-1273 versus mRNA-1273.529 Booster Regimen in a Rhesus Macaque SARS-CoV-2 Omicron Challenge Model

Study design

The study was conducted in 16 Indian-origin rhesus macaques (3 female and 13 male; 4 to 8 years old). Eight animals were immunised intramuscular with 100 µg mRNA-1273 at Week 0 and Week 4, administered in 1 mL/dose into the right quadricep. At Week 41, the eight animals were split into groups of 4 and boosted with 50 µg mRNA-1273 or 50 µg mRNA-1273.529. In addition, eight naïve animals of the control group were immunised with 50 µg control non-translating mRNA (UNFIX-01) at the time of

boost (week 41). Macaques were challenged at Week 45 (4 weeks after the booster) with a total dose of 1×10^6 PFU of SARS-CoV-2 Omicron. The viral inoculum was administered as 7.5×10^5 PFU in 3 mL intratracheal and 2.5×10^5 PFU in 1 mL intranasal in a volume of 0.5 mL distributed evenly into each nostril.

Table 6: Treatment Regimen for Study VRC-20-857

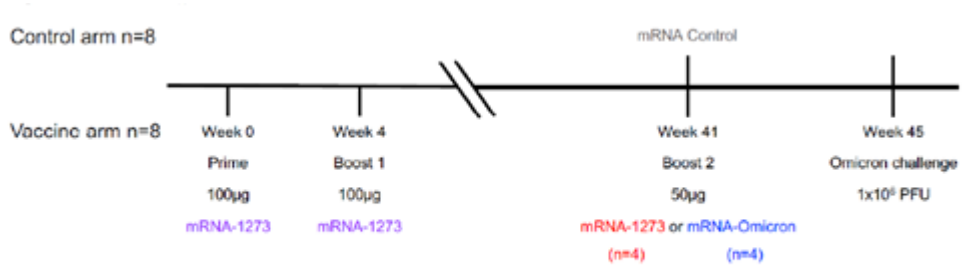
Group	Number of Animals	Vaccination Schedule			Pre-Challenge Collection	Challenge (Week 45)[a]	Post-Challenge Collection
		Prime Dose 1 (Week 0)	Prime Dose 2 (Week 4)	Boost (Week 41)			
1	8	N/A	N/A	UNFIX-01 50 µg	Sera: Weeks 6, 41, 43 NW, BAL: Weeks 8, 39, 43	SARS-CoV-2 Omicron	BAL: Days 2, 4, 8 NS: Days 1, 2, 4, 8 Oral Swab: Day 2 Lung tissue: Day 8[b]
2	4	mRNA-1273 100 µg	mRNA-1273 100 µg	mRNA-1273 50 µg			
3	4			mRNA-1273.529 50 µg			

Abbreviations: BAL = bronchoalveolar lavage; mRNA = messenger RNA; N/A = not applicable; NS = nasal swab(s); NW = nasal wash(es); PFU = plaque-forming units; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; UNFIX-01 = untranslated factor 9.

[a] Animals were challenged with a total dose of 1×10^6 PFU of SARS-CoV-2 Omicron. The viral inoculum was administered as 7.5×10^5 PFU in 3 mL intratracheally and 2.5×10^5 PFU in 1 mL intranasally in a volume of 0.5 mL distributed evenly into each nostril.

[b] Two out of 4 animals from groups 2 and 3 were euthanized for tissue collection.

Figure 18: Experimental Schema for Study VRC-20-857



Abbreviations: mRNA = messenger RNA; PFU = plaque-forming units.
Source: Gagne et al 2022b, Supplemental Figure 1

Sera were collected pre-boost (Week 6 and Week 41) and post-boost (Week 43) and were assessed for total IgG antibody response to variant SARS-CoV-2 S- and receptor-binding domain (RBD)-derived antigens (Wuhan-1 D614G, B.1.351, B.1.617.2 and B.1.1.529). The binding antibody responses were measured by mean square displacement (MSD) V-Plex. Neutralising antibodies were measured for Wuhan-1 D614G, B.1.351, B.1.617.2, and B.1.1.529 by a live virus assay and a lentiviral pseudovirus neutralisation assay. In addition, the antibody avidity for Wuhan-1 and Omicron was measured over time following immunisation. Moreover, nasal washes and BAL fluid were collected at pre-boost at Week 8, Week 39, and post-boost at Week 43 to assess upper and lower airway mucosal antibody response. The IgG binding antibody response was measured by ELISA and the neutralisation capacity of the produced antibodies was evaluated by an ACE2 receptor inhibition assay, which was used as a surrogate for antibody function.

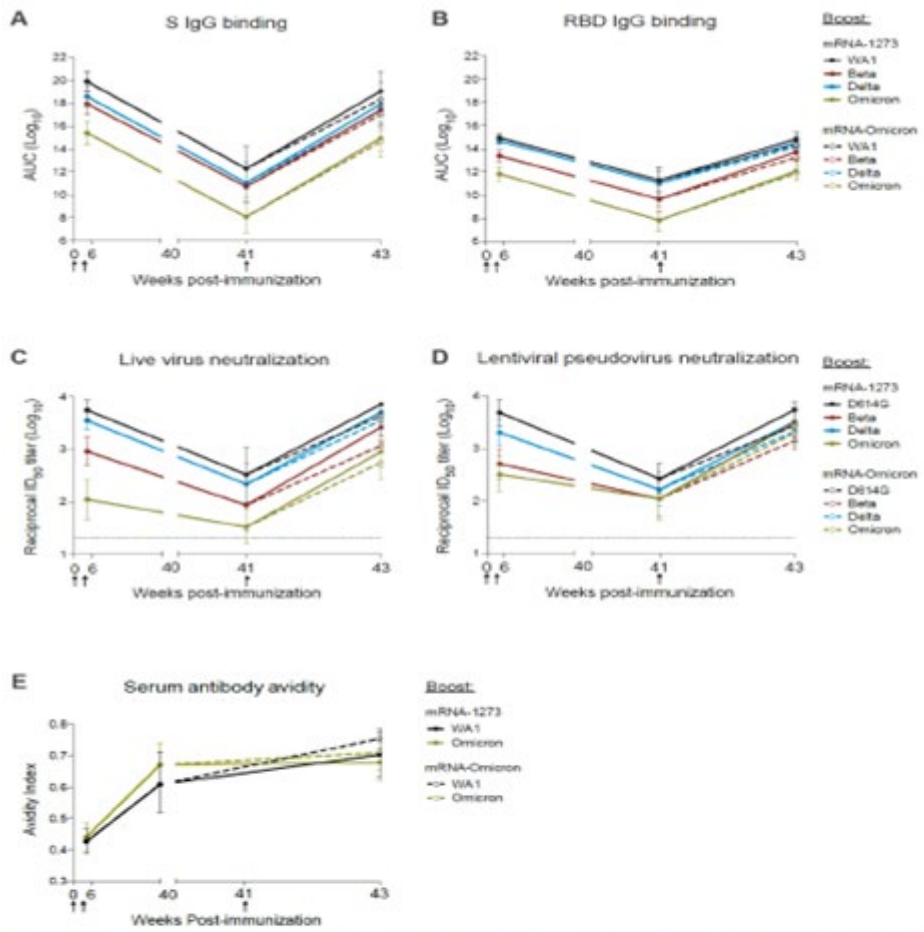
Furthermore, B-cell binding to S-2P was measured at Week 6, Week 41, and Week 43. To further explore the effect of boosting on anamnestic B-cell responses, the activation status of S-binding memory B-cells was phenotyped. In addition, T-cell responses were measured.

Four weeks after administration of either boost, animals were challenged with 1×10^6 PFU via both IT and IN routes. Two days after challenge, oral swabs were collected. BAL fluid was collected 2, 4, and 8

days after challenge. Nasal swabs were collected 1, 2, 4, and 8 days after challenge. In addition, SARS-CoV-2 sgRNA copy numbers were measured to determine the extent of viral replication. To assess lung pathology in the NHPs, 2 of the animals of each group were euthanised 7 to 9 days after the Omicron challenge, and lung tissue was processed for histopathology and immunohistochemistry.

Results

Figure 19: Serum Antibody Responses Following mRNA-1273 Primary Series and Boost



Abbreviations: AUC = area under the curve; CI = confidence interval; ID_{50} = reciprocal 50% inhibitory dilution; IgG = immunoglobulin G; LOD = limit of detection; mRNA = messenger RNA; NHP = non-human primate; RBD = receptor-binding domain; S = spike; WA1 = USA-WA1/2020.

Note: Circles represent geometric means (A-D) or arithmetic means (E). Error bars represent 95% CI. Assay LOD is indicated by dotted lines. The break in the X-axis indicates a change in scale without a break in the range depicted. Responses to variants are color-coded as WA1 or D614G (black), Beta (red), Delta (blue), and Omicron (green). Arrows represent time points of immunizations. Following the boost at Week 41, mRNA-1273-boosted NHPs are indicated by solid lines and mRNA-1273.529-boosted NHPs are indicated by dashed lines. Four NHPs were included per boost group.

Source: Gagne et al 2022b, Figure 1

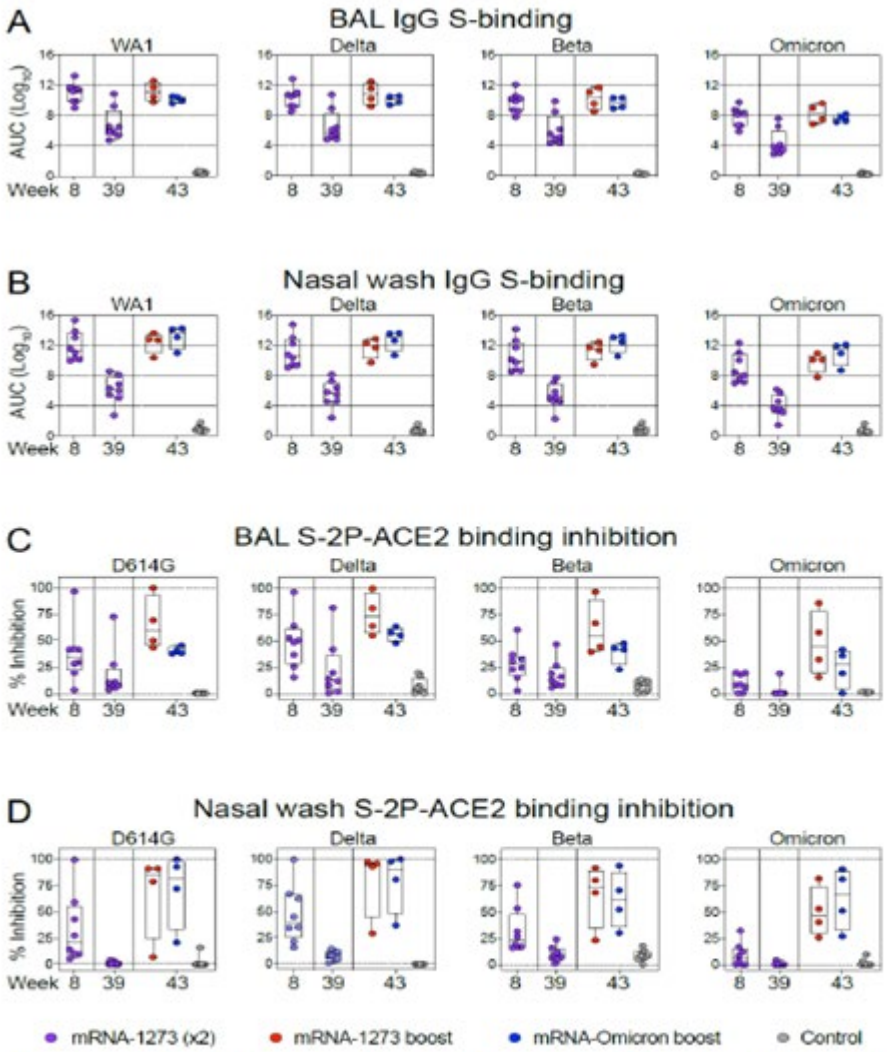
2 weeks after the second dose of the primary vaccine regimen, S- and RBD-specific IgG antibody titres were measured in serum by MSD V-Plex. Overall, the S-specific IgG antibody titres against SARS-CoV-2 Wuhan-1, Beta, Delta and Omicron were higher than the RBD-specific IgG antibody titres against those variants. In addition, the S and RBD-specific IgG antibody titres against all tested SARS-CoV-2 variants decreased markedly by week 41 before boosting. 2 weeks after boosting with mRNA-1273 or mRNA-1273.529, S- and RBD-specific IgG binding antibody titres increased for all SARS-CoV-2 variants and titre levels were similar to the titre levels at week 6. For both S- and RBD-specific IgG antibody titres, the vaccines induced the highest titres against SARS-CoV-2 variant Wuhan-1 followed by Delta, then Beta and lowest titre level for Omicron. For both S- and RBD-specific IgG binding antibody titres, the titre

levels of all four SARS-CoV-2 variants were slightly lower in mRNA-1273.529 boosted animals than in mRNA-1273 boosted animals.

Similar to the IgG binding antibody titre levels, neutralising antibody titres were highest to Wuhan-1 D614G followed by Delta, then Beta, then Omicron variant when measured by live virus neutralisation assay and lentiviral pseudovirus neutralisation assay after the 2 mRNA-1273 doses of the primary vaccine regime. Also, the neutralising antibody titres against all tested SARS-CoV-2 variants decreased significantly by Week 41. The measured neutralising antibody titres of Omicron were slightly lower when tested by the live virus neutralisation assay and measured neutralising antibody titres of Beta were slightly higher when tested by the live virus neutralisation assay. Following a boost with mRNA-1273 or mRNA-1273.529, neutralising antibody titres to Wuhan-1 D614G and Delta increased and were similar to those measured at Week 6. Neutralising antibody titres to Beta and Omicron variants were even higher than they had been measured at Week 6.

Serum antibody avidity following immunisation was measured over time for Wuhan-1 and Omicron variants to assess whether the increased neutralising titres after the boost could be attributed to antibody maturation. Antibody avidity to Wuhan-1 and Omicron Spike-2P significantly increased from Week 6 (2 weeks after the 2nd mRNA-1273 dose) to Week 40. Antibody avidity to Omicron Spike-2P increased faster than to Wuhan-1. After the boost with mRNA-1273 or mRNA-1273.529, the antibody avidity to Wuhan-1 was slightly higher compared to Omicron.

Figure 20: Mucosal Antibody Response to Boosting with mRNA-1273 or mRNA-1273.529



Abbreviations: ACE2 = angiotensin-converting enzyme 2; AUC = area under the curve; BAL = bronchoalveolar lavage; IgG = immunoglobulin G; mRNA = messenger RNA; NHP = non-human primate; S = spike; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; WA1 = USA-WA1/2020.

Note: Circles indicate individual NHPs. Boxes represent the interquartile range with the median denoted by a horizontal line. Dotted lines are for visualization purposes and denote 4-$\log_{10}$ increases in binding titers (A-B) or 0% and 100% inhibition (C-D). Eight controls and 8 vaccinated NHPs were split into 2 cohorts post-boost.

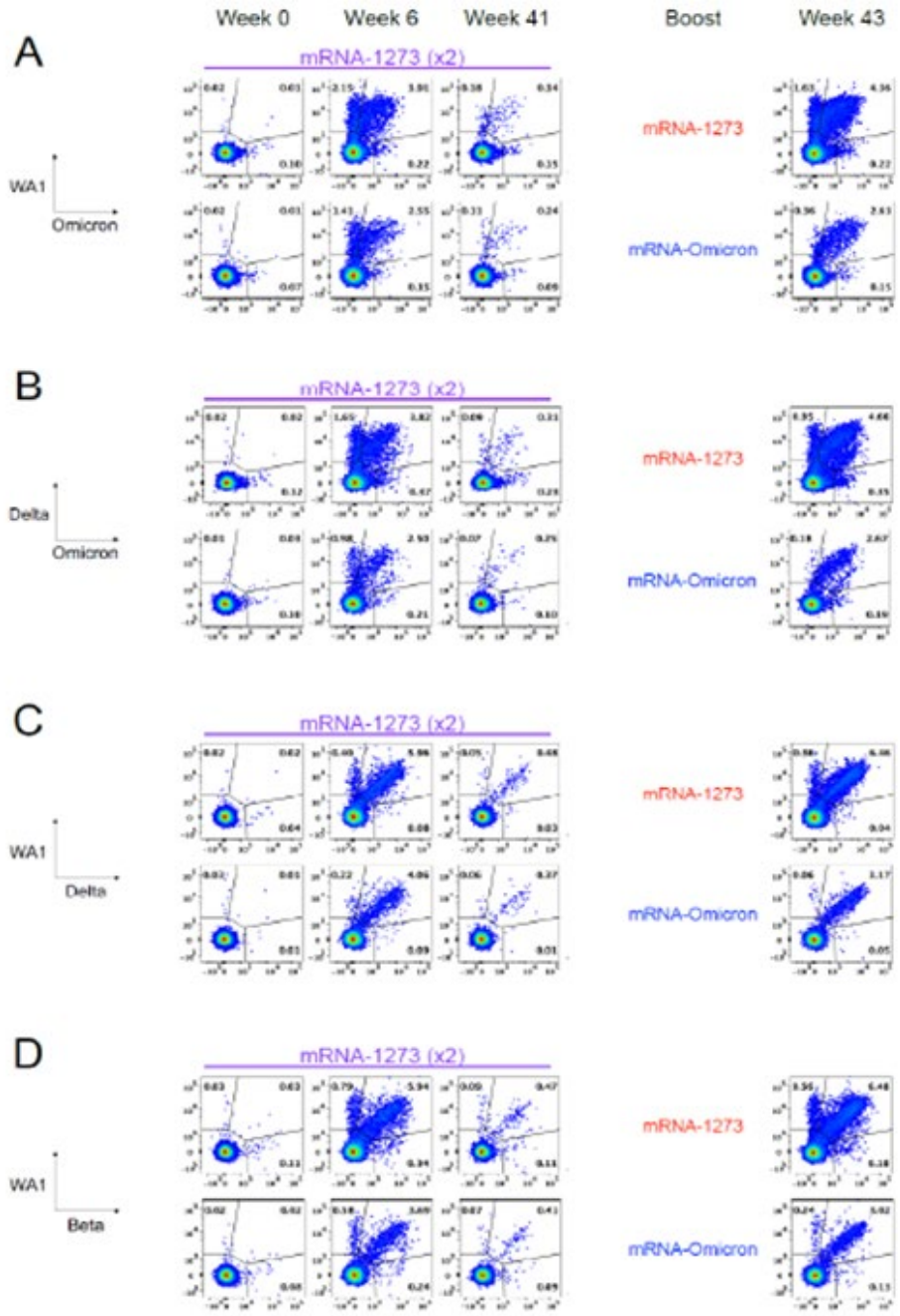
Source: Gagne et al 2022b, Figure 2

The upper and lower airway mucosal S-specific IgG antibody responses were analysed by MSD V-Plex after two doses of mRNA-1273 (primary vaccine regimen) and after boosting with mRNA-1273 or mRNA-1273.529. Overall, S-specific IgG antibody titres against all SARS-CoV-2 variants were slightly higher in nasal wash compared to bronchoalveolar lavage (BAL) fluid. Similar to the serum IgG antibody titre, the titres decreased significantly after the primary vaccine series until week 39. After boosting with mRNA-1273 or mRNA-1273.529, the mucosal IgG antibody titre increased for all SARS-CoV-2 variants. In general, BAL fluid and nasal wash S-specific IgG antibody titres were highest in Wuhan-1, then slightly lower in Delta, then Beta, and finally Omicron, after the primary vaccine series and after boosting. After boosting, the mucosal IgG antibody titres did not differ remarkably between mRNA-1273 and mRNA-1273.529 booster.

An ACE2 inhibition assay was applied, which was used as a surrogate for neutralisation capacity of the vaccines. BAL fluid expressed 25% to 50% median ACE2 binding inhibition for all variants at Week 8, except for Omicron S-2P, in which binding inhibition was low to undetectable. There was a remarkable

decrease in ACE2 binding inhibition for all variants by Week 39, followed by an increase after either the mRNA-1273 or mRNA-1273.529 boost. Despite an increase in ACE2 inhibition of Omicron S-2P following the boost, it was still lower than all the other variants. In nasal wash samples, the ACE2 binding inhibition was similar than for BAL fluid at week 8, but very low to undetectable at Week 39. After administration of either boost, there was an increase in ACE2 S-2P binding inhibition across all variants, but slightly lower for Omicron. After boosting with either mRNA vaccine, the ACE2 binding inhibition in BAL and nasal wash was higher than the initial peak after the primary vaccine series at Week 8.

Figure 21: Memory B-Cell Specificities Following Immunization and Boosting



Abbreviations: mRNA = messenger RNA; WA1 = USA-WA1/2020.
Source: Gagne et al 2022b, Figure 3

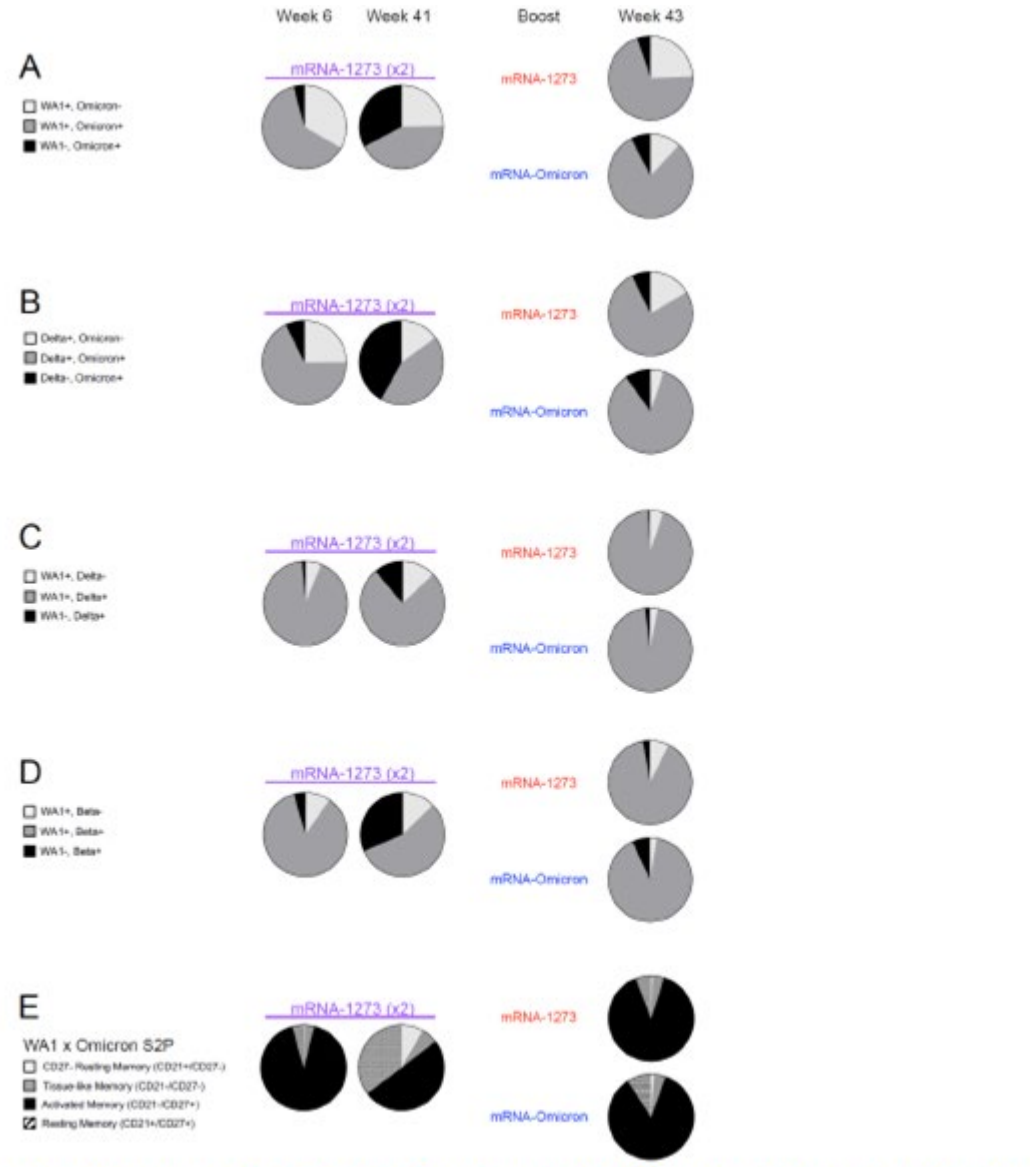
B-cell binding was measured using different SARS-CoV-2 variants to assess mobilisation of cross-reactive memory B-cells. At Week 6, Wuhan-1-specific and Wuhan-1/Omicron-cross-reactive S-2P-specific memory B-cell responses were detected. However, the total S-specific memory B-cell compartment

decreased significantly by Week 41. At Week 43, an expansion of the total S-specific memory B-cell compartment (similar to that observed at Week 6) was observed following either boost.

After boosting with mRNA-1273 and mRNA-1273.529, the majority of S-2P–specific memory B-cells were dual-specific for Wuhan-1 and Omicron. In addition, high Wuhan-1 specific memory B-cells were detected in mRNA-1273 boosted animals. In contrast, only very low amount of Omicron-specific and Wuhan-1-specific memory B-cells were detected in mRNA-1273.529 boosted animals.

Furthermore, vaccination with mRNA-1273 and mRNA-1273.529 induced only low Delta and Beta specific S-2P-specific memory B-cell responses in the NHPs.

Figure 22: Expansion of Cross-Reactive S-2P-Specific Memory B-Cells Following Boosting



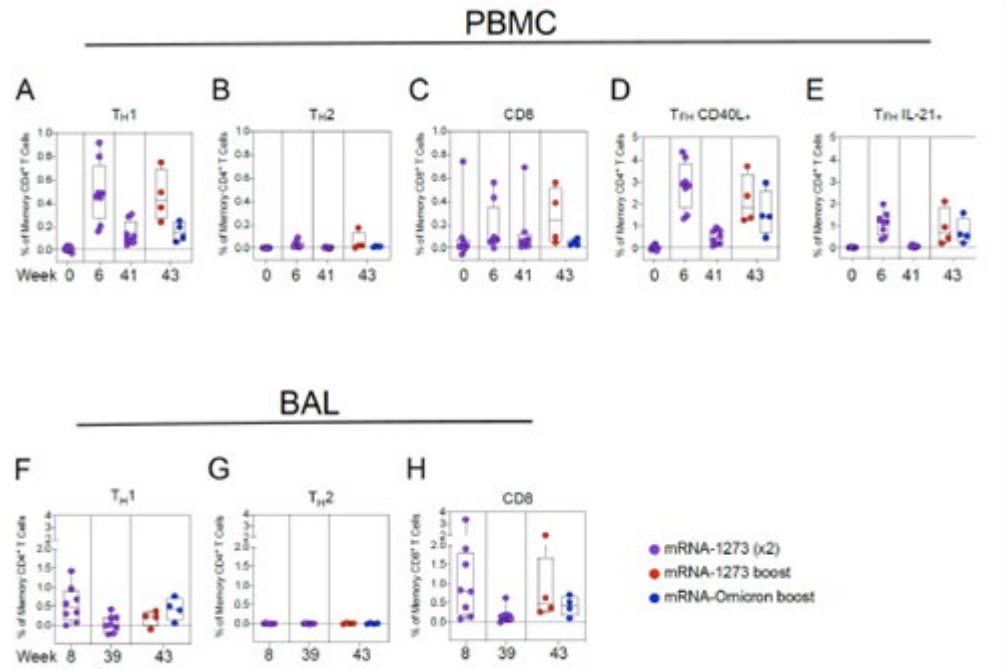
Abbreviations: CD = cluster of differentiation; mRNA = messenger RNA; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; WA1 = USA-WA1/2020.

Source: Gagne et al 2022b, Figure 4.

To further explore the effect of boosting on anamnestic B-cell responses, the activation status of S-binding memory B-cells was phenotyped. Wuhan-1 S-2P– and/or Omicron S-2P-binding memory B-cells predominantly had an activated memory phenotype immediately after both the second and third doses.

At week 41 prior to booster dose, the relative amount of resting memory B-cells and CD27-resting memory B-cells increased.

Figure 23: Both mRNA-1273 or mRNA-1273.529 Boost T-Cell Responses to S Peptides



Abbreviations: BAL = bronchoalveolar lavage; CD = cluster of differentiation; IL = interleukin; mRNA = messenger RNA; PBMC = peripheral blood mononuclear cell; Tfh = T-follicular helper; Th1 = T helper 1; Th2 = T helper 2.

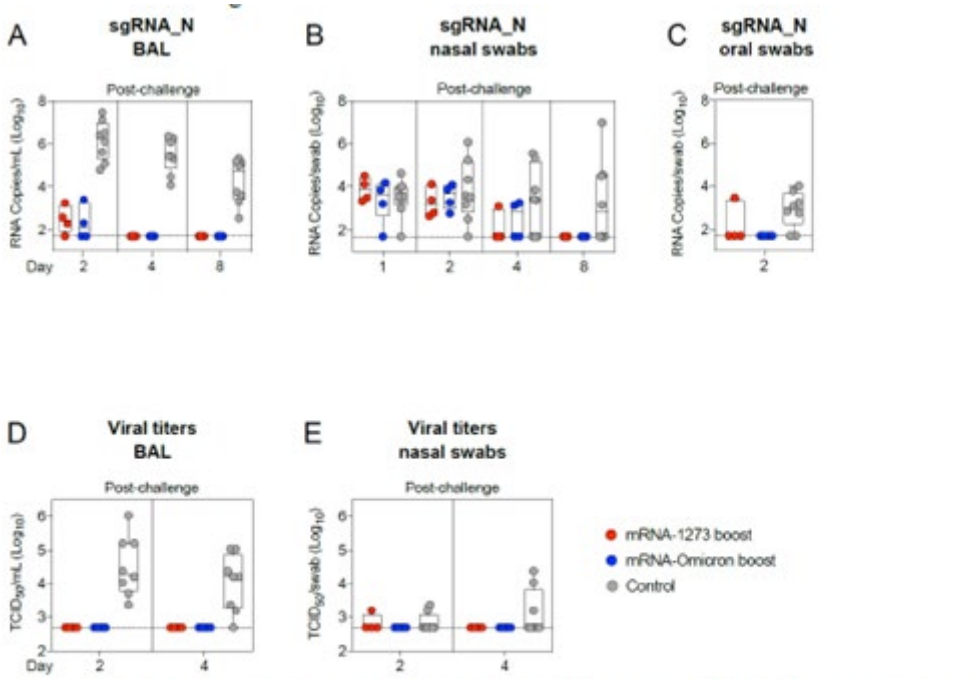
Source: Garne et al 2022b. Supplementary Figure 6

Two doses of mRNA-1273 caused low Th1 and high Tfh CD40L and Tfh IL21 levels in blood samples of NHPs. In addition, low levels of CD8+ T-cell response was observed in two vaccinated animals. However, one animal showed already detectable CD8+ T-cell levels prior vaccination (week 0). Overall, the T-cell responses significantly decreased until week 41 (before booster). Administration of mRNA-1273 or mRNA-1273.529 boost significantly increased Tfh responses. In addition, mRNA-1273 booster increased the Th1-response in all four animals and CD8+ T-cell response in two animals.

In BAL fluid, Th1 and CD8+ T-cell responses were detected at Week 8, which decreased to extreme low T-cell levels at Week 39 (pre-boost). At week 43, these T-cell responses were increased with either the mRNA-1273 or the mRNA-1273.529 boost (Week 43).

There was little to no response from Th2 T-cells in BAL fluid or blood samples after the primary mRNA-1273 vaccine series or after the booster with mRNA-1273 or mRNA-1273.529.

Figure 24: Boosting Provides Equivalent Protection in the Lungs Against Omicron Challenge



Abbreviations: BAL = bronchoalveolar lavage; LOD = limit of detection; mRNA = messenger RNA; NHP = non-human primate; sgRNA_N = subgenomic RNA encoding for the N gene; $TCID_{50}$ = median tissue culture infectious dose.

Note: Circles indicate individual NHPs. Boxes represent the interquartile range with the median denoted by a horizontal line. Assay LOD is indicated by dotted lines. Eight controls and 4 vaccinated NHPs were included per boost cohort.

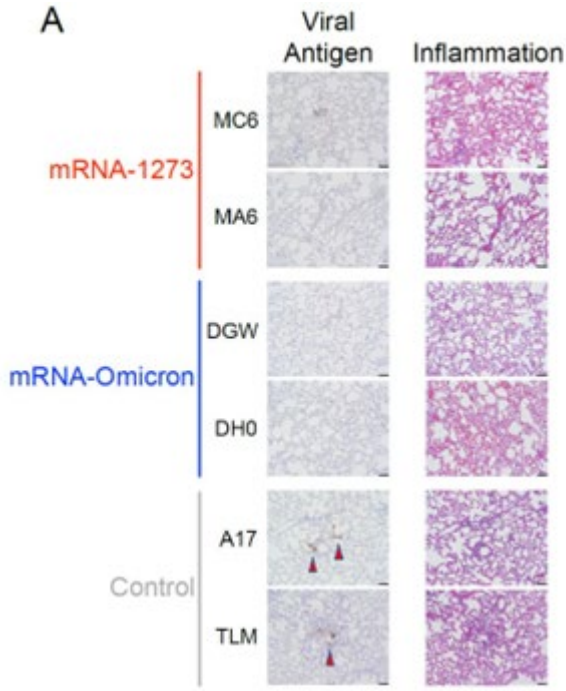
Source: Gagne et al 2022b, Figure 5

Four weeks after administration of either boost, NHPs were challenged with $1x10^6$ PFU of SARS-CoV-2 Omicron both IT and IN. After the challenge, BAL fluid, nasal swabs and oral swabs were collected and the extent of viral replication was measured by copy numbers of SARS-CoV-2 sgRNA using q-RT-PCR.

Control NHPs had high sgRNA copy numbers (10E6 copies/mL) in BAL fluid at Day 2 post-infection, which decreased over time (10E4 copies/ml on Day 8). In addition, sgRNA copy numbers were also detected in nasal swabs (Day1 to Day8) and oral swabs of control animals (Day 2). In BAL fluid, vaccinated NHPs had mean copy numbers of 3×10^2 and 2×10^2 sgRNA copies/mL for the mRNA-1273 and mRNA-1273.529 cohorts on Day 2, respectively. In addition, sgRNA was detected in nasal swabs of all vaccinated NHPs on Day 1 and Day 2, in one mRNA-1273 and two mRNA-1273.529 boosted NHPS on Day 4, and in oral swabs of one mRNA-1273 boosted animal on Day 2. All vaccinated NHPs had undetectable levels of sgRNA in BAL by Day 4 and in nasal swabs by Day 8 post-infection.

In TCID50 assay, no virus was detected in the BAL fluid of any vaccinated NHPs, while almost all control NHPs had detectable virus on Day 2 and Day 4 post-infection. One mRNA-1273 boosted NHP had detectable virus load in the nasal swab on Day 2. In the unvaccinated control animals, 2 out of 8 NHPs on Day 2 and 3 out of 8 NHPs on Day 4 had detectable virus in the nose.

Figure 25: Viral Antigen and Pathology in the Lungs After Challenge



Abbreviations: Lc = left cranial lobe; mRNA = messenger RNA; Rc = right caudal lobe; Rmid = right middle lobe.
Antigen scoring legend: - = no antigen detected; +/- = rare to occasional foci; + = occasional to multiple foci; ++ = multiple to numerous foci; +++ = numerous foci.
Inflammation scoring legend: - = minimal to absent inflammation; +/- = minimal to mild inflammation; + = mild to moderate inflammation; ++ = moderate to severe inflammation; +++ = severe inflammation.
Source: Gagne et al 2022b, Figure 6

Following Omicron challenge, two NHPs from each of the vaccinated groups and four NHPs from the control group were euthanised on Day 8. Variable amounts of nucleocapsid antigen were detected in the lungs of two control NHPs. Viral antigen was often associated with the alveolar capillaries and occasional nearby immune cells. In contrast, there was no evidence of viral antigen in the lung samples of any vaccinated NHP.

Animals from both boost groups exhibited histopathologic alterations that were classified as minimal to mild or mild to moderate. Inflammation was characterised by mild and patchy expansion of alveolar capillaries, generalised alveolar capillary hypercellularity, mild and regional type II pneumocyte hyperplasia, and (less frequently) scattered collections of immune cells within some alveolar spaces.

Control NHPs had moderate to severe pathology. Lung sections from controls included moderate and often diffuse alveolar capillary expansion, diffuse hypercellularity, moderate type II pneumocyte hyperplasia, and multiple areas of perivascular cellular infiltration (cuffing).

CHMP comment

For this study, Indian rhesus macaques vaccinated with mRNA-1273 or Omicron-matched mRNA-1273.529 booster were analysed for immunogenicity and efficacy against SARS-CoV-2 Omicron infection. This animal model is known for susceptibility to SARS-CoV-2 infection and is thus a suitable model for challenge studies. However, the animal sexes were not equal distributed with only 3 female and 13 male rhesus macaques in total. In addition, the age distribution was wide with 4 to 8 years old animals. Furthermore, the groups size was small with 4 animals per vaccine group and only 2 animals per vaccine group were histo-pathologically analysed after the SARS-CoV-2 Omicron challenge. Thus, no meaningful statistic evaluation of the results could be made.

For this study, a dose schedule comparable to the recommended clinical dose schedule with this vaccine was applied (primary vaccination regime 4 weeks apart, booster vaccination about 10 months after 1st dose). Furthermore, the vaccines were administered via the recommended human route and the human vaccine doses were used (100 µg mRNA for primary series, 50 µg for booster). However, mRNA-1273.214 was not analysed as booster, although, this vaccine variant is the article for this approval.

This study showed that one mRNA-1273.529 booster vaccination is not superior to one mRNA.1273 booster vaccination in NHP regarding immunogenicity. Both booster vaccine candidates induced similar high serum and mucosal S- and RGB-specific IgG antibody titres and neutralising antibody responses. Both were highest effective against the Wuhan-1 variant, then Delta, then Beta and then Omicron.

Many Wuhan/Omicron cross-reactive memory B-cells were detected after mRNA-1273 and mRNA-1273.529 booster vaccination. In addition, high amount of Wuhan-specific memory B-cells were detected in mRNA-1273 and slightly less in mRNA-1273.529 boosted NHPs. As expected, only little amount of Omicron-specific memory B-cells were detected in mRNA-1273 boosted cells. However, the amount of Omicron-specific memory B-cells were even smaller in mRNA-1273.529 boosted cells. Further analysis showed, that activated memory cells were the predominant phenotype after booster with either vaccine.

Furthermore, both booster vaccines induced comparable T-cell responses in NHPs. They both showed similar Tfh CD40L+ and Tfh IL21+ activation in PBMC. But, mRNA-1273.529 induced slightly lower Th1 and CD8+ T-cell responses than mRNA-1273 in blood cells. However, mRNA-1273.529 induced slightly higher Th1 and CD8+ T-cell responses than mRNA-1273 in BAL. In addition, Th2 T-cell response was not induced by either vaccine, not in PBMC nor in BAL fluid. Overall, the vaccine-inducted T-cell response was relatively low after booster with mRNA-1273 or mRNA-1273.529. These data are in line with the data from the initial MAA of Spikevax (mRNA-1273).

Both boosters showed similar, partly protection from SARS-CoV-2 Omicron infection. The viral titre in BAL and nasal swabs of vaccinated NHPs were at the lower detection limit in contrast to control animals that showed detectable viral titres. Furthermore, viral sgRNA in BAL was only detected on Day 2 in both vaccinated groups, in nasal swabs from Day 1 to Day 4, and in oral swabs only in one mRNA-1273 boosted animal. The histopathology of lung tissue showed similar results. The two analysed NHPs per vaccine group showed no viral antigen in lung tissue, but minimal to mild inflammation in lung tissue was observed in both vaccine groups. Thus, one booster dose of either vaccine showed comparable efficacy with partly protection from SARS-CoV-2 Omicron infection. Therefore, no superior in efficacy against Omicron infection was shown for one booster dose of mRNA-1273.529 in NHPs.

4.1.3. CHMP's overall conclusions on pharmacology

The MAH conducted several pharmacodynamics studies with Omicron-matched mRNA vaccines in mice and one study in rhesus macaques. All studies were non-GLP compliant, which is accepted. In these studies, the MAH analysed the immunogenicity of the monovalent Omicron-matched vaccine mRNA-

1273.529 and the bivalent Omicron-matched vaccine mRNA-1273.214 and compered them with the parent vaccine mRNA-1273. In addition, the efficacy of these vaccines was evaluated by challenging mice or NHPs with Wuhan or BA.1 SARS-CoV-2 infection.

mRNA-1273, mRNA-1273.529 and mRNA-1273.214 could demonstrate robust Spike protein-specific IgG antibody responses against different SARS-CoV-2 variants (Wuhan-1, Omicron (BA.1, BA.2, BA.1.1), Beta and/or Delta) in blood. In one BALB/c mice and one NHP study, it could be also shown that mRNA-1273 and mRNA-1273.529 induce RBD-specific IgG antibody responses against Wuhan-1, Omicron BA.1, Beta and Delta SARS-CoV-2. However, the Omicron IgG titre was slightly lower in both vaccine groups compared to the titre of the other variants. Moreover, mRNA-1273, mRNA-1273.529 and mRNA-1273.214 induced neutralising antibody titres against several SARS-CoV-2 variants but differed remarkably in their specificity. The bivalent Omicron-matched vaccine mRNA-1273.214 showed neutralising antibody titres against Wuhan-1 and the two Omicron variants BA.1 and BA.2 in mice (Beta and Delta were not tested). The monovalent Omicron-matched vaccine mRNA-1273.529 showed neutralising antibody titres against BA.1, BA.1.1 and BA.2, but only very low levels against Wuhan, Beta and Delta in mice. However, in NHPs, one booster dose of mRNA-1273.529 could induce moderate neutralising antibody responses against Wuhan, Delta, Beta and Omicron. mRNA-1273 showed neutralising antibody titres against Wuhan, Delta and Beta in mice, and Wuhan, Omicron, Delta and Beta in NHPs. In mice, neutralising antibody titres were generally low after one (booster) dose but reached robust levels after a second dose of the vaccine variant. In NHPs, one booster dose already induced stronger neutralising antibody titres.

All vaccine variants induced only low T-cell responses in mice and NHPs. In addition, one booster dose of an Omicron-matched vaccine did not induce significant memory B-cell activation in mice and NHP lymph nodes. However, increased levels of Wuhan-specific memory B-cells were observed after a booster dose with mRNA-1273 in mice and NHPs. A second booster dose with mRNA-1273.529 in BALB/c mice did also not increase the Omicron-specific memory B-cell level in mice. This result might be also due to too low amount of booster vaccine (0.25 µg).

mRNA-1273 and/or mRNA-1273.529 vaccinated mice and NHPs showed partly protection against Wuhan or Omicron SARS-CoV-2 infection. The viral load was significantly reduced in the upper and lower respiratory tract of the vaccinated study animals compared to the negative control animals. In addition, pro-inflammatory markers and inflammation in lung tissue were reduced in the vaccinated animals. However, significant differences in efficacy against Wuhan or Omicron infection were not observed in mice and NHPs.

Overall, the submitted pharmacodynamics data package is sufficient for the type II variation of Spikevax regarding bivalent Omicron-matched Spikevax mRNA-1273.214. However, the studies had some limitations. Most of the immunogenicity studies and all challenge studies were not conducted with mRNA-1273.214. Instead, the parent vaccine mRNA-1273 was compared with the monovalent Omicron-matched vaccine mRNA-1273.529. This can be accepted since mRNA-1273.214 is a 1:1 mix of mRNA-1273 and mRNA-1273.529. Thus, significant pharmacodynamics differences are not expected, especially not for protection against SARS-CoV-2 variants infections (which were not tested with mRNA-1273.214). In addition, challenge studies were conducted only with Wuhan-1 and Omicron BA.1. Challenge studies with other SARS-CoV-2 VoCs were not conducted, e.g. with Omicron BA.5 or Delta. Another limitation was that only females were used for the mice pharmacodynamics studies. It might be possible that male mice would show a different immunogenicity reaction. Regarding to the significant differences of spike-specific IgG antibody responses in female and male rats of the repeat-dose toxicity study, it can be assumed that the mRNA-1273-induced immune responses differs generally between female and male rodents. Any conclusion about sex-based differences in immunogenicity of mRNA-1273 in NHPs cannot be drawn because the sexes were not equally distributed in this study.

4.2. Pharmacokinetics

4.2.1. Pharmacokinetic studies

One non-GLP compliant biodistribution study with mRNA-1647 in SD rats has been conducted. This study has been assessed already for the initial MAA of Spikevax (mRNA-1273). Here, the MAH submitted an amendment of this study. Moreover, metabolites of SM-102 were identified in one in vitro and one in vivo study, both non-GLP.

Table 7: Summary of Nonclinical PK Program for mRNA-1273

Study Type	Test Article	Species, Strain	Method of Administration, Dose	GLP	Report Number
Biodistribution					
Single-dose tissue distribution study	mRNA-1647[a]	Rat, Sprague Dawley	IM injection dose of 100 µg on Day 1	No	5002121 Amendment 2
Metabolite Identification					
In vitro metabolite profiling and identification	SM-102	Rat, monkey, and human hepatocytes	In vitro, 10 µM	No	NCS-BA-2022-010
In vivo metabolite profiling and identification	SM-102	Rat, Sprague Dawley	IV, 0.7 mg/kg	No	QV-0236-DA-RE

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

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

4.2.2. Distribution

Study 5002121 (Amendment No. 2): A Single Dose Intramuscular Injection Tissue Distribution Study of mRNA-1647 in Male Sprague-Dawley Rats

Study design

This non-GLP compliant biodistribution study was already evaluated in the Spikevax MAA. For this grouped Type II variation, the MAH submitted an amendment of this study report including editorial changes and correction of study results.

Summary of changes:

Item or Section(s)	Justification
Final Amended Report 1	
2. SUMMARY	To correct the average value of terminal half-life for the muscle (i.e. injection site) based on the results of the toxicokinetic evaluation.
8.5. Toxicokinetic Evaluations	To correct the average value of terminal half-life for the muscle (i.e. injection site) based on the results of the toxicokinetic evaluation.
Toxicokinetic Report	To include a clarification page to correct the average value of terminal half-life for the muscle (i.e. injection site) based on the results of the toxicokinetic evaluation.
Final Amended Report 2	
RESPONSIBLE PERSONNEL	To modify the name of the individual scientist at the sponsor test site since the updated toxicokinetic analysis/interpretation was performed by another scientist.
4.11. Laboratory Evaluations	To correct the sub-sections numbering. The table of content was updated to reflect the change in Section 4.11. Due to software limitations, some additions and deletions have not been strikethrough or underlined.
8.5. Toxicokinetic Evaluations	To correct the mRNA-1647 tissue-to-plasma $AUC_{(0-t)}$ ratios for highly exposed tissues (injection site muscle) based on the correction performed in the toxicokinetic report.
Deviations	To include a minor Study Plan deviation due to DocuSign® software that was used to sign the Study Plan Amendment 03.
Bioanalytical Report	To include the Final Bioanalytical Report Amendment 01 since the mRNA concentrations were clarify and results were updated.
Toxicokinetic Report	To remove the justification memo since the changes were updated directly in the toxicokinetic report and to include the TK Final Report Amendment 01.

In brief, the tissue distribution of mRNA-1647 was analysed in this study, when given one dose of 100 µg by intramuscular injection to male SD rats. In addition, the toxicokinetic characteristics of mRNA-1647 were determined.

Results

Mean plasma concentrations of mRNA-1647 were quantifiable up to 24 hours. All six mRNA-1647 constructs, gB, gH, gL, UL130, UL131A, and UL128 levels measured in plasma and tissues demonstrated nearly identical pharmacokinetic behaviour. The highest mRNA-1647 exposure was observed in muscle (i.e. site of injection), followed by proximal (popliteal) lymph nodes, axillary distal lymph nodes and spleen, suggesting the mRNA-1647 distribution to the circulation by lymph flow. All other tissues tested, except for kidney and eye, have demonstrated exposures comparable or below that measured in plasma. Exposure observed for the eye was only slightly higher than that in plasma while no mRNA-1647 constructs were detected at any time point in the kidney.

Concentrations of mRNA-1647 were quantifiable in the majority of tissues examined and in plasma at the first time point collected (i.e. 2 hours post-dose) and peak concentrations were reached between 2 and 24 hours post-dose in tissues with exposures above that of plasma. The t1/2 of mRNA-1647 was reliably estimated in muscle (i.e. site of injection), proximal popliteal and axillary distal lymph nodes and spleen with average values for all construct t1/2 of 14.9, 34.8, 31.1, and 63.0 hours, respectively.

The average for all constructs, mRNA-1647 tissue-to-plasma AUC(0-t) ratios for highly exposed tissues were 1010 (not 939, as written in the previous version of this study report), 201, 62.8, and 13.4 for muscle (i.e. injection site), the lymph nodes (proximal popliteal and axillary distal) and spleen, respectively.

CHMP comment

The changes are accepted.

4.2.3. Metabolism

Specific metabolism studies with mRNA-1273 or mRNA-1273 variant vaccines have not been conducted. However, the metabolism and elimination of the amino-lipid component SM-102 in mRNA-1273 have been examined in vivo (Study QV-0236-DA-RE) and in vitro (Study NCS-BA- 2022-010).

Study NCS-BA-2022-010: Identification and profiling of metabolites of SM-102 in Rat, Monkey and Human Hepatocytes

Study design

The purpose of this non-GLP compliant study was to evaluate the metabolism of SM-102 in cryopreserved primary hepatocytes prepared from 6 male Sprague-Dawley rat, 5 male cynomolgus monkey and 5 female and 5 male humans, and to qualitatively characterise the in vitro metabolism of SM-102 following incubation of SM-102-containing LNPs with hepatocytes from these species. 10 µM of SM-102 was used as final concentration in this metabolism study.

The metabolite profiling was performed by Liquid chromatography–high resolution mass spectrometry. Chromatographic and mass spectral data were collected at 0h, 4h and 24h and were compared to the corresponding control samples. SM-102 was incubated with denatured hepatocytes to identify potential metabolism-related components, such as interfering signals or chemical degradation products.

Results

Table 8: Summary of Metabolite Profiling of SM-102 in Human, Rat and NHP Hepatocytes

Metabolite ID	RT	Observed m/z	Theoretical m/z	Error	Elemental Composition	Proposed Biotransformation	Human	Rat	NHP
M1	3.2	204.1591	204.1592	-0.48	C10H22NO3	N Dealkylation + Hydrolysis	Y	Y	Y
M3	6.2	290.1961	290.1962	0.34	C14H28NO5	Ester Hydrolysis (2X)+ B-oxidation(2X)	Y*	N	Y
M4	6.9	318.2269	318.2275	1.8	C16H32NO5	Ester Hydrolysis (2X)	Y	Y	Y
M6	15.1	528.4614	528.4623	1.7	C31H62NO5	Ester Hydrolysis(1X) + B-oxidation (1X)	Y	Y	Y
M7	16.4	556.4934	556.4936	0.35	C33H66NO5	Ester Hydrolysis(1X)	Y	Y	Y
Parent	19.2	710.6660	710.6657	0.42	C44H88NO5	NA	Y	Y	Y

- Labels for metabolites are consistent with In vivo Rat Met ID – Y – Detected , N Below Detection limit
- M2, M5, M8-M12(*In vivo*) were not detected in *in vitro samples*

Confirmed based on RT and HRMS – Low signal for MS/MS

- Abundances (EIC signal) of all the metabolites are at least similar or less in human hepatocytes compared to rat and NHP hepatocytes

SM-102 and 5 metabolites (M1, M3, M4, M6 and M7) were detected by UPLC-MS/MS in human and NHP hepatocytes that were incubated with 10 µM of SM-102-containing LNPs. 4 metabolites (M1, M4, M6 and M7) were detected in rat hepatocytes incubated with 10 µM SM-102-containing LNPs, but metabolite M3 was not detected in rat hepatocytes. In general, the Extracted Ion Chromatograms for all the metabolite signals were higher in rat and NHP hepatocytes compared to the signals in human hepatocytes. SM-102 metabolites were formed by ester hydrolysis, ester hydrolysis with beta-oxidation chain shortening or N-dealkylation followed by ester hydrolysis. No human-specific metabolites were detected.

Study QV-0236-DA-RE: Metabolite Profile and Identification of SM-102 in Rat Plasma, Urine and Bile Following IV Infusion of SM-102 containing Lipid Nanoparticles to Male Sprague Dawley Rats

Study design

In this non-GLP compliant study, complete SM-102 was quantified and metabolites of SM-102 were identified in plasma, bile and urine samples from Sprague Dawley rats (n=3). The metabolites were analysed by LC-HRMS and SM-102 was quantified by LC/MS/MS.

Urine, bile and plasma samples used for metabolite profiles

Group No.	Matrix	Sex	Time Point (minutes)	Animal No. Pooled
1	Urine	Male	Predose	Spare
1	Urine	Male	0-2 hr	1-3
1	Urine	Male	2-6 hr	1-3
1	Urine	Male	6-24 hr	1-3
1	Bile	Male	Predose	Spare
1	Bile	Male	0-2 hr	1-3
1	Bile	Male	2-6 hr	1-3
1	Bile	Male	6-24 hr	1-3
1	Plasma	Male	Predose	1-3
1	Plasma	Male	2 hr	1-3
1	Plasma	Male	6 hr	1-3
1	Plasma	Male	24 hr	1-3

The metabolite profile of SM-102 was obtained in rat plasma, urine and bile at various time points (2h, 6h and 24h) after intravenous administration of an SM-102-containing LNP to bile duct cannulated rats. To achieve this, m/z derived from the predicted metabolites [M+H] were used to extract the ion chromatograms.

Results

In addition to the unchanged SM-102, 12 metabolites were observed in rats after intravenous administration of SM-102.

Table 9: Identification of Metabolites

ID	Retention Time (min)	Observed m/z	Proposed Formula $[M+H]^+$	Theo. m/z $[M+H]^+$	Error (ppm)	Proposed Biotransformation
M1	3.5	204.1595	C10H22NO3	204.1594	-0.48	N Dealkylation + Ester Hydrolysis
M2	3.9	262.1654	C12H24NO5	262.1649	1.91	Ester Hydrolysis (2X) + β-oxidation(2X)
M3	5.3	290.1962	C14H28NO5	290.1963	0.68	Ester Hydrolysis (2X)+ β-oxidation(2X)
M4	6.1	318.2279	C16H32NO5	318.2275	1.25	Ester Hydrolysis (2X)
M5	15.98	442.4263	C27H56NO3	442.4255	1.8	N-Dealkylation elimination of straight chain ester
M6	15.94	528.4633	C31H62NO5	528. 4623	1.9	Ester Hydrolysis(1X) + β-oxidation (1X)
M7	16.00	556.4942	C33H66NO5	556.4936	1.1	Ester Hydrolysis(1X)
M8	13.92	572.4898	C33H66NO6	572.4885	2.3	Ester Hydrolysis(1X) + Aliphatic Hydroxylation

M9	13.75	544.4590	C31H62NO5	544.4572	3.3	Ester Hydrolysis(1X) + β-oxidation (1X)+ Hydroxylation
M10	13.83	542.4430	C31H60NO5	544.4430	2.7	Ester Hydrolysis(1X) + β-oxidation (1X)+ Hydroxylation + Dehydrogenation
M11	17.85	724.6461	C44H86NO5	724.6450	1.5	Aliphatic Hydroxylation + Dehydrogenation
M12	15.02	833.5324	C41H77N4O11S	833.5304	2.4	β-oxidation + GSH conjugation

Metabolism of SM-102 in rats occurs primarily by hydrolysis of the ester groups followed by β-oxidation of the resulting aliphatic acidic linkers. Additionally, low abundance oxidative metabolites of ester-hydrolysed SM-102 fragments were detected.

Table 10: Summary of Parent SM102 in Plasma, Urine and Bile

Time (h)	Mean Plasma Conc. (ng/mL) [Stdev]	Pooled Bile Conc. (ng/mL)	Pooled Urine Conc. (ng/mL)
2	22500 [3500]	3140	BQL
6	1730 [1240]	2029	BQL
24	41.7 [36.0]	291	BQL

High levels of complete SM-102 were found in plasma at 2 hours (mean 22,500 ng/mL), which declined to 42 ng/mL by 24 hours. Similarly, bile had the highest levels of SM-102 detected at 2 hours (3,140 ng/mL), which declined to 291 ng/mL by 24 hours. In contrast, SM102 could not be detected in urine samples at any time point.

Table 11: Metabolites of SM102 in Plasma

Metabolite ID	m/z	RT (min)	Plasma 2 hour		Plasma 6 hour		Plasma 24 hour	
			EIC Area (Counts)	% Area	EIC Area (Counts)	% Area	EIC Area (Counts)	% Area
M1	204.1594	3.51	4.29E+04	0.00%	2.37E+04	0.01%	0.00E+00	0.00%
M2	262.1649	3.92	ND	ND	ND	ND	ND	ND
M3	290.1962	5.1	4.07E+05	0.02%	1.18E+05	0.07%	5.66E+03	0.06%
M4	318.2275	6.1	2.30E+05	0.01%	1.30E+05	0.08%	3.76E+04	0.40%
M5	442.4255	15.98	8.91E+04	0.00%	6.74E+04	0.04%	3.92E+04	0.41%
M6	528.4623	15.91	4.75E+06	0.23%	2.29E+06	1.39%	6.81E+04	0.71%
M7	556.4936	16	6.49E+06	0.31%	2.21E+06	1.34%	6.62E+04	0.69%
M8	572.4885	13.91	2.10E+04	0.00%	1.52E+04	0.01%	ND	ND
M9	544.4572	13.73	ND	ND	ND	ND	ND	ND
M10	542.4415	13.83	ND	ND	ND	ND	ND	ND
M11	724.645	17.86	8.65E+04	< 0.01 %	8.13E+03	< 0.01%	4.84E+03	0.05%
M12	833.5304	15.02	ND	ND	ND	ND	ND	ND
Parent SM102	710.6657	19.3	2.09E+09	99.42%	1.60E+08	97.05%	9.30E+06	97.67%

ND- Below Detection Limit

Unchanged SM-102 was the dominant chemical species in plasma at all tested time points. Furthermore, eight metabolites appeared in plasma from 2 to 6 h including multiple species of ester-hydrolysed, β-oxidised and hydroxylated metabolites. By 24h post-dose, unchanged SM-102, one mono acidic ester hydrolysis metabolite (M7), diacidic ester hydrolysis metabolite (M4), and their corresponding β-oxidation

products (M3 and M6) were also detected. In addition, N-dealkylation of the straight chain ester linker of SM-102 (M5) was detected. In general, all metabolite concentrations in plasma declined over time.

Table 12: Metabolites of SM-102 in Urine Pools

Metabolite ID	m/z	RT (min)	Urine 0-2 hr		Urine 2-6 hr		Urine 6-24 hr	
			EIC Area (Counts)	% Area	EIC Area (Counts)	% Area	EIC Area (Counts)	% Area
M1	204.1594	3.51	3.89E+07	35.4%	1.90E+08	32.6%	1.23E+08	36.7%
M2	262.1649	3.92	1.52E+07	13.8%	7.80E+07	13.3%	3.64E+07	10.9%
M3	290.1962	5-5.5	1.36E+07	12.4%	2.59E+08	44.4%	1.36E+08	40.6%
M4	318.2275	6.1	4.24E+07	38.5%	5.62E+07	9.6%	3.93E+07	11.7%
M5	442.4255	15.98	ND Detected (Abundance similar to Predose samples)	NA	ND	NA	ND	NA
M6	528.4623	15.91	ND	NA	ND	NA	ND	NA
M7	556.4936	16	ND	ND	ND	ND	ND	ND
M8	572.4885	13.91	ND	ND	ND	ND	ND	ND
M9	544.4572	13.73	ND	ND	ND	ND	ND	ND
M10	542.4415	13.83	ND	ND	ND	ND	ND	ND
M11	724.645	17.86	ND	ND	ND	ND	ND	ND
M12	833.5304	15.02	ND	ND	ND	ND	ND	ND
Parent SM102	710.6657	19	1.04E+04	0.01%	3.64E+04	0.01%	9.25E+04	0.03%

Beside of parent SM-102, four metabolites could be detected in the urine including diacids (M2-M4) and a monoacidic secondary amine (M1). These metabolites are relatively more hydrophilic and have in general a lower molecular weight compared to those found in bile and plasma samples. In general, the metabolite concentration did not change remarkably over time.

Table 13: Metabolites of SM-102 in Bile

Metabolite ID	m/z	RT	Bile 0-2h		Bile 2-6h		Bile 6-24h	
			EIC Area (Counts)	% Area	EIC Area (Counts)	% Area	EIC Area (Counts)	% Area
M1	204.1594	3.51	1.45E+06	0.32%	1.28E+06	0.17%	1.17E+05	0.20%
M2	262.1649	3.92	1.74E+07	3.79%	7.46E+06	1.00%	1.00E+06	1.73%
M3	290.1962	5-5.5	1.89E+08	41.11%	4.60E+08	61.97%	2.37E+07	40.75%
M4	318.2275	6.1	3.05E+07	6.64%	3.84E+07	5.17%	3.84E+06	6.62%
M5	442.4255	15.98	4.58E+06	4.16%	1.01E+07	1.73%	6.13E+05	0.18%
M6	528.4623	15.91	2.38E+07	5.19%	3.06E+07	4.12%	2.25E+06	3.88%
M7	556.4936	16	2.65E+07	5.77%	2.47E+07	3.33%	1.46E+06	2.52%
M8	572.4885	13.91	4.46E+06	0.97%	1.18E+07	1.59%	1.51E+05	0.26%
M9	544.4572	13.73	5.17E+06	1.13%	1.34E+07	1.80%	3.63E+05	0.62%
M10	542.4415	13.83	4.67E+06	1.02%	1.69E+07	2.28%	4.90E+05	0.84%
M11	724.645	17.86	6.17E+06	1.34%	8.69E+06	1.17%	5.11E+04	0.09%
M12	833.5304	15.02	1.25E+06	0.27%	7.86E+06	1.06%	1.01E+06	1.73%
Parent SM102	710.6657	19	1.44E+08	31.46%	1.11E+08	14.97%	2.30E+07	39.69%

Unchanged SM-102 and twelve metabolites were identified in the bile. In addition to those metabolites found in urine and plasma, multiple step β-oxidation products (M2, M3, M6, M9, M10), β-oxidation +GSH conjugation product (M12) and hydroxylated metabolites (M8, M9, M10, and M11) were detected. In general, the metabolite concentration declined slightly over time.

CHMP comment

Both metabolism pharmacokinetic studies were non-GLP. The in vitro study in rat, NHPs and human hepatocytes showed that SM-102 metabolites were formed mainly by ester hydrolysis, ester hydrolysis with β-oxidation chain shortening or N-dealkylation followed by ester hydrolysis. In this study, no human-specific metabolites were detected.

The in vivo study in rats showed that complete SM-102 was the dominant species in plasma. The SM-102 concentration declined significantly over time. SM-102 was also found in the bile, which declined slightly over time. However, only traces of SM-102 were found in urine. In addition, primary metabolites in plasma were formed by ester hydrolysis and/or β-oxidation, which were cleared via both the renal and hepatic routes of elimination. In urine and bile, relative high metabolite concentrations were still detectable after 24 hours.

4.2.4. CHMP's overall conclusions on pharmacokinetics

The MAH conducted two non-GLP pharmacokinetic studies to evaluate the metabolism of SM-102, which is included in the LNP-formulation of Spikevax mRNA-1273 and mRNA-1273.529. SM-102 was already approved in the EU as ingredient of the SARS-CoV-2 vaccine Spikevax. The conducted pharmacokinetic studies showed that no human-specific metabolites were detected. In addition, the detected metabolites declined in plasma over time and were cleared by the renal or hepatic route.

In addition, the MAH submitted an amendment of the biodistribution study 5002121 with mRNA-1647. Mainly, editorial changes and correction of data were made.

Overall, the pharmacokinetic data package is acceptable. Because mRNA-1273.214 is based on the same mRNA vaccine-platform and LNP-formulation than mRNA-1273 and mRNA-1647, no additional pharmacokinetic studies with mRNA-1273.214 are needed.

4.3. Toxicology

4.3.1. Repeat-dose toxicity

Study 2308-245: 8-week Toxicity Study of mRNA-1273 Following Intramuscular Injection in Rats with a 2-Week Recovery Period

Study design

The safety of mRNA-1273 was assessed in a GLP-compliant repeat-dose toxicity study. In this study, 7 weeks old Sprague Dawley (SD) rats were vaccinated intramuscularly with three doses of mRNA-1273 at a dose level of 40 µg/dose with a volume of 0.2 mL/dose. As control, 20 mM Tris buffer with 8% (w/v) sucrose was used. The dose formulations of mRNA-1273 were within specifications, which were confirmed by a GLP-compliant ion exchange high performance liquid chromatography analysis. The MAH chose SD rats as an animal model because they are commonly used for toxicity evaluation of various classes of chemicals and a large historical database of these animal models is available. The animals were divided into two groups: group 1 rats were injected with the control article and group 2 rata were injected with mRNA-1273. mRNA-1273 or the control article were administered on Day 1, Day 29 and Day 57. The doses were administered into the left quadriceps on Day 1, right quadriceps on Day 29 and left biceps femoris muscle on Day 57. The administration route, intramuscular, was the intended human clinical route. In each group, 10 rats/sex were analysed and euthanised one day after the last vaccination (terminal euthanasia, Day 58) and 5 rats/sex were analysed and euthanised after a 2 week-recovery period (recovery euthanasia, Day 72).

Group No.	Test Material	Dose Level (µg/dose)	Dose Volume (mL/dose)	Dose Concentration (µg/mL)	Main Study		Recovery Study	
					No. of Males	No. of Females	No. of Males	No. of Females
1	Control	0	0.2	0	10	10	5	5
2	mRNA-1273	40		200	10	10	5	5

No. = Number.

Results

The animals were checked for mortality and general cage side observation was conducted at least twice daily. After initiation of dosing, one control male was found dead on Day 1 and was replaced with an alternate male. The cause of the death was undetermined because the animal had no previous clinical signs or indications of distress. This dead control animal was not pathologically analysed because the MAH considered this case as incidental. Another male control animal was found dead on Day 60. This animal was analysed pathologically, but the cause of death remained undefined as the animal showed no previous clinical signs or indications of distress. Nevertheless, this death was considered as not vaccine-related.

A general clinical observation was done once daily, where the animals were observed within their cages and a detailed clinical observation was done during the acclimation period, prior to randomisation on Day 1, and weekly thereafter throughout the study on dosing days. For a detailed clinical observation, the animals were removed from the cage. The animals were observed on skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, nervous system effects including tremors, convulsions, reactivity to handling

and unusual behaviour. No mRNA-1273-related clinical findings were observed. Only few transient clinical findings were detected, which were not dose-responsive and were considered incidental and/or commonly seen within this animal strain, age and species.

For local toxicity analysis, the injection sites were observed for erythema and oedema pre-dose, 24 and 72 hours post-dose and weekly on non-dosing weeks. Oedema were observed at injection sites in the left and right hind limb in all mRNA-1273 vaccinated animals at approximately 24 hours post-dose, which recovered within 2-4 days after injection. In addition, two male mRNA-1273 vaccinated animals showed transient erythema at the injection site on the left hind limb on Day 58.

The individual body weights were collected at receipt, prior to randomisation (Day -1), and once weekly during the study. The body weight changes were calculated for all animals between each weighting interval and for the entire dosing and recovery period. The food consumption was also calculated weekly. mRNA-1273-related body weight and body weight gain effects were not observed as well as no vaccine related effects on food consumption. The ophthalmology was analysed pre-treatment and prior to each scheduled necropsy. mRNA-1273-related ophthalmologic changes were not observed during this study. The rectal body temperature was measured prior to each dose, and 6 and 24 hours post each dose. No mRNA-1273-related body temperature changes were observed.

The clinical pathology evaluation was conducted prior to scheduled terminal (Day 58) or recovery necropsy (Day 72). Haematology, coagulation marker, clinical chemistry and different acute phase proteins were analysed in the study animals.

After the last vaccine injection on Day 58, white blood cells were increased in mRNA-1273-vaccinated males (1.47-fold) and females (1.42-fold). In particular, neutrophils (males: 4.46-fold, females: 5.29-fold), eosinophils (males: 2.08-fold, females: 3.82-fold), basophils (males: 2.03-fold, females: 1.48-fold) and large unstained cells (males: 3.14-fold, females: 3.16-fold) were increased in the mRNA-1273-vaccinated animals. In addition, lymphocytes (males: 0.85-fold, females: 0.8-fold), reticulocytes (males: 0.7-fold, females: 0.8-fold) and platelets (males: 0.81-fold) were decreased in mRNA-1273-vaccinated animals. Most of these changes were transient and were not observed after the recovery period. However, eosinophils were still increased (males: 1.9-fold, females: 2.12-fold) at Day 72, but showed a trend to recovery. Also, lymphocyte counts in males were still slightly reduced (0.88-fold) after the recovery period.

One day after the 3rd mRNA-1273 dose, fibrinogen was markedly increased in vaccinated males (2.62-fold) and females (3.49-fold). In addition, prothrombin time and activated partial thromboplastin time were increased in vaccinated females (PT: 1.06-fold, APTT: 1.31-fold). All coagulation changes were transient and resolved within the recovery period.

At the terminal collection on Day 58, albumin (males: 0.96-fold, females: 0.88-fold) and albumin/globulin ratio (males: 0.76-fold, females: 0.75-fold) were slightly transiently decreased in mRNA-1273 vaccinated animals. Furthermore, globulin was slightly increased in mRNA-1273 vaccinated males (1.25-fold) and females (1.18-fold). In addition, aspartate aminotransferase and alanine aminotransferase were increased in mRNA-1273 vaccinated females (AST: 1.37-fold, ALT: 2.26-fold), which were resolved at the end of the recovery period. In contrast, mRNA-1273 vaccinated males showed normal AST and ALT levels at Day 58 but increased levels at the end of the recovery period (AST: 1.3-fold, ALT: 1.88-fold). These findings in males were incidential and the increased AST and ALT levels were found in a single male. Moreover, urea nitrogen and creatinine were slightly increased in mRNA-1273-vaccinated males (UREAN: 1.15-fold, CREAT: 1.19-fold). Urea was still slightly increased in males after the recovery period (1.15-fold), but creatinine levels were normal in vaccinated males after the recovery period.

Different acute phase proteins were analysed in the study animals. At the terminal collection, alpha-2-macroglobulin was markedly increased in mRNA-1273 vaccinated males (78.99-fold) and females (36.71-

fold). After the recovery periode, the mean of alpha-2-macroglobulin was less increased in males (5.71-fold) and females (1.72-fold) due to still markedly increased levels in two males and two females of the mRNA-1273 group. However, two control males and two control females of the recovery group showed also increased alpha-2-macroglobulin levels. In addition, mRNA-1273 vaccinated males (9.84-fold) and females (8.95-fold) showed increased alpha-1-acid glycoprotein levels at Day 58. This finding were transient and were resolved after the recovery period. Moreover, C-reactive protein levels were increased in males at the terminal collection (2.67-fold) and after the recovery period (2.26-fold). However, the CRP levels were very heterogenic in mRNA-1273 and control animals of the main and the recovery study.

The immunogenicity of mRNA-1273 vaccine was analysed in an ELISA with serum samples of control and mRNA-1273 vaccinated rats. The serum IgG antibody analysis was not GLP-compliant. This assay should show the IgG antibody response against the SARS-CoV-2 pre-fusion stabilised Spike Protein (S2P) antigen. The samples were collected on Day 1 pre-dose, and on Day 58 and Day 72 prior to necropsy. After three doses of 40 µg mRNA-1273, vaccinated rats showed a strong IgG antibody titre against the S2P antigen in vaccinated rats on Day 58 prior termination (males: 90,130.4 antibody units/mL, females: 271,604.2 antibody units/mL) and on Day 72 at the end of recovery (males: 270,963.8 antibody units/mL, females: 700,298.6 antibody units/mL). At Day72, the antibody titres were even higher. However, the IgG antibody titres on Day 58 and on Day 72 were significantly higher in females than in males. The antibody titres of the control animals were under the detection limit.

After scheduled euthanasia on Day 58 and Day 72, an autopsy of the study animals was conducted including gross pathology, organ weight analysis and microscopic pathology.

At Day 58 (terminal euthanasia), dark discoloration in the left gastrocnemius muscle adjacent to the injection site were observed in two mRNA-1273 vaccinated males that were considered as vaccine related by the MAH. However, similar finding were also observed in one control female. Other observed gross findings were considered as incidental, commonly observed in this strain and age of rats and/or were observed in control and vaccinated rats of this study. In the recovery euthanasia animals, no mRNA-1273-related gross findings were observed.

In the terminal euthanasia animals, slightly increased liver weights (males: 1.1-fold absolute, females: 1.2-fold absolute) and spleen weights (males: 1.2-fold absolute, females: 1.3-fold absolute) were measured, which affected the absolute weight as well as relative to body and brain weight. In addition, adrenal gland weight was slightly increased in vaccinated males (1.16-fold absolute) and prostate gland was slightly increased in males (1.12-fold absolute). In vaccinated females of the main study group, the heart weight was slightly increased (1.14-fold absolute) and the ovary weight was increased (1.32-fold absolute). The latter finding was due to one female which heart weight was significantly increased. In the recovery animals, increased liver, spleen, adrenal gland, prostate gland and heart weights were not observed in the vaccinated animals. However, the absolute thymus weight in vaccinated males was 1.9-fold increased due to a single male, which showed a significant heavier thymus. In addition, the thyroid/parathyroid weight was slightly decreased in recovery males (0.85-fold absolute) and females (0.83-fold absolute). Moreover, the uterus/cervix weight was slightly decreased in recovery females (0.79-fold, absolute).

In terminal euthanasia animals, minimal increased haematopoietic cells were observed in the bone marrow of vaccinated females, minimal to moderate increased cellularity and minimal neutrophilic infiltration in iliac and inguinal lymph nodes of vaccinated males and females with a higher incidence and more severe of increased cellularity in iliac lymph nodes in males. In addition, minimal to moderate mixed and mononuclear cell inflammation were observed in the fascia of the sciatic nerve of vaccinated males and females. Moderate mixed cell inflammation was observed in the myofiber and fascia of the left gastrocnemius muscle of two vaccinated males and minimal haemorrhage in the left gastrocnemius muscle of one vaccinated male. Mixed cell inflammation at the injection sites were observed in vaccinated

males and females, which were minimal to mild in the 1st injection site and mild to marked in the 3rd injection site. In addition, minimal to moderate haemorrhage was observed in the 3rd injection site of 1 male and 2 females of the vaccinated group. Furthermore, in the heart, minimal to mild mixed cell inflammation were observed in 2 vaccinated males and 1 control male, as well as minimal mononuclear cell infiltration in 3 vaccinated males, 2 control males and 1 vaccinated female. In addition, minimal haemorrhage was observed in the lung of 3 vaccinated males. One female was observed with a minimal necrosis in the liver on Day 58 and minimal to mild mononuclear cell infiltrations were observed in livers of females of mRNA-1273 and control group on Day 58.

After the recovery, one vaccinated female showed still minimal increased cellularity in the iliac lymph node. Four vaccinated females and one control male showed minimal mononuclear cell infiltration and fascia at the sciatic nerve. Four vaccinated females and one vaccinated male showed minimal to mild mononuclear cell infiltration at the third injection site. Overall, the microscopic findings were significantly reduced in the recovery animals, which showed full or partly recovery of the findings observed at terminal euthanasia on Day 58.

CHMP comment

In this GLP-compliant repeat-dose toxicity study, rats were vaccinated intramuscularly 3x with 40 µg mRNA-1273. The intramuscular administration route was the same than the human administration route. Also, the formulation and concentration of the vaccine was comparable to the human vaccine formulation. However, the administered dose level was lower than the maximum human dose. The human adult dose level of mRNA-1273 is 100 µg for the primary vaccine series (2x doses) and 50 µg for the booster vaccination. The MAH justified the lower administered dose levels because 40 µg/dose in 0.2mL is the maximum injectable dose for this animal model. This justification can be accepted because the administered dose in the animal model exceeds already the dose:body weight ratio in rats (0.3 kg) compare to human (50 kg).

The selected animal model, SD rats, is acceptable due to a large historical data set and the number of animals per group is adequate.

The number of administered mRNA-1273 doses (3 doses) to rats can be accepted since this number of doses (2x primary series mRNA-1273 + 1x booster mRNA-1273) is recommended for most healthy adults. However, also a 4th dose of mRNA-1273 is already accepted for human use. It has to be noted, that mRNA-1273 is already approved in several countries, including in the EU, and clinical studies did not indicate significant severe toxicity issues.

Toxicity study with the bivalent Omicron mRNA-1273.214 or the monovalent mRNA-1273.529 vaccine have been not conducted. This is accepted, because both Omicron variant vaccines are based on the same vaccine platform as the parent vaccine mRNA-1273 including the same composition of lipid nanoparticles. Thus, a significant difference in toxicity aspects is not expected.

Acceptable local toxicity was shown. Oedema were observed at the injection sites in all mRNA-1273 vaccinated animals, which recovered within few days. Erythema was only observed in two vaccinated males at the 3rd injection site. These findings indicate an acute inflammation and is commonly observed after vaccination.

The local toxicity signs are in line with laboratory and pathological findings in the mRNA-1273 vaccinated animals. Increased white blood cell types, increased fibrinogen, decreased albumin/globulin ratio and increased acute phase protein concentrations are signs of an inflammatory response. Secondary to the inflammation response, the reticulocyte counts were decreased in the terminal euthanasia animals. In addition, necropsy of the vaccinated animals showed minimal to moderate inflammation at the injection

sites. Most of these findings were transient and not observed in the recovery animals. However, eosinophils were still slightly increased and minimal inflammation at the 3rd injection site of few animals were observed, indicating a subchronic inflammation. Moreover, lower lymphocytes counts were observed in vaccinated animals on Day 58. This might be stress-related.

In addition, some vaccinated animals showed minimal haemorrhage at the injection sites or organs. These findings might be associated with minimal decreased platelets, increased prothrombin time and increased activated partial thromboplastin time. However, these findings were mainly observed in the terminal euthanasia group and not in the recovery group indicating a transient condition.

The acute phase protein alpha-2-macroglobulin was markedly increased in mRNA-1273 vaccinated males and females, which is probably a sign of acute inflammation. After the recovery periode, alpha-2-macroglobulin was less increased in males and females due to still markedly increased levels in two males and two females of the mRNA-1273 group. The increased alpha-2-macroglobulin concentrations in the recovery animals were considered vaccine related by the MAH. However, two control males and two control females of the recovery group showed also increased alpha-2-macroglobulin levels. Thus, the increased alpha-2-macroglobulin concentrations in the recovery animals are rather incidential findings or signs of general stress and probably not mRNA-1273-related.

In vaccinated females, increased AST and ALT concentrations were observed at terminal analysis on Day 58. In contrast, vaccinated males showed normal AST and ALT levels at the terminal analysis but increased concentrations after the recovery period. These findings might be associated with the increased liver weights in the vaccinated animals on Day 58 necropsy. However, severe pathological findings were not observed in the liver of these animals. Only one female had a minimal necrosis on Day 58 necropsy and minimal to mild mononuclear cell infiltrations were observed in livers of females of mRNA-1273 and control group on Day 58 necropsy.

Furthermore, minimal to moderate increased cellularity was observed in iliac and inguinal lymph nodes in both sexes of vaccinated animals on Day 58 necropsy. These findings were resolved after the recovery, except in one female, which still showed minimal increased cellularity in the iliac lymph node. The findings in the lymph nodes might be associated with the increased spleen weight of vaccinated animals on Day 58 necropsy. These results are signs of an activated immune response typically observed after vaccination.

The MAH could also demonstrate strong immunogenicity of 3 doses mRNA-1273 in rats. However, the S2P-specific IgG antibody titres on Day 58 and Day 72 were higher in females than in males indicating a stronger immune response in females.

4.3.2. CHMP's overall conclusions on toxicology

Overall, mRNA-1273 was well tolerated in rats and no severe or unexpected vaccine-related mortalities, clinical signs, severe changes in body weight or pathological findings were observed. All observed findings recovered fully or showed signs of improvement.

Overall, the toxicity data package is acceptable. Because mRNA-1273.214 is based on the same mRNA vaccine-platform and LNP-formulation than mRNA-1273, and it is assumed that toxicity findings are primary based on the LNPs, no additional toxicity studies with mRNA-1273.214 are needed.

In non-clinical pharmacodynamic studies in mice and NHPs, bivalent mRNA-1273.214 and monovalent mRNA-1273.529 Omicron-matched SARS-CoV-2 vaccines show sufficient immunogenicity against several VoC of SARS-CoV-2, including IgG binding antibody and neutralising antibody responses. In addition, one booster dose of mRNA-1273.529 shows protective effects against Wuhan and Omicron BA.1 infection by reducing significantly the viral load in the upper and lower respiratory tract of mice and NHPs.

Furthermore, a repeat-dose toxicity study with mRNA-1273 and two pharmacokinetic studies with SM-102 in rats showed no severe findings.

From a non-clinical perspective, mRNA-1273.214 is considered approvable.

5. Clinical Efficacy aspects

5.1. Methods – analysis of data submitted

Statistical methods

Sample size (Part G)

The target enrolment was to be approximately 375 participants. It was assumed that 20% of participants were to be excluded from the PP Set for Immunogenicity – SARS-CoV-2 negative. With approximately 300 participants in Part G and 300 participants in Part F, Cohort 2 in the PP Set for Immunogenicity and SARS-CoV-2 negative, there is approximately 71% global power to demonstrate the primary immunogenicity objectives with alpha of 0.025 (2-sided) at each time point (Day 29 and Day 91). The assumptions were:

- the true GMR against the variant (B.1.1.529) is 1.5
- the true GMR against ancestral SARS-CoV-2 is 1
- the standard deviation of the log-transformed titre is 1.5

The non-inferiority margin for GMR was set to 0.67.

- the true SRR against B.1.1.529 as a second booster dose is 90% regardless of vaccine, i.e., SSR difference is 0

The non-inferiority margin for SRR difference was set to -10%.

Randomisation and Blinding (masking)

The study was a single arm multi-cohort open-label study; no randomisation or blinding was performed.

Statistical hypotheses and multiplicity control (Part G)

50 µg mRNA-1273.214 as the second booster dose was to be compared to 50 µg mRNA-1273 as the second booster dose (active control arm in Part F, Cohort 2). For the primary objective on immune response, there were 8 hypotheses in total. Four hypotheses were to be tested at Day 29:

A. H_1^1: 50 µg mRNA-1273.214, as a second booster dose, **against the variant B.1.1.529** is non-inferior to the second booster dose of (50 µg) mRNA-1273 against B.1.1.529 based on the **GMT ratio at Day 29** with a non-inferiority margin of 1.5.

B. H_1^2: 50 µg mRNA-1273.214, as a second booster dose, **against the variant B.1.1.529** is non-inferior to the second booster dose of (50 µg) mRNA-1273 against B.1.1.529 based on the difference in **SRR at Day 29** with a non-inferiority margin of 10%.

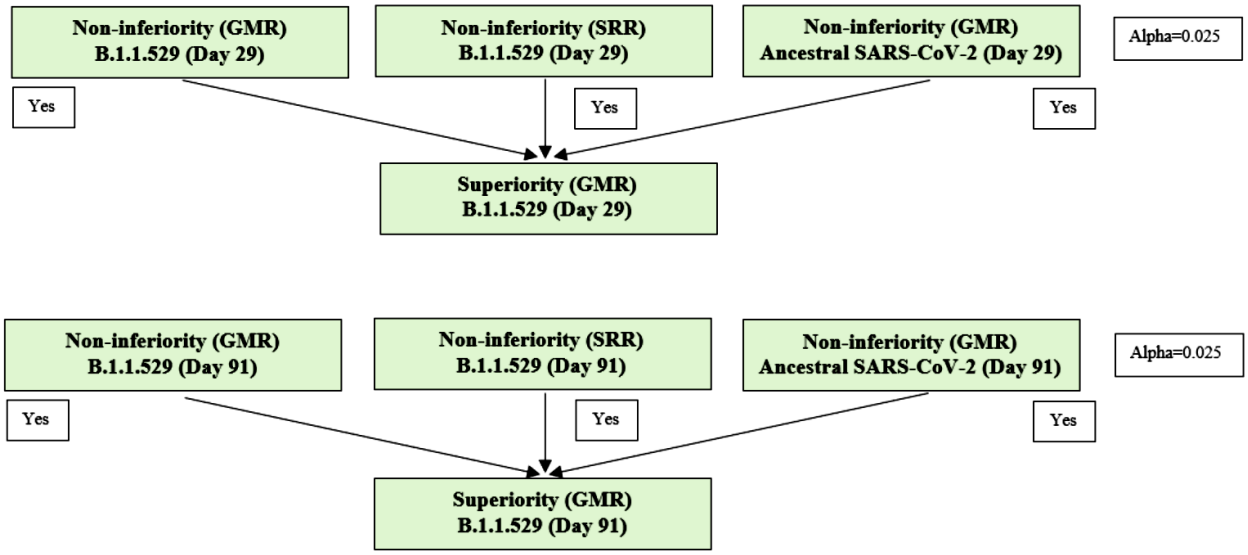
C. H_1^3: 50 µg mRNA-1273.214, as a second booster dose, **against ancestral SARS-CoV-2** is non-inferior to the second booster dose of (50 µg) mRNA-1273 against ancestral SARSCoV-2 based on the **GMT ratio at Day 29** with a non-inferiority margin of 1.5.

D. H_1^4: 50 µg mRNA-1273.214, as a second booster dose, **against the variant B.1.1.529** is **superior** to the second booster dose of (50 µg) mRNA-1273 against B.1.1.529 based on the **GMT ratio at Day 29**.

Analogously, these four hypotheses (labelled as H_1^5 to H_1^8) were to be tested at Day 91 in the same order.

For the primary immunogenicity objective, an alpha of 0.05 (two-sided) was to be allocated to the two time points (Day 29 and Day 91). Day 29 and Day 91 each were to have an alpha of 0.025 (two-sided) for hypotheses testing. The primary immunogenicity objective was to be considered met if non-inferiority against B.1.1.529 based on GMR, SRR difference, and non-inferiority against ancestral SARS-CoV-2 based on GMR were demonstrated either at Day 29 or Day 91.

The following figure depicts the hypotheses testing strategy.



For the key secondary immunogenicity objective, there were 2 hypotheses to be tested (Day 29 and analogously at Day 91 (H_1^{10}) will each have alpha of 0.025 [two-sided] for hypotheses testing):

E. H_1^9: 50 µg mRNA-1273.214, as a second booster dose, **against ancestral SARS-CoV-2** is non-inferior to the booster dose of (50 µg) mRNA-1273 against ancestral SARS-CoV-2 based on the difference in **SRR at Day 29** with a non-inferiority margin of 10%.

Analysis sets:

The analysis sets are described in the table below; the same definitions across Parts A (1, 2), B, C, D, F, and G were to apply when applicable.

Set	Description
Full Analysis Set (FAS)	The FAS consists of all participants who receive investigational product (IP).
Modified Intent-to-Treat (mITT) Set	The mITT Set consists of all participants in the FAS who have no serologic or virologic evidence of prior SARS-CoV-2 infection (both negative RT-PCR test for SARS-CoV-2 and negative serology test based on bAb specific to SARS-CoV-2 nucleocapsid) pre-booster, ie, all FAS participants with baseline SARS-CoV-2 negative status at pre-booster.
Per-Protocol (PP) Set for Immunogenicity	The PP Set for Immunogenicity consists of all participants in the FAS who received the planned dose of study vaccination and no major protocol deviations that impact key or critical data. The PP Set will be used as the primary analysis set for analyses of immunogenicity for immunobridging.
PP Set for Immunogenicity - SARS-CoV-2 negative (PPSI-Neg)	Participants in the PPSI who have no serologic or virologic evidence of SARS-CoV-2 infection at baseline, ie, who are SARS-CoV-2 negative, defined by both negative RT-PCR test for SARS-CoV-2 and negative serology test based on bAb specific to SARS-CoV-2 nucleocapsid PPSI-Neg will be the primary analysis set for analyses of immunogenicity for between booster comparisons.
Solicited Safety Set	The Solicited Safety Set consists of all participants who receive IP and contribute any solicited AR data. The Solicited Safety Set will be used for the analyses of solicited ARs. Participants will be included in the study arm corresponding to the dose of IP that they actually received.
Safety Set	The Safety Set consists of all participants who receive IP. The Safety Set will be used for all analyses of safety except for the solicited ARs. Participants will be included in the study arm corresponding to the dose of IP that they actually received.
Per-Protocol Set for Efficacy	The PP Set for Efficacy consists of all participants in the FAS who receive the planned dose of study vaccination, who are SARS-CoV-2 negative at baseline (ie, have a negative RT-PCR test for SARS-CoV-2 and a negative serology test based on bAb specific to SARS-CoV-2 nucleocapsid at baseline), and have no major protocol deviations that impact key or critical data.

Primary immunogenicity analyses

The 50 µg mRNA-1273.214 booster dose (second booster dose) from Part G was to be assessed with respect to mRNA-1273 booster dose (second booster dose) from Part F, Cohort 2. The analysis methods described in Part F Cohort 1 were to be used for Part G as well. Essentially the following analyses were defined:

An analysis of covariance (ANCOVA) model was to be used to assess the difference in immune response with antibody titers at Day 29 post-booster as dependent variable, and treatment group age group (< 65, ≥ 65 years) and pre-booster antibody titer level as independent variables. The GMT was to be estimated by the geometric least squares mean (GLSM) from the model and its corresponding 95% was to be provided for each group. The GMR (ratio of GMTs) was to be estimated by the ratio of GLSM from the model together with the corresponding 95% CIs. Non-inferiority in GMR was to be considered demonstrated if the lower bound of the 97.5% of the GMR was ≥ 0.67 based on the non-inferiority margin of 1.5. If the lower bound of the CI was > 1 superiority was considered to be demonstrated.

The number and percentage (rate) of participants achieving sero-response at Day 29 was to be summarised with 95% CI calculated using the Clopper-Pearson method for each group. The difference of

SRR between treatment groups was to be calculated with 95% CI based on Miettinen-Nurminen method. Non-inferiority in SRR was to be considered demonstrated if the lower bound of the 97.5% of the SRR difference was > -10% based on the non-inferiority margin of 10%.

Other Immunogenicity Analyses

SARS-CoV-2-specific bAb and nAb were to be assessed at multiple time points. For each of the antibodies of interest, e.g., levels of SARS-CoV-2–specific bAb and SARS-CoV-2-specific nAb, the GMT or level with corresponding 95% CI at each time point, and global mean fold rise (GMFR) of post-baseline/baseline titres or levels with corresponding 95% CI at each post-baseline time point was to be provided for each arm. The 95% CIs were to be calculated based on the t-distribution of the log-transformed values then back-transformed to the original scale for presentation.

Subgroup analyses

Immunogenicity was to be assessed in the following subgroups:

- Age (18 to < 65, and ≥ 65 years)
- Sex (female, male)
- Baseline/pre-booster SARS-CoV-2 status (negative, positive) if there is enough number of pre-booster positives
- Race and ethnicity group (non-Hispanic White, communities of colour)

Safety may be assessed for the same subgroups.

Interim and final analyses

Interim analyses were to be conducted based on safety and immunogenicity data collected through Day 29. The interim analyses were potentially to be conducted either after all participants in G had completed their Day 29 visit assessments, and/or after subsequent time-point visits (Day 91). The final analysis of all endpoints was planned to be performed after all participants have completed all planned study procedures. The final CSR is planned to include full analyses of all safety and immunogenicity through Day 366 (Month 12).

CHMP comment

Sample size

While the assumptions for the individual analyses are understood, the global power cannot be reproduced. It is noted that the CV or the average titre (log(μ)), which would usually be needed for sample size planning was not provided but only the expected GMR and standard deviation (log(σ)). In fact, the log(σ) value seems to be rather low and hence increases the power. Given that this is a planning issue and other assumptions might have been made, this not considered an issue of relevance for this procedure.

Randomisation and Blinding (masking)

The MAH did not plan for any protective measures such as central (blinded) assessment of samples or a firewall to reduce bias and to avoid data driven decisions within an ongoing study. This is not considered state-of-the-art. The lack of randomisation complicates the interpretation of effectiveness data.

Statistical Hypothesis and Multiplicity Control

The provided multiple testing approach equally splits the significance level between time points (Day 29 and Day 91). At each analysis time point 4 hypotheses were tested in a pre-defined order. It is understood that the first three NI-hypothesis (H^1 to H^3 and H^5 to H^7, respectively) were considered as co-primary, i.e., the study would only be considered successful if all these null hypotheses were rejected at

least at one visit. The additional superiority hypothesis for Omicron (B.1.1.529) was tested in a hierarchical fashion if the other hypothesis were rejected. This is methodologically acceptable and endorsed.

It is noted, however, that this testing approach was only introduced late with Amendment 6. Given additional information provided upon request, the modification is considered acceptable and well justified (see Conduct of Study for more details).

Analysis sets

The analysis sets are overall endorsed. They lack clarity and their role is not always clear, though. For example, it is not fully clear what the difference the PPSI-neg and the PPS for efficacy is. For the sake of this procedure this is however not of key relevance.

Primary immunogenicity analyses

The primary analysis model for GMR was based on an ANCOVA model adjusting for age group and pre-booster Ab-titre (of the assay corresponding to the outcome of interest). Titres were log-transformed in the model and results were back-transformed to the original scale, which is endorsed. This can be seen from the sample size considerations and the actual analyses but was not specified in the primary analysis methods (which is not endorsed). Titres between Part F, Cohort 2 (second booster with mRNA-1273) were used as comparator for Part G. This is endorsed.

SRR and SRR differences were computed without adjustment. This is not optimal in this study design but acceptable given the minor role of SRR as compared to GMT/GMR.

The NI-margins (1.5 or analogously 0.67 for GMR, and -10% for SRR) are acceptable. Subsequent superiority testing for Omicron specific Abs is considered of high relevance and hence endorsed as well.

Lack of definition of the primary endpoints / estimands

It is noted that nowhere in the protocol the primary endpoints were well-defined. It is not clear based on which assay and at which cut-off the primary immunogenicity hypotheses were to be tested. Only in Cohort A.1 it was stated that "*Pseudotyped virus neutralising antibody will be used as the basis to assess non-inferiority in immune response. The assays that will be used to assess the immune response to vaccination will be described in the SAP.*" No corresponding definition was made for any other cohort. Whether PsVNA50 or PsVNA80 was planned to be used as primary was defined nowhere. The SAP lacks the promised description of assays to be used.

In the presented Clinical Overview, ID50 nAb titres as measured by the PsVNA with were used. As there are other assays (e.g. the MSD multiplex for bAbs), and other cut-offs (ID80 for PsVNA) this might have been a data driven choice. It seems that after the fact this is not an issue as results seem well aligned. However, at the planning stage this potentially increases flexibility and the chance to win (in any of the endpoints). This hence might inflate the type 1 error. Overall, given the results and endpoints in previous procedures the issue is considered minor at this stage. A better planning and pre-specification is strongly advised for future trials, though.

Subgroup analyses

Subgroups age, sex, baseline SARS-CoV-2 status and race/ethnicity as pre-specified in the protocol are considered acceptable. Results in the key subgroups age and SARS-CoV-2 status were provided. Results for sex as well as race/ethnicity were provided upon request.

Conduct of study (key changes)

- Arm F was first introduced with **Amendment 4** (04 Jan 2022). At that time the mRNA-1273 arm in Cohort 2 was not yet included but two doses (50 and 100µg) of mRNA-1273.529 were to be studied as either first (cohort 1) or second booster (cohort 2; making it 4 arms altogether).
- With **Amendment 5** (10 Feb 2022) the higher dose of 100µg in both cohorts of Arm F was removed, the mRNA-1273 arm in Cohort 2 and Part G were added.
- With **Amendment 6** (17 Mar 2022) the statistical testing approach for Part G was modified by moving the GMT superiority hypothesis from the second to the fourth place in the hierarchy and by adding a non-inferiority hypothesis for SRR as second hypothesis instead. The same hypotheses were newly added for Day 91 and alpha was equally split between Day 29 and Day 91.
- **Amendment 7** (26 Apr 2022) introduced a new Subpart A.2 (second booster dose with mRNA-1273.214 after a booster dose with mRNA-1273.211), which is not of interest to the current procedure.

CHMP comment

Upon request all protocols starting with the first relevant Amendment 4 and all SAP versions were provided allowing a better understanding of the changes in the ongoing study.

It is noted that the modification of the hierarchy in primary hypothesis was made quite late with Amendment 6 (17 Mar 2022) in an open label study and given that the data cut-off (27 Apr 2022) was only shortly after these changes. The MAH further provided the number of subjects enrolled, with Day 29 visit and with available immunogenicity data by the date of PA6 upon request (see Table below). This data shows that the changes are apparently not data driven and hence acceptable.

Table 2: Number of Participants and Immunogenicity Data Available by Protocol Amendment 6 (Mar 17, 2022)

Study Group	Number of participants enrolled	Number of participants with Day 29 visit	Number of Participants with available immunogenicity data
Part F (Cohort 2: mRNA-1273)	379	29	0
Part G	279	0	0

The addition of primary endpoints at Day 91 was justified upon request with accumulating data from a related (first) booster cohort using a Beta-containing bivalent vaccine candidate (mRNA-1273.211) which showed enhanced nAb persistence against various strains (Beta, Omicron, Delta and the ancestral virus). As this would indeed be considered as a clinically relevant benefit *per se* the inclusion of a later time point to the primary endpoints is well understood. Overall, the changes to the hierarchy and regarding multiplicity adjustment are understood and acceptable.

5.2. Results

Vaccine efficacies was inferred from immunological endpoints, mostly titres of neutralising antibodies. No efficacy/effectiveness studies have been conducted in support of the present variation procedure.

Results are provided in a structure as below:

I. mRNA-1273.214 50 µg (P205 Part G) Compared with mRNA-1273 50 µg (P205 Part F), combined with:
II. Addendum A: Immunogenicity Comparison of mRNA-1273.214 50 µg with mRNA-1273 50 µg Against the Omicron Subvariants BA.4, BA.5
III. Exploratory Immunogenicity Analyses
    a. Observed Neutralising Antibody Titers for Beta and Delta Variants on First 50 Participants
    b. Observed Neutralising Antibody Titers for Omicron Subvariants BA.4/5 after the 50 µg mRNA-1273.214 Booster Dose
    c. Summary of Binding Antibody Geometric Mean Titers to Ancestral SARS-CoV-2, Alpha, Beta, Delta, Gamma, and Omicron after the mRNA-1273.214 50 µg and mRNA-1273 50 µg Second Booster Doses Variants
    d. mRNA-1273.211 50 µg (P205 Part A) Compared to mRNA-1273 50 µg (P201 Part B)
    e. Summary of Observed Neutralising Antibody Titers for the Ancestral SARS-CoV-2 and Beta, Delta, and Omicron Variant
    f. Summary of Neutralising Antibody Geometric Titer (ID_{50}) Ratio for Ancestral SARS-CoV-2, Beta, Delta, and Omicron Variants
    g. Summary of Seroresponse Rate Differences to Ancestral SARS-CoV-2, Beta, Delta, and Omicron Variants
IV. SARS-CoV-2 Infection and Symptomatic Infection
    a. SARS-CoV-2 Incidence Rates After the mRNA-1273.214 and mRNA-1273 Booster Vaccines
    b. SARS-CoV-2 Infections After the mRNA-1273.211 Booster Vaccine

Primary and key secondary immunogenicity objectives are addressed by I. "mRNA-1273.214 50 µg (P205 Part G) Compared with mRNA-1273 50 µg (P205 Part F)" and II. "Addendum A: Immunogenicity Comparison of mRNA-1273.214 50 µg with mRNA-1273 50 µg Against the Omicron Subvariants BA.4, BA.5" for results at Day 29. There are no results provided from Day 91, yet.

Results are presented as provided by the MAH:

mRNA-1273.214 50 µg (P205 Part G) Compared with mRNA-1273 50 µg (P205 Part F)

The primary immunogenicity objective of Study mRNA-1273 P205 Part G was to compare the immunogenicity of mRNA-1273.214 50 µg when administered as a second booster dose (in adults who previously received the 2-dose primary mRNA-1273 100 µg vaccine series and a booster dose of 50 µg mRNA-1273) to the immunogenicity of mRNA-1273 50 µg as the second booster (in adults who previously received the 2-dose primary mRNA-1273 100 µg vaccine series and a booster dose of 50 µg mRNA-1273) in Study P205 Part F, Cohort 2.

In the primary analysis set (PPSI-Neg), the observed GMT (95% CI) against Omicron GMTs pre-booster were 298.1 (258.8, 343.5) and increased to 2372.4 (2070.6, 2718.2) at 28 days after the booster dose for mRNA-1273.214 and geometric mean fold rise GMFR (95% CI) for the GMTs at 28 days after the booster compared to pre booster was 8.0 (7.2, 8.8). In comparison, the GMT (95% CIs) was 1473.5 (1270.8, 1708.4) in the mRNA-1273 at 28 days after the booster dose and GMFR (95% CI) was 4.4 (4.0, 5.0). The observed GMT (95% CI) against ancestral pre-booster were 1266.7 (1120.2, 1432.5) and increased to 5977.3 (5321.9, 6713.3) at 28 days after the booster dose for mRNA-1273.214 and GMFR

(95% CI) for the GMTs at 28 days after the booster compared to pre-booster was 4.7 (4.4, 5.1). In comparison, the GMT (95% Cis was 5649.3 (5056.8, 6311.2) in the mRNA-1273 group at 28 days after the booster dose and GMFR (95% CI) was 3.7 (3.4, 4.0).

In the primary analysis set (PPSI - Neg), estimated neutralising antibody GMTs (95% CI) against ancestral SARS-CoV-2 (D614G) adjusted for pre-booster titer and age group were 6422.3 (5990.1, 6885.7) and 5286.6 (4887.1, 5718.9) 28 days after the mRNA-1273.214 and mRNA 1273 booster doses, respectively, and the GMR (97.5% CI) was 1.22 (1.08, 1.37), meeting the pre specified criterion for non-inferiority (lower bound of CI ≥0.67) (Table 10).

The Omicron SRRs (95% CI) were 100% (98.9, 100) and 99.2% (97.2, 99.9), 28 days after the mRNA-1273.214 and mRNA-1273 booster doses, respectively, and the SRR difference (97.5% CI) was 1.5% (-1.1, 4.0) meeting the non-inferiority criterion (lower bound of CI >-10%).

The neutralising antibody GMTs (95% CI) against Omicron were 2479.9 (2264.5, 2715.8) and 1421.2 (1283.0, 1574.4) 28 days following the mRNA 1273.214 and mRNA-1273 booster doses, respectively, and the GMR (97.5% CI) was 1.75 (1.49, 2.04) met the pre-specified superiority criterion (lower bound of CI >1).

Therefore, all primary immunogenicity endpoints were met based on the pre-specified testing sequence. The SRR (95% CI) against the ancestral SARS-CoV-2 (D614G) was 100% (98.9, 100 and 98.6, 100) 28 days after the mRNA 1273.214 and mRNA-1273 booster doses, respectively, with an SRR difference of 0. Therefore, the key secondary immunogenicity objective was also met (Table 14).

Table 14: Ancestral SARS-CoV-2 (D614G) and Omicron Neutralizing Antibody Titers (ID50) mRNA-1273.214 50 µg and mRNA-1273 50 µg Administered as Second Booster Doses – Per-Protocol Immunogenicity – SARS-Cov-2 Negative Set (Primary Analysis)

	Omicron Variant		Ancestral SARS-CoV-2	
Antibody: PsVNA nAb ID_{50} titers	P205 Part G mRNA-1273.214 50 µg (N=334)	P205 Part F mRNA-1273 50 µg (N=260)	P205 Part G mRNA-1273.214 50 µg (N=334)	P205 Part F mRNA-1273 50 µg (N=260)
Pre-booster, n	334	260	334	260
Observed GMT (95% CI)[a]	298.127 (258.753, 343.492)	332.023 (282.047, 390.854)	1266.743 (1120.190, 1432.469)	1520.998 (1352.766, 1710.151)
Day 29, n	334	260	334	260
Observed GMT (95% CI)[a]	2372.424 (2070.634, 2718.200)	1473.462 (1270.849, 1708.379)	5977.257 (5321.897, 6713.320)	5649.331 (5056.848, 6311.231))
Observed GMFR (95% CI)[a]	7.958 (7.181, 8.819)	4.438 (3.971, 4.960)	4.719 (4.358, 5.109)	3.714 (3.420, 4.034)
GLSM [Estimated GMT] (95% CI)[b]	2479.890 (2264.472, 2715.801)	1421.243 (1282.975, 1574.412)	6422.323 (5990.117, 6885.714)	5286.626 (4887.065, 5718.855)
GMR (97.5% CI)[b]	**1.745 (1.493, 2.040)**		**1.215 (1.078, 1.370)**	
Seroreponse, N1	333	258	334	260
Seroresponse rate, n (%)[c]	333 (100)	256 (99.2)	334 (100)	260 (100)
95% CI[d]	(98.9, 100.0)	(97.2, 99.9)	(98.9, 100.0)	(98.6, 100.0)
Difference in seroresponse rates (97.5%)[e]	**1.5 (-1.1, 4.0)**		**0**	

Abbreviations: CI = confidence interval; GLSM = geometric least squares mean; GMFR = geometric mean fold-rise; GMR = geometric mean ratio; GMT = geometric mean titer; ID_{50} = 50% inhibitory dilution; LLOQ = lower limit of quantification; nAb = neutralizing antibodies; PsVNA = pseudotyped virus neutralization assay; SARS-CoV-2 = severe acute respiratory syndrome-2;.

n = number of participants with non-missing data at the corresponding timepoint.

N1 = number of participants with non-missing data at pre-vaccination baseline and the corresponding timepoint.

[a] 95% CI is calculated based on the t-distribution of the log-transformed values or the difference in the log-transformed values for GM value and GM fold-rise, respectively, then back transformed to the original scale for presentation.

[b] Based on ANCOVA modeling; the model includes adjustment for treatment group, pre-booster antibody titers, and age groups.

[c] Seroresponse at a participant level is defined as a change from below the LLOQ to equal or above 4 x LLOQ if the participant's baseline is below the LLOQ, or at least a 4-fold rise if the baseline is equal to or above the LLOQ. For participants without pre-Dose 1 antibody titer information, seroresponse is defined as >= 4*LLOQ for participants with negative SARS-CoV-2 status at their pre-dose 1 of the primary series, and these titers are imputed as <LLOQ at pre-dose 1 of primary series. For participants without SARS-CoV-2 status information at pre-dose 1 of primary series, their pre-booster SARS-CoV-2 status is used to impute their SARS-CoV-2 status at their pre-dose 1 of primary series.

[d] 95% CI is calculated using the Clopper-Pearson method.

[e] 97.5% CI was calculated by stratified Miettinen-Nurminen method adjusted by age group. The SRR difference is a calculated common risk difference using inverse-variance stratum weights and the middle point of Miettinen-Nurminen confidence limits of each one of the stratum risk differences. The stratified Miettinen-Nurminen estimate of the CI cannot be calculated when the seroresponse rate in both groups is 100%, absolute difference is reported.

Source: Tables 14.2.1.1.14.8, Table 14.2.2.1.1.8, and Table 14.2.2.1.2.8.

A sensitivity analysis which excluded participants who had SARS-CoV-2 infection after the booster dose and up to Day 29 was also performed and the results were consistent with the primary analysis. A supportive immunogenicity analysis was also performed taking into account all participants regardless of evidence of prior SARS-CoV-2 infection (PPSI population regardless of SARS CoV-2 infection status at pre-booster baseline) and results were consistent with the primary immunogenicity analysis (Table 52). The GMR and SRR difference results are summarised below.

• **Ancestral SARS-CoV-2 (D614G):** The Day 29 GMR for mRNA1273.214 50 µg booster dose versus the mRNA-1273 50 µg booster dose was 1.237 (97.5% CI: 1.117, 1.369) (lower bound CI >1). The estimated SRR difference 50 µg mRNA-1273.214 booster dose and the mRNA-1273 50 µg booster dose against the ancestral SARS-CoV-2 was 0% (97.5% CI: cannot be calculated) at Day 29.

• **Omicron:** The Day 29 GMR for mRNA-1273.214 50 µg booster dose versus the mRNA-1273 50 µg booster dose was 1.781 (97.5% CI: 1.557, 2.037), (lower bound CI >1). The estimated SRR difference 50 µg mRNA-1273.214 booster dose and the mRNA-1273 50 µg booster dose against the Omicron variant was 1.2% (97.5% CI: -1.3, 3.7) at Day 29 (lower bound of CI > -10%).

Table 15: Ancestral SARS-CoV-2 and Omicron Neutralizing Antibody Titers (ID50) mRNA-1273.214 50 µg and mRNA-1273 50 µg Administered as Second Booster Doses – Per-Protocol Immunogenicity Set (Participants with and without prior SARS-Cov-2 Infection) (Supportive Analysis)

	Omicron Variant		Ancestral SARS-CoV-2	
Antibody: PsVNA nAb ID50 titers	P205 Part G mRNA-1273.214 50 µg (N=428)	P205 Part F mRNA-1273 50 µg (N=367)	P205 Part G mRNA-1273.214 50 µg (N=428)	P205 Part F mRNA-1273 50 µg (N=367)
Pre-booster, n	428	367	428	367
Observed GMT (95% CI)[a]	432.051	511.984	1603.353	1944.781
	(372.466, 501.168)	(433.386, 604.836)	(1420.264, 1810.045)	(1725.353, 2192.116)
Day 29, n	428	367	428	367
Observed GMT (95% CI)[a]	3070.379	1932.785	6619.010	6047.489
	(2685.375, 3510.581)	(1681.186, 2222.037)	(5941.728, 7373.494)	(5465.873, 6690.994)
Observed GMFR (95% CI)[a]	7.107	3.775	4.128	3.110
	(6.484, 7.789)	(3.422, 4.165)	(3.840, 4.438)	(2.877, 3.361)
GLSM [Estimated GMT] (95% CI)[a]	3232.516 (2951.832, 3539.890)	1815.135 (1650.045, 1996.743)	6555.689 (6122.337, 7019.715)	5301.367 (4931.769, 5698.663)
GMR (97.5% CI)[a]	**1.781 (1.557, 2.037)**		**1.237 (1.117, 1.369)**	
Seroreponse, N1	380	342	383	347
Seroresponse rate, n (%)[c]	380 (100)	340 (99.4)	383 (100)	347 (100)
95% CI[d]	(99.0, 100.0)	(97.9, 99.9)	(99.0, 100.0)	(98.9, 100.0)
Difference in seroresponse rates (97.5%)[e]	**1.2 (-1.3, 3.7)**		**0 (-, -)**	

Abbreviations: CI = confidence interval; GLSM = geometric least squares mean; GMFR = geometric mean fold-rise; GMR = geometric mean ratio; GMT = geometric mean titer; ID50 = 50% inhibitory dilution; LLOQ = lower limit of quantification; nAb = neutralizing antibodies; PSVNA = pseudotyped virus neutralization assay; SARS-CoV-2 = severe acute respiratory syndrome-2.

n = number of participants with non-missing data at the corresponding timepoint.

N1 = number of participants with non-missing data at pre-vaccination baseline and the corresponding timepoint.

[a] 95% CI is calculated based on the t-distribution of the log-transformed values or the difference in the log-transformed values for GM value and GM fold-rise, respectively, then back transformed to the original scale for presentation.

[b] Based on ANCOVA modeling; the model includes adjustment for treatment group, baseline SARS-CoV-2 infection status, pre-booster antibody titers, and age groups.

[c] Seroresponse at a participant level is defined as a change from below the LLOQ to equal or above 4 x LLOQ if the participant's baseline is below the LLOQ, or at least a 4-fold rise if the baseline is equal to or above the LLOQ. For participants without pre-Dose 1 antibody titer information, seroresponse is defined as >= 4*LLOQ for participants with negative SARS-CoV-2 status at their pre-dose 1 of the primary series, and these titers are imputed as <LLOQ at pre-dose 1 of primary series. For participants without SARS-CoV-2 status information at pre-dose 1 of primary series, their pre-booster SARS-CoV-2 status is used to impute their SARS-CoV-2 status at their pre-dose 1 of primary series.

[d] 95% CI is calculated using the Clopper-Pearson method.

[e] 97.5% CI was calculated by stratified Miettinen-Nurminen method adjusted by age group and baseline SARS-CoV-2 infection status. The SRR difference is a calculated common risk difference using inverse-variance stratum weights and the middle point of Miettinen-Nurminen confidence limits of each one of the stratum risk differences. The stratified Miettinen-Nurminen estimate of the CI cannot be calculated when the seroresponse rate in both groups is 100%, absolute difference is reported.

Source: Table 14.2.1.1.10.8, Table 14.2.2.1.3.8, and Table 14.2.2.1.4.8.

In addition, a pre-planned subgroup immunogenicity analysis was also performed to assess the consistency of the immunogenicity results in participants with evidence of prior SARS-CoV-2 infection at pre-booster (Table 12). Based on this subgroup analysis, results in participants with SARS-CoV-2 infection prior to the booster vaccination are consistent with the immunogenicity results of the primary analysis in that mRNA-1273.214 elicited higher neutralising antibody responses compared to mRNA-1273. The GMR and SRR difference results are summarised below.

• Ancestral SARS-CoV-2 (D614G): The Day 29 GMR for mRNA1273.214 50 µg booster dose versus the mRNA-1273 50 µg booster dose was 1.272 (97.5% CI: 1.070, 1.512). The estimated SRR difference 50 µg mRNA-1273.214 booster dose and the mRNA-1273 50 µg booster dose against the ancestral SARS CoV-2 was 0% (97.5% CI: cannot be calculated) at Day 29.

• Omicron: The Day 29 GMR for mRNA-1273.214 50 µg booster dose versus the mRNA-1273 50 µg booster dose was 1.898 (97.5% CI: 1.499, 2.403). The estimated SRR difference 50 µg mRNA-1273.214 booster dose and the mRNA-1273 50 µg booster dose against the Omicron variant was 0% (CI: cannot be calculated) at Day 29.

Table 16: Ancestral SARS-CoV-2 and Omicron Neutralizing Antibody Titers (ID50) mRNA-1273.214 50 µg and mRNA-1273 50 µg Administered as Second Booster Doses – Per-Protocol Immunogenicity SARS-Cov-2 Positive Set (Subgroup Analysis)

	Omicron Variant		Ancestral SARS-CoV-2	
Antibody: PsVNA nAb ID_{50} titers	P205 Part G mRNA-1273.214 50 µg (N=94)	P205 Part F mRNA-1273 50 µg (N=98)	P205 Part G mRNA-1273.214 50 µg (N=94)	P205 Part F mRNA-1273 50 µg (N=98)
Pre-booster, n	94	98	94	98
Observed GMT (95% CI)[a]	1614.640 (1149.671, 2267.658)	1558.360 (1088.941, 2230.136)	3703.953 (2793.198, 4911.670)	3637.972 (2742.046, 4826.629)
Day 29, n	94	98	94	98
Observed GMT (95% CI)[a]	7676.226 (5618.245, 10488.050)	3885.596 (2877.774, 5246.367)	9509.727 (7345.948, 12310.856)	7003.503 (5592.574, 8770.390)
Observed GMFR (95% CI)[a]	4.754 (3.954, 5.716)	2.493 (2.058, 3.021)	2.567 (2.245, 2.936)	1.925 (1.649, 2.247)
GLSM [Estimated GMT] (95% CI)[b]	7669.159 (6470.661, 9089.642)	4041.480 (3375.056, 4839.493)	9891.516 (8732.181, 11204.771)	7776.531 (6813.034, 8876.285)
GMR (97.5% CI)[b]	**1.898 (1.499, 2.403)**		**1.272 (1.070, 1.512)**	
Seroreponse, N1	47	76	49	79
Seroresponse rate, n (%)[c]	47 (100)	76 (100)	49 (100)	79 (100)
95% CI[d]	(92.5, 100.0)	(95.3, 100.0)	(92.7, 100.0)	(95.4, 100.0)
Difference in seroresponse rates (97.5%)[e]	**0 (-, -)**		**0 (-, -)**	

Abbreviations: CI = confidence interval; GLSM = geometric least squares mean; GMFR = geometric mean fold-rise; GMR = geometric mean ratio; GMT = geometric mean titer; ID_{50} = 50% inhibitory dilution;LLOQ = lower limit of quantification; nAb = neutralizing antibodies; PSVNA = pseudotyped virus neutralization assay; SARS-CoV-2 = severe acute respiratory syndrome-2;.

n = number of participants with non-missing data at the corresponding timepoint.

N1 = number of participants with non-missing data at pre-vaccination baseline and the corresponding timepoint.

[a] 95% CI is calculated based on the t-distribution of the log-transformed values or the difference in the log-transformed values for GM value and GM fold-rise, respectively, then back transformed to the original scale for presentation.

[b] Based on ANCOVA modeling; the model includes adjustment for treatment group, pre-booster antibody titers, and age groups.

[c] Seroresponse at a participant level is defined as a change from below the LLOQ to equal or above 4 x LLOQ if the participant's baseline is below the LLOQ, or at least a 4-fold rise if the baseline is equal to or above the LLOQ. For participants without pre-Dose 1 antibody titer information, seroresponse is defined as >= 4*LLOQ for participants with negative SARS-CoV-2 status at their pre-dose 1 of the primary series, and these titers are imputed as <LLOQ at pre-dose 1 of primary series. For participants without SARS-CoV-2 status information at pre-dose 1 of primary series, their pre-booster SARS-CoV-2 status is used to impute their SARS-CoV-2 status at their pre-dose 1 of primary series.

[d] 95% CI is calculated using the Clopper-Pearson method.

[e] 97.5% CI was calculated by stratified Miettinen-Nurminen method adjusted by age group. The SRR difference is a calculated common risk difference using inverse-variance stratum weights and the middle point of Miettinen-Nurminen confidence limits of each one of the stratum risk differences. The stratified Miettinen-Nurminen estimate of the CI cannot be calculated when the seroresponse rate in both groups is 100%, absolute difference is reported.

Source: Table 14.2.1.1.12.8, Table 14.2.2.1.9.8, and 14.2.2.1.10.8.

Addendum A: Immunogenicity Comparison of mRNA-1273.214 50 µg with mRNA-1273 50 µg Against the Omicron Subvariants BA.4, BA.5

The purpose of this addendum is to provide updated information on the neutralising antibody response against the Omicron subvariants BA.4, BA.5 elicited by the Omicron BA.1-containing bivalent vaccine mRNA-1273.214 50 µg (study mRNA-P205 part G) and by mRNA-1273 50 µg (mRNA-P205 part F, cohort 2) and to perform a GMR-based comparison of the antibody response between the two groups. The

Overview - .214 M2.5, Section 2.5.5.2.1.1.2; included the neutralising antibody titers only after the mRNA-1273.214 booster dose (and not after the mRNA-1273 booster dose) because the antibody results for the mRNA-1273 were not available at the time the overview was prepared. The Omicron subvariants BA.4, BA.5 have currently become the dominant circulating subvariants in multiple geographies.

Based on the results provided in the addendum:

i. mRNA-1273.214 50 µg elicited superior neutralising antibody responses against the Omicron subvariants BA.4, BA.5 compared to mRNA-1273 50 µg (nominal alpha of 0.05).

ii. The BA.4, BA.5 neutralising antibody response was consistently higher in the mRNA-1273.214 group compared to the mRNA-1273 group in participants with and without prior SARS-CoV-2 infection.

iii. The BA.4, BA.5 neutralising antibody response was consistent across age groups, 18-65 years old and above 65 years.

Overall, the results in the Overview - .214 M2.5 and in the addendum indicate that the bivalent Omicron BA.1-containing vaccine mRNA-1273.214 50 µg elicits superior neutralising antibody responses against Omicron BA.1 and BA.4, BA.5, compared to mRNA-1273 50 µg, regardless of prior SARS-CoV-2 infection and across age groups. Table A presents the summary of the observed neutralising antibody GMTs and GMFRs against the Omicron BA.4, BA.5 for participants who received either the mRNA-1273.214 50 µg booster vaccine (part G) or mRNA-1273 50 µg booster vaccine (part F) as a second booster dose (4th dose). All participants previously received 2 doses of mRNA-1273 100 µg as the primary series vaccine followed by the booster dose of mRNA-1273 50 µg dose as the 1st booster vaccine) in the PPSI, PPSI – Neg, and PPSI – Pos populations. In the primary analysis set (PPSI – Neg, reflecting participants without prior SARS-CoV-2 infection), the observed GMTs (95% CI) against Omicron BA.4, BA5 GMTs (95% CI) pre-booster were 115.6 (98.5, 135.6) and increased to 727.4 (632.8, 836.1) at 28 days after the booster dose for mRNA-1273.214 with a GMFR (95% CI) of 6.3 (5.7, 6.9). The GMTs (95% CIs) were 139.7 (119.5, 163.3) pre-booster and 492.1 (431.1, 561.9) in the mRNA-1273 at 28 days after the booster dose and GMFR (95% CI) was 3.5 (3.2, 3.9). The GMR (95% CI) for the comparison of mRNA-1273.214 50 µg booster dose with the mRNA-1273 50 µg booster dose was 1.69 (1.51, 1.90) with the lower bound of the CI > 1. In the PPSI – Pos population (reflecting participants with prior SARS-CoV-2 infection), the pre-booster GMTs (95% CI) against Omicron BA.4, BA.5 subvariant were 719.5 (531.6, 973.9) which increased to 2337.4 (1825.5, 2992.9) 28 days after the mRNA-1273.214 booster dose with a GMFR (95% CI) of 3.2 (2.8, 3.8). The GMTs (95% CIs) were 609.1 (448.1, 828.1) pre-booster and 1270.8 (987.3, 1635.8) in the mRNA-1273 group at 28 days after the booster dose and GMFR (95% CI) was 2.1 (1.8, 2.4). The GMR (95% CI) for the comparison of mRNA-1273.214 50 µg booster dose with the mRNA-1273 50 µg booster dose was 1.60 (1.34, 1.91) with a lower bound of the CI > 1.

Lastly, in the PPSI population (participants with and without prior SARS-CoV-2 infection), the pre-booster GMT (95% CI) against Omicron BA.4, BA.5 subvariant was 172.7 (147.4, 202.3) which increased to 940.6 (826.3, 1070.6) 28 days after the mRNA-1273.214 booster dose with a GMFR (95% CI) of 5.4 (5.0, 5.9). The GMTs (95% CIs) were 209.3 (179.5, 244.1) pre-booster and 645.4 (570.1, 730.6) in the mRNA-1273 group at 28 days after the booster dose and GMFR (95% CI) was 3.1 (2.8, 3.3). The GMR (95% CI) for the comparison of mRNA-1273.214 50 µg booster dose with the mRNA-1273 50 µg booster dose was 1.68 (1.52, 1.84) with a lower bound of the CI > 1.

Therefore, in all participants, regardless of prior SARS-CoV-2 infection, mRNA-1273.214 50 µg elicited superior neutralising antibody responses against the Omicron subvariants BA.4, BA.5 compared to mRNA 1273 50 µg (nominal alpha of 0.05). The MAH claims that results were also consistent across age groups, 18-65 years old and above 65 years.

Table 17: Summary of Neutralizing Antibody-Geometric Mean Titers for Omicron BA.4, BA.5 Variant - Comparison Between mRNA-1273.214 50 µg and mRNA-1273 50 µg Booster Doses

	Omicron BA.4, BA.5 Variant					
	PPSI		PPSI - Neg		PPSI - Pos	
Antibody: PsVNA nAb ID_{50} titers	P205 Part G mRNA-1273.214 50 µg (N=428)	P205 Part F mRNA-1273 50 µg (N=367)	P205 Part G mRNA-1273.214 50 µg (N=334)	P205 Part F mRNA-1273 50 µg (N=260)	P205 Part G mRNA-1273.214 50 µg (N=94)	P205 Part F mRNA-1273 50 µg (N=98)
Pre-booster, n[a]	428	367	334	260	94	98
Observed GMT (95% CI)[a,b]	172.716	209.307	115.590	139.683	719.542	609.123
	(147.449, 202.313)	(179.475, 244.097)	(98.507, 135.635)	(119.510, 163.260)	(531.639, 973.857)	(448.078, 828.051)
Day 29, n[a]	427	367	333	260	94	98
Observed GMT (95% CI)[a,b]	940.567	645.365	727.427	492.126	2337.435	1270.823
	(826.319, 1070.611)	(570.113, 730.551)	(632.846, 836.143)	(431.053, 561.853)	(1825.510, 2992.918)	(987.277, 1635.804)
Observed GMFR (95% CI)[a,b]	5.444	3.083	6.299	3.523	3.249	2.086
	(5.005, 5.922)	(2.842, 3.345)	(5.739, 6.913)	(3.212, 3.864)	(2.780, 3.795)	(1.795, 2.425)
GLSM [Estimated GMT]	985.376	588.359	776.447	458.282	2246.251	1406.894
(95% CI)[b]	(914.769, 1061.434)	(544.078, 636.244)	(719.488, 837.915)	(420.621, 499.316)	(1975.519, 2554.085)	(1227.880, 1612.006)
GMR (95% CI)[b]	**1.675**		**1.694**		**1.597**	
	(1.521, 1.844)		**(1.511, 1.900)**		**(1.336, 1.909)**	

Abbreviations: CI = confidence interval; GLSM=geometric least squares mean; GMFR = geometric mean fold-rise (post-baseline/baseline titers); GMT = geometric mean titer; ID_{50} = 50% inhibitory dilution; LOD = limit of detection; mRNA = messenger ribonucleic acid; nAb = neutralizing antibody; PPSI = per-protocol set for immunogenicity; PPSI – Neg = per-protocol Set for immunogenicity – SARs-CoV-2 Negative at baseline; PPSI – Pos = per-protocol Set for immunogenicity – SARS-CoV-2 Positive at baseline; PsVNA = pseudotyped virus neutralization assay.

Note: antibody values reported as below the lower limit of detection are replaced by 0.5 x LOD.

N1 = number of participants with non-missing data at pre-vaccination baseline and the corresponding timepoint.

[a] Number of subjects with non-missing data at the timepoint (baseline or post-baseline).

[b] 95% CI is calculated based on the t-distribution of the log-transformed values or the difference in the log-transformed values for GM value and GM fold-rise, respectively, then back transformed to the original scale for presentation.

Source: Table 5, Table 6, Table 7, Table 8, Table 9, and Table 10.

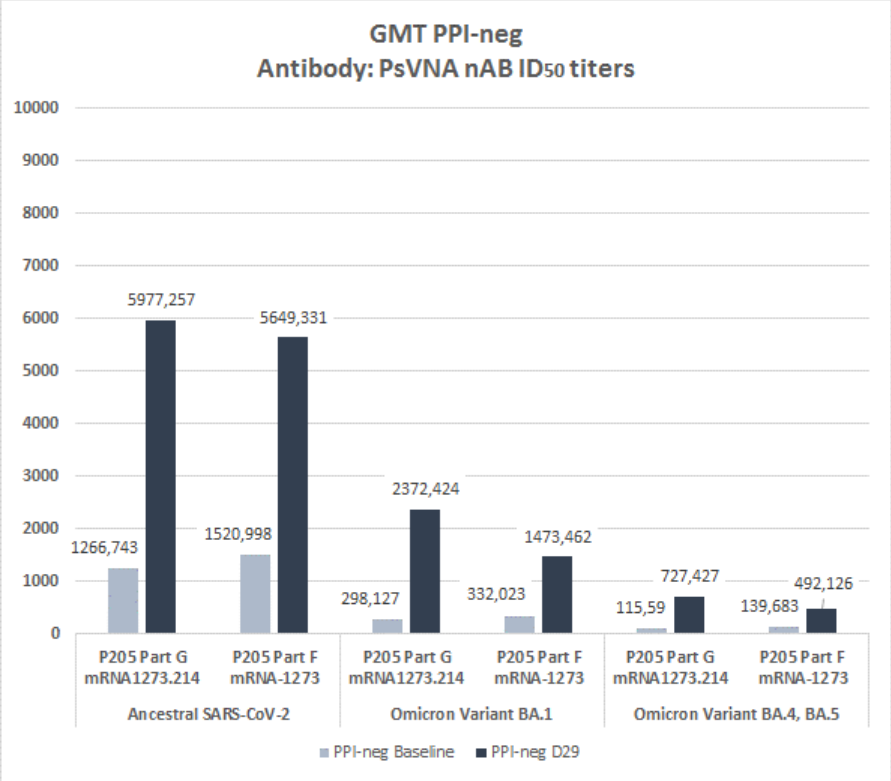
CHMP comment:

Of note, the Clinical Overview 2.5.3 Overview of Clinical Pharmacology, p. 36 reads: *"The Omicron BA.4 and BA.5 sub-lineages were chosen for the development of a research-grade pseudovirus neutralisation assay using a spike-pseudotyped virus designated BA.4/BA.5 given that the BA.4 and BA.5 sub lineages have an identical spike sequence. The assay was performed in a manner consistent with the BA.1 pseudovirus neutralisation assay. However, range, dilutional linearity, precision and limits of quantification have yet to be determined."* Upon request the MAH confirmed that PsVNAs against delta variant and Omicron variants were not validated at the time of testing but were considered to be qualified "fit-for-purpose". As a general rule, providing results from assays that are not validated for the purpose of a MAA is not acceptable. Given the short timelines for generation of results against Omicron variants BA.4, BA.5 together with prior experience with the validated PsVNA against D614G, results from the PsVNA against Omicron variants can be considered acceptable for the time being. The MAH is requested to provide the PsVNA validation reports for Omicron variants BA.1 and BA.4, BA.5 once available. The reports are expected to be signed prior to further analyses for Day 91 interim analysis. **(REC)**

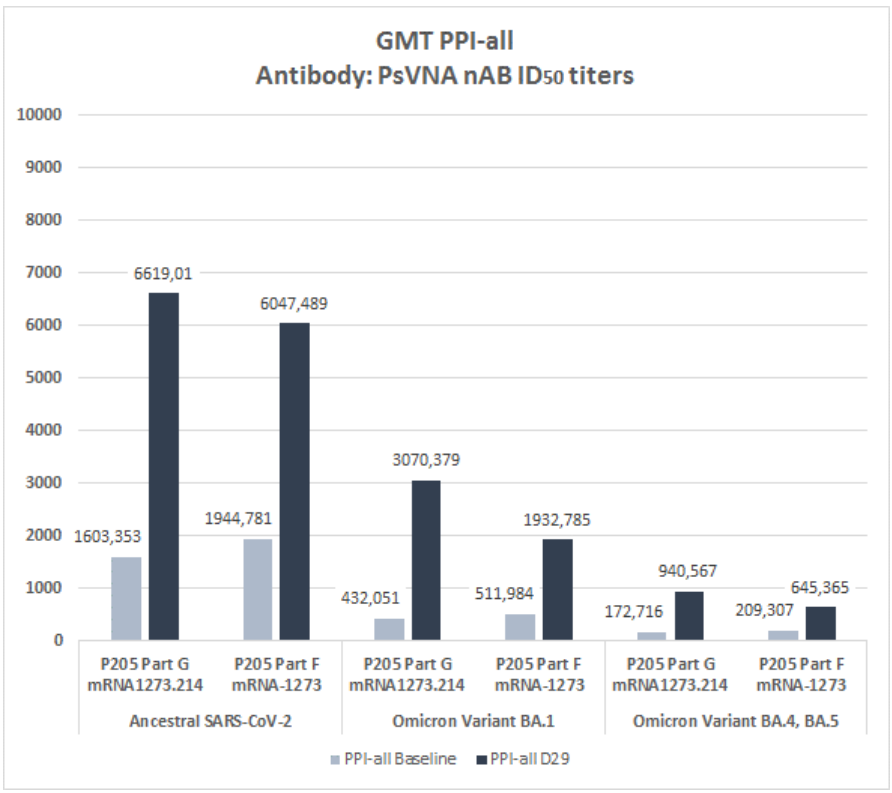
It needs to be stated that key results such as comparison of age groups, race and sex are presented only in raw format in accessory tables. The referenced tables in the Clinical Addendum A could not be found.

The figures below depict the provided results (without CIs). The first three figures show the nAB GMTs in the different Per-Protocol-Immunogenicity-Sets (PPIS) for subjects negative to SARS-CoV-2 N-protein

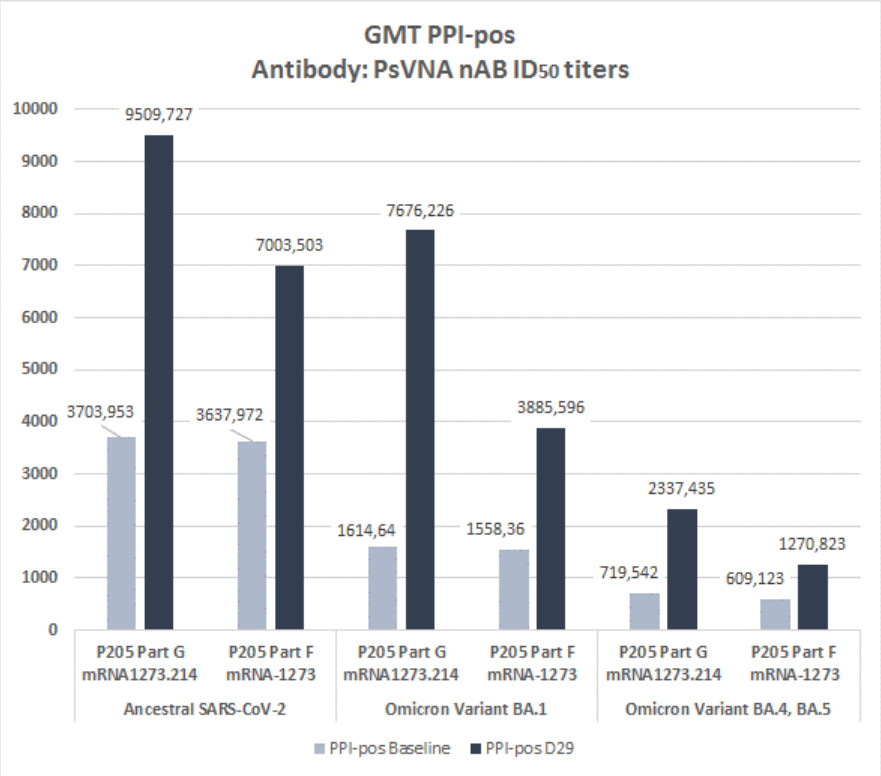
(PPIS-neg), all subjects regardless of prior SARS-CoV-2 infection (PPIS-all) and subjects positive to prior SARS-CoV-2 infection (PPIS-pos).



Data source: Clinical Overview, table 10; Clinical Overview Addendum A, table A



Data source: Clinical Overview, table 11; Clinical Overview Addendum A, table A

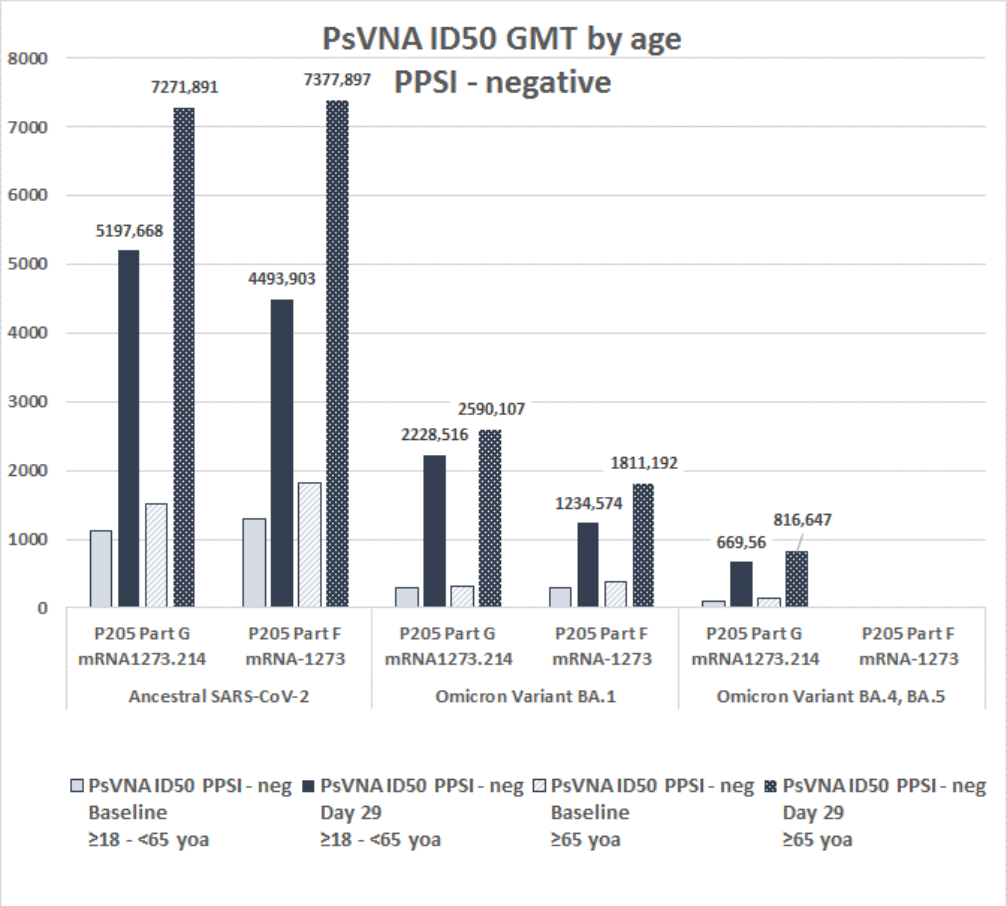


Data source: Clinical Overview, table 12; Clinical Overview Addendum A, table A

In all these population settings booster vaccination with mRNA-1273.214 results in higher nAB GMTs against the ancestral SARS-CoV-2 strain and Omicron variants BA.1 and BA.4, BA.5 compared to booster vaccination with mRNA-1273.

nAB GMTs after booster vaccination with mRNA-1273.214 are lower for BA.1 and again lower against BA.4, BA.5 as compared to nAB GMTs against ancestral SARS-CoV-2. Booster vaccination with mRNA-1273.214 efficiently elicits nAB GMTs against the ancestral SARS-CoV-2, however, it is rather unexpected that mRNA-1273.214 elicits higher nAB GMTs against ancestral SARS-CoV-2 as compared to booster vaccination with mRNA-1273.

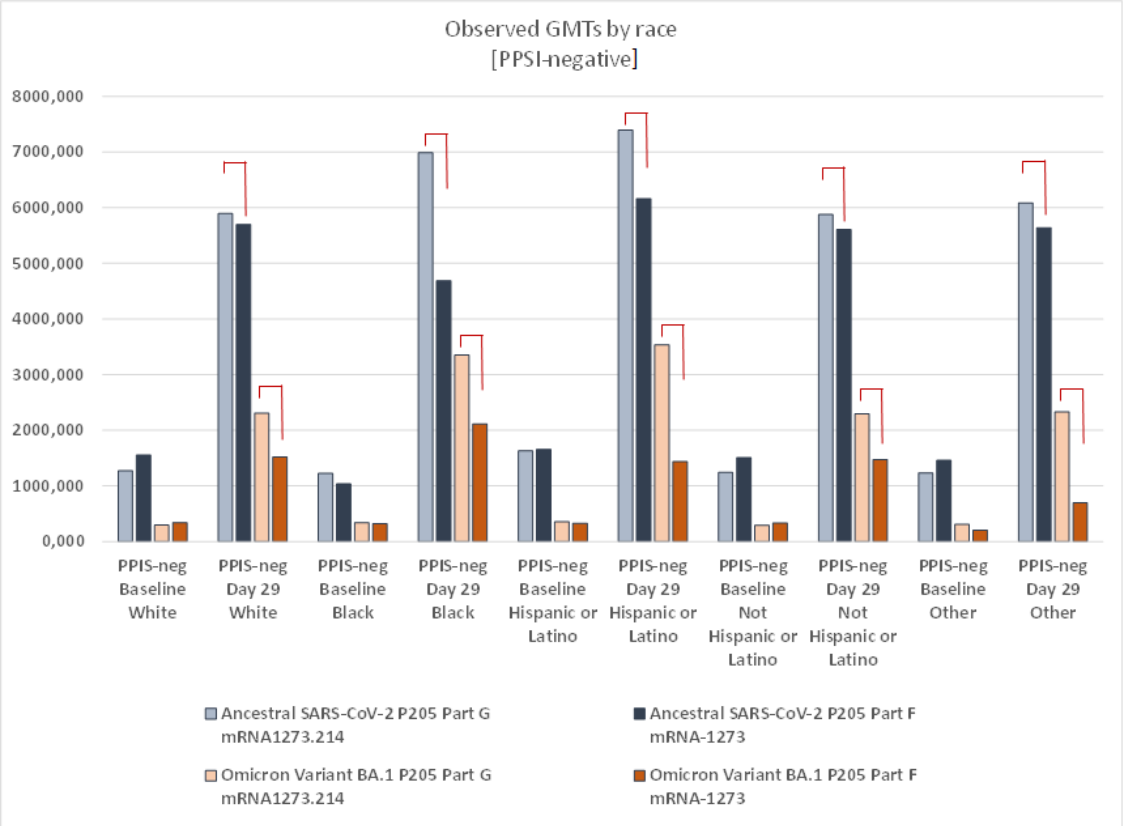
The figure below depicts the results provided for nAB GMTs at Baseline and on Day 29 in the age groups ≥18 to <65 years of age as compared to ≥65 years of age (PPIS-neg). There are no sub-group (age) results available on nAB GMTs against variant Omicron BA.4, BA.5 after vaccination with mRNA-1273.



Data source: mrna-1273-p205-interim-analysis-part-f-part-g-tables; table 14.2.1.1.4.8

There is a tendency of higher nAB GMTs in the elderly population ≥65 years of age. This is rather unexpected and difficult to interpret, but might be at least partly explainable by the fact that also the baseline titers are somewhat higher in elderly. Most importantly, these results confirm that also in the elderly population a very good immune response increase is induced by the 2nd booster dose.

The figure below illustrates the observed GMTs by race in the PPIS-negative.

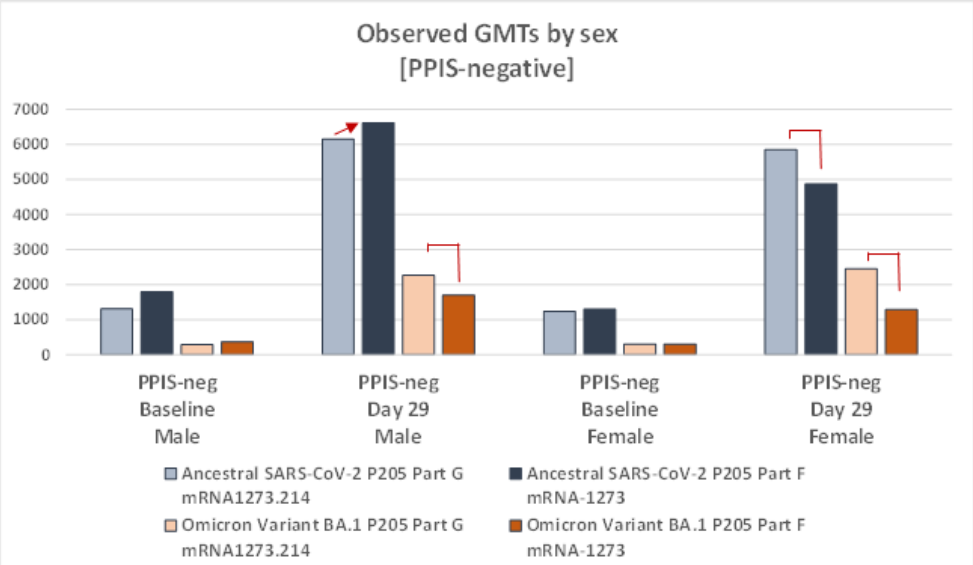


Data source: t140201012008; t140201012108; t140201012208; t140201012308 (additional tables provided by the MAH upon request).

In PPIS-negative the pattern of nAB GMTs is overall comparable throughout all sub-groups. Vaccination with mRNA-1273.214 and mRNA-1273 increases the nAB GMTs against ancestral SARS-CoV-2 and variant Omicron BA.1 in all sub-groups as compared to baseline GMTs. In all sub-groups in the PPIS-negative nAB GMTs after vaccination with mRNA-1273.214 were higher as compared to GMTs after vaccination with mRNA-1273. The PPIS-negative excludes the influence of prior SARS-CoV-2 infection on the nAB GMTs.

The pattern of nAB GMTs in PPIS-all (results not provided in a figure) is basically comparable in White, Hispanic or Latino, and Not Hispanic or Latino groups. In the group of Black subjects and the group of Other the nAB GMTs after vaccination with mRNA-1273 are higher compared to nAB GMTs after vaccination with mRNA-1273.214. The numbers of subjects in these groups were rather small (N=30; N=26 and N=22; N=25, respectively) and prior infection with SARS-CoV-2 would have significant impact on the nAB GMTs in these small groups which may account for these differences. Therefore, results from analysis of PPIS-negative is considered more meaningful for a sub-group analysis.

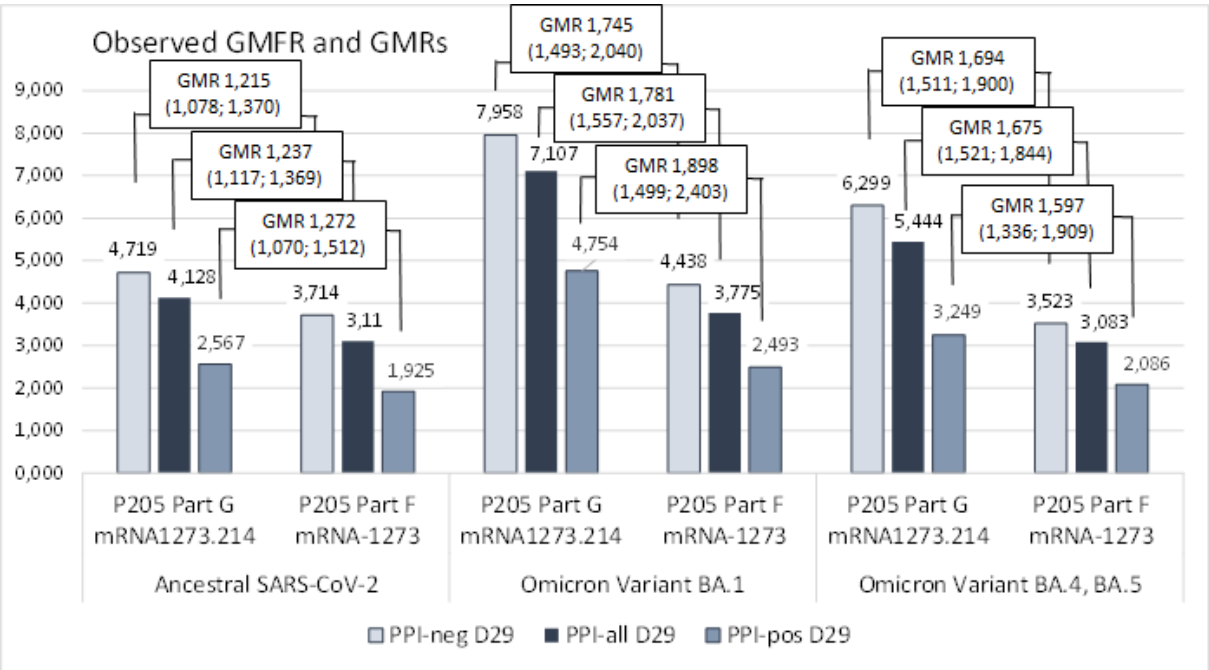
The figure below shows the observed nAB GMTs in PPIS-neg by sex.



Data source: t140201012508, table 14.2.1.1.25.8

Vaccination with mRNA-1273.214 or mRNA-1273 elicits an increase in nAB GMTs in both sub-groups, male and female. In female the pattern of nAB GMTs after vaccination with mRNA-1273.214 or mRNA-1273 is as expected, with higher nAB GMTs after vaccination with mRNA-1273.214. Vaccination with mRNA-1273 results in higher nAB GMTs on Day 29 in male compared to nABs after vaccination with mRNA-1273.214 (red arrow). Results from the PPIS-negative are shown which excludes an influence on prior SARS-CoV-2 infection. The numbers of the sub-groups are not obviously too small (N=145 vs. N=126). The CIs are largely overlapping for nAB GMTs on Day 29 in male (GM Level 6144.836; 95% CI 5081.693; 7430.401 vs. GM Level 6616.689; 95% CI 5618.470; 7792.260), indicating a comparable capacity in eliciting nAB GMTs by any of the vaccines. While this concerns the question on non-inferiority of mRNA-1273.214 against ancestral SARS-CoV-2 in male population this is not considered an issue. Nonetheless, it would have been expected to be discussed by the MAH.

The figure below depicts the observed Geometric Mean Fold Ratios (GMFRs) pre-booster vs. Day 29 post booster vaccination, and Geometric Mean Ratios between vaccinations with mRNA-1273.214 vs. mRNA-1273 for the different PPISs.



Data source: Clinical Overview, table 10; Clinical Overview, table 11; Clinical Overview, table 12; Clinical Overview Addendum A, table A

nAB GMTs and corresponding GMFRs are consistently higher after vaccination with mRNA-1273.214 as compared to vaccination with mRNA-1273 against the ancestral SARS-CoV-2 strain and Omicron variants BA.1 and BA.4, BA.5. Values for GMFRs depend on the baseline values and therefore the nominal reduction of GMFRs from PPIS-neg > PPIS-all > PPIS-pos is expected.

The primary definition of seroresponse is defined as ≥ 4 × LLOQ for those with pre-dose 1 of primary series baseline < LLOQ; ≥ 4-foldrise for those with pre-dose 1 of primary series baseline ≥ LLOQ. For Part F Cohort 2 and Part G, seroresponse rate (SRR) are based on the primary definition when comparing with the mRNA-1273 primary series. Therefore, seroresponse is considered for those subjects who have a GMT of ≥ 4 x LLOQ in this interim analysis. This definition is not appropriate for the characterisation of a booster vaccination and resulting SRR positivity cannot be considered clinically meaningful.

In summary, provided results indicate that the bivalent Omicron BA.1-containing vaccine mRNA-1273.214 50 µg elicits superior neutralising antibody responses against Omicron BA.1 and BA.4, BA.5, compared to mRNA-1273 50 µg, regardless of prior SARS-CoV-2 infection.

Exploratory Immunogenicity Analyses

Observed Neutralising Antibody Titers for Beta and Delta Variants on First 50 Participants

An exploratory immunogenicity analysis to assess neutralisation against the Beta and Delta variants was performed based on the first 50 enrolled participants in each group (mRNA-1273.214 50 µg in Study P205 Part G and mRNA-1273 50 µg in P205 Part F). Only 50 participants were included without selection of representativeness to the whole population in order to expedite the availability of the data. The exploratory summary results are shown for a subset of the group of participants without evidence of prior SARS-CoV-2 infection (n=33 for mRNA-1273.214 50 µg, n=40 for mRNA-1273 50 µg) at pre-booster

(Table 55) and for all 50 participants regardless of SARS-CoV-2 infection status at pre-booster (Table 56). The booster of 50 µg of mRNA-1273.214 elicited a neutralising antibody response against these two variants that are not contained in the vaccine. While the pre-booster titers for Beta and Delta were lower for the mRNA-1273.214 compared to mRNA-1273 in the group of participants without evidence of prior SARS-CoV-2 infection, the MAH claims that the post-booster titers between the two vaccines are comparable (overlapping confidence intervals), and the GMFR was numerically higher in the mRNA-1273.214 group versus the mRNA-1273 arm. The whole cohorts were able to be tested for multiple variants in the MSD assay because the multiplex nature of this assay allowed a higher throughput and more timely data availability.

The MAH explains that these exploratory analysis results (Beta, Delta) are to be interpreted with caution due to the small group of participants and differences in pre-booster titer in Beta and Delta between two treatment groups. This small dataset was intended to provide directional information.

Table 18: Summary of Observed Neutralizing Antibody Geometric Mean Titers for Beta, and Delta (Second Booster Dose: mRNA-1273.214 50 µg, mRNA-1273 50 µg) first 50 Participants – Per-Protocol Immunogenicity SARS-CoV-2 Negative Set (Exploratory Analysis)

	Beta Variant		Delta Variant	
	P205 Part G	P205 Part F	P205 Part G	P205 Part F
Antibody: PsVNA nAb ID_{50} titers	mRNA-1273.214 50 µg (N=33)	mRNA-1273 50 µg (N=40)	mRNA-1273.214 50 µg (N=33)	mRNA-1273 50 µg (N=40)
Pre-booster, n	33	40	33	40
Observed GMT (95% CI)[a]	291.260 (173.425, 489.158)	693.590 (496.037, 969.821)	392.726 (260.456, 592.170)	715.545 (549.066, 932.502)
Day 29, n	33	40	33	40
Observed GMT (95% CI)[a]	1327.475 (907.255, 1942.331)	1390.721 (1002.376, 1929.520)	1512.304 (1048.400, 2181.479)	2128.682 (1600.252, 2831.609)
Observed GMFR (95% CI)[a]	4.558 (3.295, 6.303)	2.005 (1.571, 2.559)	3.851 (2.938, 5.047)	2.975 (2.448, 3.615)

Abbreviations: CI = confidence interval; GMFR = geometric mean fold-rise; GMT = geometric mean titer; ID_{50} = 50% inhibitory dilution; nAb = neutralizing antibody; PsVNA = pseudotyped virus neutralization assay; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2.

n = number of participants with non-missing data at the corresponding timepoint.

[a] 95% CI is calculated based on the t-distribution of the log-transformed values or the difference in the log-transformed values for GM value and GM fold-rise, respectively, then back transformed to the original scale for presentation.

Source: Table 14.2.1.1.18.8.

Table 19: Summary of Observed Neutralizing Antibody Geometric Mean Titers for Beta, and Delta, Variants (Second Booster Dose: mRNA-1273.214 50 µg, mRNA-1273 50 µg) First 50 Participants – Per-Protocol Immunogenicity Set) (Exploratory Analysis)

	Beta Variant		Delta Variant	
	P205 Part G	P205 Part F	P205 Part G	P205 Part F
Antibody: PsVNA nAb ID_{50} titers	mRNA-1273.214 50 µg (N=50)	mRNA-1273 50 µg (N=50)	mRNA-1273.214 50 µg (N=50)	mRNA-1273 50 µg (N=50)
Pre-booster, n	50	50	50	50
Observed GMT (95% CI)[a]	448.679 (283.195, 710.863)	770.536 (551.625, 1076.320)	598.255 (395.688, 904.524)	773.553 (577.559, 1036.057
Day 29, n	50	50	50	50
Observed GMT (95% CI)[a]	1306.211 (879.438, 1940.089)	1511.978 (1064.586, 2147.386)	1600.456 (1134.907, 2256.976)	2191.205 (1643.139, 2922.07
Observed GMFR (95% CI)[a]	2.911 (2.015, 4.205)	1.962 (1.534, 2.509)	2.675 (2.098, 3.411)	2.833 (2.278, 3.523)

Abbreviations: CI = confidence interval; GMFR = geometric mean fold-rise; GMT = geometric mean titer; ID_{50} = 50% inhibito dilution; nAb = neutralizing antibody; PsVNA = pseudotyped virus neutralization assay; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2.

n = number of participants with non-missing data at the corresponding timepoint.

[a] 95% CI is calculated based on the t-distribution of the log-transformed values or the difference in the log-transformed valu for GM value and GM fold-rise, respectively, then back transformed to the original scale for presentation.

Source: Table 14.2.1.1.17.8.

CHMP comment:

In P205 part F and part G binding antibody titers against different variants (ancestral, Alpha, Beta, Delta, Gamma and Omicron) have been analysed for the per-protocol immunogenicity set while neutralising antibodies against variants Beta and Delta have been analysed in samples from <50 subjects. The results are controversial in particular for variant Delta. The MAH was asked if additional analyses are planned on neutralising antibodies against variants Beta and Delta.

MAH response: *The MAH would like to clarify that testing against the Beta and Delta variant in small groups (mRNA-1273.214: n=33; mRNA-1273, n=40) was intended to confirm neutralisation by mRNA-1273.214 against these two variants and these results were not intended for comparisons between groups. The pre-booster titers of these two small groups are different. Specifically, the mRNA-1273 group pre-booster titers are numerically higher (Beta: x2.38 higher [694 vs. 291]; Delta: x1.82 higher [716 vs. 393]), and given that there is a strong correlation between pre-booster and Day 29 titers, it is not suitable to directly compare the Day 29 titers for the mRNA-1273 and the mRNA-1273.214 groups. In sum, no conclusions can be drawn from the small group exploratory neutralising antibody data for Beta and Delta. We are unable to accommodate full cohort testing for mRNA-1273 and mRNA-1273.214 for in the Beta and Delta PsVNA assays due to the relatively low throughput of the assay. Testing for Omicron BA. 4/5 (provided already) and Omicron BA 2.75 (in development) in PsVNA have been prioritised instead. The testing of the full cohorts using the multi-plex MSD assay which includes Beta, Delta as well as Alpha and Gamma is tended to provide assurance about the benefit of mRNA-1273.214 across variants.*

It is understood that due to the nature of the PsVNA there are capacity constraints and analysis of currently circulating VOCs such as Omicron BA.2.75 shall be prioritised. This explanation and ranking is endorsed. It is noted that vaccination with both mRNA.1273 and mRNA-1273.214 does elicit nABs against SARS-CoV-2 variants Beta and Delta. The extent to which nABs against variants Beta and Delta are

elicited remains unknown from this analysis but would roughly be expected to be in the same range due to overlapping CIs.

Summary of Binding Antibody Geometric Mean Titers to Ancestral SARS-CoV-2, Alpha, Beta, Delta, Gamma, and Omicron after the mRNA-1273.214 50 µg and mRNA-1273 50 µg Second Booster Doses Variants

An immunogenicity analysis was performed based on binding antibody data after the mRNA-1273.214 50 µg and mRNA-1273 50 µg second booster doses (Day 29) from the full cohorts to evaluate the binding antibody response against multiple variants, including variants that are not contained in the mRNA 1273.214 booster vaccine (Alpha, Gamma, Beta, and Delta). The results indicate that mRNA-1273.214 elicited a broad binding antibody response against multiple variants. mRNA-1273.214 booster elicited a higher antibody response compared to mRNA-1273 booster.

Table 57 and Figure 32 binding antibody immunogenicity data after the second booster doses (Day 29) of mRNA-1273.214 50 µg and mRNA-1273 50 µg against the ancestral SARS-Cov-2, Alpha, Beta, Delta, Gamma and Omicron for all participants regardless of evidence of prior SARS-CoV-2 infection (per protocol immunogenicity set regardless of participants being SARS-CoV-2 positive or negative at pre booster). The following summarises the GMR results shown in Table 17:

- Ancestral SARS-CoV-2: The Day 29 GMR for mRNA1273.214 50 µg booster dose versus the mRNA1273 50 µg booster dose was 1.138 (95% CI: 1.068, 1.213).
- Alpha: The Day 29 GMR for mRNA1273.214 50 µg booster dose versus the mRNA1273 50 µg booster dose was 1.165 (95% CI: 1.093, 1.241).
- Beta: The Day 29 GMR for mRNA1273.214 50 µg booster dose versus the mRNA1273 50 µg booster dose was 1.142 (95% CI: 1.071, 1.217).
- Delta: The Day 29 GMR for mRNA1273.214 50 µg booster dose versus the mRNA1273 50 µg booster dose was 1.095 (95% CI: 1.031, 1.163).
- Gamma: The Day 29 GMR for mRNA1273.214 50 µg booster dose versus the mRNA1273 50 µg booster dose was 1.160 (95% CI: 1.087, 1.238).
- Omicron: The Day 29 GMR for mRNA1273.214 50 µg booster dose versus the mRNA1273 50 µg booster dose was 1.232 (95% CI: 1.149, 1.321).

mRNA-1273.214 50 µg booster dose met superiority at Day 29, compared to mRNA-1273 50 µg, based on GMR (lower bound 95% CI >1) at nominal alpha of 0.05 for all variants tested. An analysis with binding antibody immunogenicity was also performed with participants with no evidence of prior SARS CoV-2 infection at pre-booster and the results were consistent with the analysis performed with all participants (regardless of prior SARS-CoV-2 infection) (Table 58).

Table 20: Summary of Binding Antibodies Specific to SARS-CoV-2 Spike Protein by MSD Assay Based on Pre-Booster (Second Booster Dose: mRNA-1273.214, mRNA-1273) Per-Protocol Immunogenicity Set (Regardless of SARS-CoV-2 Infection Status at Pre-booster Baseline)

	Omicron Variant		Ancestral SARS-CoV-2		Alpha Variant	
	P205 Part G	P205 Part F	P205 Part G	P205 Part F	P205 Part G	P205 Part F
Antibody: MSD (VAC123) bAb	mRNA-1273.214 50 µg (N=428)	mRNA-1273 50 µg (N=367)	mRNA1273.214 50 µg (N=428)	mRNA-1273 50 µg (N=367)	mRNA-1273.214 50 µg (N=428)	mRNA-1273 50 µg (N=367)
Pre-booster, n	420	360	423	365	423	364
Observed GM level (95% CI)[a]	54956.411 (49719.089, 60745.423)	58640.356 (53338.964, 64468.656)	282635.391 (257514.098, 310207.342)	299028.620 (274089.724, 326236.658)	207586.582 (188619.042, 228461.500)	223595.094 (204442.975, 244541.373)
Day 29, n	384	334	405	353	400	350
GLSM (95% CI)	187825.636 (178053.923, 198133.627)	152402.936 (144175.281, 161100.118)	797187.847 (758938.563, 837364.833)	700606.075 (665933.827, 737083.555)	610201.169 (581170.416, 640682.074)	523953.176 (498178.435, 551061.449)
GMR (95% CI)	**1.232 (1.149, 1.321)**		**1.138 (1.068, 1.213)**		**1.165 (1.093, 1.241)**	

	Beta Variant		Delta Variant		Gamma Variant	
	P205 Part G	P205 Part F	P205 Part G	P205 Part F	P205 Part G	P205 Part F
Antibody: MSD (VAC123) bAb	mRNA-1273.214 50 µg (N=428)	mRNA-1273 50 µg (N=367)	mRNA-1273.214 50 µg (N=428)	mRNA-1273 50 µg (N=367)	mRNA-1273.214 50 µg (N=428)	mRNA-1273 50 µg (N=367)
Pre-booster, n	423	365	423	365	423	365
Observed GM level (95% CI)[a]	127083.359 (115947.897, 139288.254)	135258.647 (123818.071, 147756.313)	181213.122 (165494.880, 198424.239)	193939.053 (177756.919, 211594.331)	138574.157 (126336.110, 151997.690)	150029.588 (137680.495, 163486.318)
Day 29, n	398	350	402	351	399	350
GLSM [Estimated GM level] (95% CI)	382057.528 (363633.803, 401414.703)	334638.433 (317956.725, 352195.353)	516916.009 (493390.181, 541563.595)	472236.684 (450020.728, 495549.364)	419812.526 (399164.797, 441528.306)	361838.508 (343419.182, 381245.757)
GMR (95% CI)	**1.142 (1.071, 1.217)**		**1.095 (1.031, 1.163)**		**1.160 (1.087, 1.238)**	

Abbreviations: ANCOVA = analysis of covariance; CI = confidence interval, GLSM = Geometric least squares mean, GMR = geometric mean ratio, GMT = geometric mean titer; LLOQ = lower limit of quantification; LS = least square; MSD = Meso Scale Discovery; n = number of participants with non-missing data at the corresponding timepoint; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; ULOQ = upper limit of quantification.

Antibody values reported as below the LLOQ are replaced by 0.5 x LLOQ. Values greater than the upper limit of quantification (ULOQ) are replaced by the ULOQ if actual values are not available.

The log-transformed antibody levels are analyzed using an ANCOVA model with the treatment variable as fixed effect, adjusting for age group (<65, ≥65 years) and pre-booster antibody titer level (in log 10 scale), and SARS-CoV-2 status. The treatment variable corresponds to each individual study arm dose. The resulted LS means, difference of LS means, and confidence intervals are back transformed to the original scale for presentation.

[a] 95% CI is calculated based on the t-distribution of the log-transformed values or the difference in the log-transformed values for GM value and GM fold-rise, respectively, then back transformed to the original scale for presentation.

Source: Table 14.2.1.2.10.8 and Table 14.2.3.1.3.8.

Table 21: Summary of Binding Antibodies Specific to SARS-CoV-2 Spike Protein by MSD Assay Based on Pre-Booster Baseline (Second Booster Dose: mRNA-1273.214, mRNA-1273) Per-Protocol Immunogenicity SARS-CoV-2

	Omicron Variant		Ancestral SARS-CoV-2		Alpha Variant	
	P205 Part G	P205 Part F	P205 Part G	P205 Part F	P205 Part G	P205 Part F
Antibody: MSD (VAC123) bAb	mRNA-1273.214 50 µg (N=334)	mRNA-1273 50 µg (N=260)	mRNA1273.214 50 µg (N=334)	mRNA-1273 50 µg (N=260)	mRNA-1273.214 50 µg (N=334)	mRNA-1273 50 µg (N=260)
Pre-booster, n	331	256	332	259	332	259
Observed GM level (95% CI)[a]	50655.927 (45583.372, 56292.960)	55961.844 (50768.815, 61686.057)	253731.553 (230052.946, 279847.322)	280186.866 (255545.533, 307204.275)	183544.862 (166028.649, 202909.056)	207233.058 (188611.265, 227693.401)
Day 29, n	292	239	313	251	308	250
GLSM (95% CI)	209858.615 (198169.736, 222236.952)	168686.787 (158454.185, 179580.187)	884978.378 (839674.114, 932727.016)	761984.392 (718960.954, 807582.401)	668710.370 (634902.081, 704318.937)	562715.291 (531543.650, 595714.950)
GMR (95% CI)	1.244 (1.143, 1.354)		1.161 (1.074, 1.256)		1.188 (1.100, 1.283)	

	Beta Variant		Delta Variant		Gamma Variant	
	P205 Part G	P205 Part F	P205 Part G	P205 Part F	P205 Part G	P205 Part F
Antibody: MSD (VAC123) bAb	mRNA-1273.214 50 µg (N=334)	mRNA-1273 50 µg (N=260)	mRNA-1273.214 50 µg (N=334)	mRNA-1273 50 µg (N=260)	mRNA-1273.214 50 µg (N=334)	mRNA-1273 50 µg (N=260)
Pre-booster, n	332	259	332	259	332	259
Observed GM level (95% CI)[a]	113986.467 (103400.922, 125655.694)	125811.778 (114408.266, 138351.921)	160021.236 (145267.097, 176273.887)	176050.555 (160244.776, 193415.341)	121087.417 (109783.228, 133555.578)	136347.211 (124198.876, 149683.817)
Day 29, n	306	252	310	252	307	252
GLSM [Estimated GM level] (95% CI)	408454.565 (387526.328, 430513.025)	354483.033 (334714.357, 375419.275)	546566.964 (520177.789, 574294.891)	494689.007 (468536.105, 522301.721)	443654.796 (420473.235, 468114.405)	374273.107 (352956.042, 396877.634)
GMR (95% CI)	1.152 (1.066, 1.245)		1.105 (1.027, 1.189)		1.185 (1.095, 1.283)	

Abbreviations: ANCOVA = analysis of covariance; CI = confidence interval, GLSM = Geometric least squares mean, GMR = geometric mean ratio, GMT = geometric mean titer; LLOQ = lower limit of quantification; LS = least square; MSD = Meso Scale Discovery; n = number of participants with non-missing data at the corresponding timepoint; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; ULOQ = upper limit of quantification.

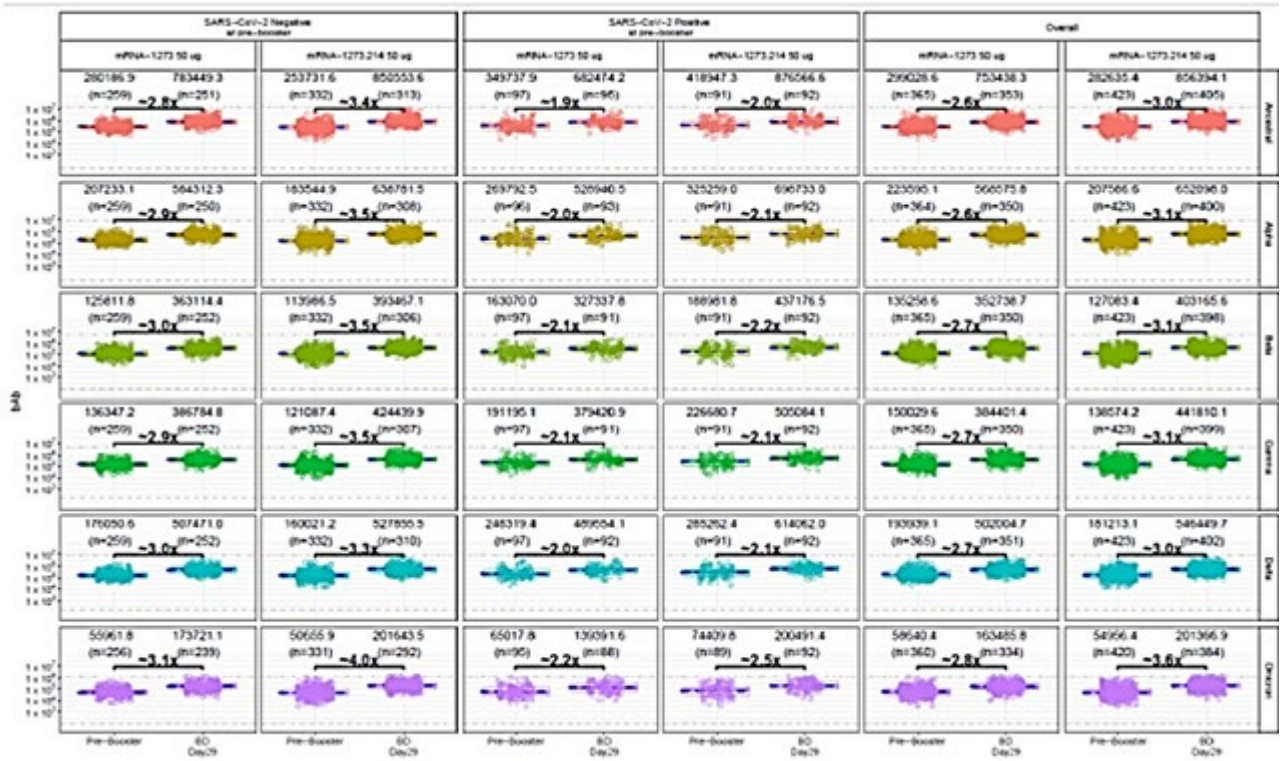
Antibody values reported as below the LLOQ are replaced by 0.5 x LLOQ. Values greater than the upper limit of quantification (ULOQ) are replaced by the ULOQ if actual values are not available.

The log-transformed antibody levels are analyzed using an ANCOVA model with the treatment variable as fixed effect, adjusting for age group (<65, ≥65 years) and pre-booster antibody titer level (in log 10 scale). The treatment variable corresponds to each individual study arm dose. The resulted LS means, difference of LS means, and confidence intervals are back transformed to the original scale for presentation.

[a] 95% CI is calculated based on the t-distribution of the log-transformed values or the difference in the log-transformed values for GM value and GM fold-rise, respectively, then back transformed to the original scale for presentation.

Source: Table 14.2.1.2.13.8 and Table 14.2.3.1.1.8.

Figure 26: Summary of Binding Antibodies Specific to SARS-CoV-2 Spike Protein by MSD Assay Based on Pre-Booster Baseline (Second Booster Dose: mRNA-1273.214, mRNA-1273) Per-Protocol Immunogenicity Populations (SARS-CoV-2 Negative, Positive, and Overall)



Source: Table 14.2.1.2.10.8 and Table 14.2.1.2.12.8.

mRNA-1273.211 50 µg (P205 Part A) Compared to mRNA-1273 50 µg (P201 Part B)

While the MAH is seeking authorisation of the bivalent Omicron-containing booster vaccine mRNA 1273.214 50 µg, the immunogenicity results of the Beta-containing bivalent booster vaccine mRNA 1273.211 50 µg are summarised in this section as supportive information. The mRNA-1273.211 results also show that a bivalent booster vaccine that retains the ancestral SARS-CoV-2 spike protein mRNA sequence and also includes the spike protein of a divergent variant can induce an enhanced and durable neutralising antibody response, compared to the booster vaccine mRNA-1273.

As previously described in Section 2.5.1.5, of the Clinical Overview the primary immunogenicity objective of Study mRNA-1273 P205 Part A was to compare the immunogenicity results of mRNA-1273.211 as the first booster dose (administered to adults who previously received the 2-dose primary mRNA-1273 vaccine series) to the immunogenicity induced after a 2-dose primary series of mRNA-1273 in Study P301; these results are presented in Module 5.3.5.1 Study P205 Part A Interim Analysis Report.

In addition, a pre-specified immunogenicity objective was to compare the antibody response of the 50 µg mRNA-1273.211 booster vaccine (P205 Part A) to the antibody response of the 50 µg mRNA-1273 booster vaccine (external comparator group from study mRNA-1273-P201 Part B). The following sections summarise this booster-to-booster comparison.

Based on the results from P205 Part A described in this section, the neutralising antibody response elicited by the mRNA-1273.211 50 µg booster vaccine against the ancestral SARS-CoV-2 and the Beta,

Omicron and Delta variants was superior compared to that after the mRNA-1273 50 µg booster dose, 28 days as well as 181 days after administration of the booster dose (nominal alpha of 0.05).

CHMP comment:

Given the overall lower nAB GMTs against variants Omicron BA.1 and Omicron BA.4, BA.5 as compared to ancestral SARS-CoV-2 nAB GMTs, investigating the durability of the immune response is of particular value. In this respect, results from P205 Part A (bivalent original / Beta variant vaccine mRNA-1273.211) can be considered supportive. The upcoming interim analysis on Day 91 for study P205 Parts F and G is considered essential to address durability of the response after vaccination with mRNA-1273.214 and shall be submitted immediately upon availability.

Table 22: Summary of Observed Neutralizing Antibody Geometric Mean Titers for Ancestral SARS-CoV-2 and Beta, Delta, Omicron Variants (Per-Protocol Set for Immunogenicity – SARS-CoV-2 Negative Set) – Comparison Between mRNA-1273.211 50 µg and mRNA-1273 50 µg Booster Doses

	Beta Variant		Ancestral SARS-CoV-2		Delta Variant		Omicron Variant	
	P205	P201	P205	P201	P205	P201	P205	P201
Antibody: PsVNA nAb ID_{50} titers	mRNA-1273.211 50 µg (N=295)	mRNA-1273 50 µg (N=149)	mRNA-1273.211 50 µg (N=295)	mRNA-1273 50 µg (N=149)	mRNA-1273.211 50 µg (N=295)	mRNA-1273 50 µg (N=149)	mRNA-1273.211 50 µg (N=295)	mRNA-1273 50 µg (N=149)
Pre-booster, n	295	149	295	149	294	149	294	147
Observed GMT (95% CI)	25.26 (22.67, 28.14)	37.54 (31.41, 44.86)	100.48 (89.40, 112.94)	150.22 (125.73, 179.49)	53.22 (47.45, 56.69)	50.65 (42.78, 59.96)	20.85 (18.78, 23.15)	20.87 (17.92, 24.30)
Day 29, n	295	149	295	149	294	149	294	147
Observed GMT (95% CI)	1032.13 (911.87, 1168.25)	920.53 (797.31, 1062.79)	2167.43 (1947.40, 2412.34)	1951.73 (1729.61, 2202.39)	1489.50 (1318.34, 1682.88)	827.77 (738.48, 927.86)	1382.84 (1186.27, 1611.98)	633.55 (531.68, 754.93)
Observed GMFR (95% CI)	40.87 (35.76, 46.70)	24.52 (20.51, 29.33)	21.57 (18.96, 24.54)	12.99 (11.04, 15.29)	27.95 (24.40, 32.02)	16.34 (13.70, 19.50)	66.29 (57.64, 76.24)	30.36 (25.04, 36.81)
Day 181, n	283	147	283	147	282	147	283	146
Observed GMT (95% CI)	327.17 (284.48, 376.27)	138.74 (115.57, 166.54)	996.03 (871.61, 1138.21)	673.27 (578.17, 784.02)	499.12 (438.28, 568.40)	392.49 (333.14, 462.41)	311.94 (264.10, 368.44)	132.82 (105.92, 166.56)
Observed GMFR (95% CI)	12.68 (11.05, 14.54)	3.72 (3.09, 4.49)	9.64 (8.34, 11.13)	4.55 (3.82, 5.42)	9.11 (7.92, 10.47)	7.81 (6.44, 9.48)	14.74 (12.74, 17.05)	6.33 (5.02, 7.98)

Abbreviations: CI = confidence interval; GMFR = geometric mean fold-rise; GMT = geometric mean titer; ID_{50} = 50% inhibitory dilution; LLOQ = lower limit of quantification; nAb = neutralizing antibody; PsVNA = pseudotyped virus neutralization assay; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; ULOQ = upper level of quantification.

N = number of participants with non-missing data at the corresponding timepoint. Antibody values reported as below the LLOQ are replaced by 0.5 x LLOQ. Values greater than the ULOQ are replaced by ULOQ if actual values are not available. Analysis is based on actual treatment group. The P201 Per-Protocol Set for Immunogenicity SARS-CoV-2 Negative Set includes participants with negative SARS-CoV-2 status and participants with missing SARS-CoV-2 status (n = 11).

Sources: Module 5.3.5.1 Study P205 Part A Interim Analysis Report Table 4.1, Table 4.2, Table 4.3, Table 4.4.

Summary of Neutralising Antibody Geometric Titer (ID50) Ratio for Ancestral SARS-CoV-2, Beta, Delta, and Omicron Variants

The booster-to-booster comparisons (50 µg mRNA-1273.211 booster vaccine from P205 Part A versus 50 µg mRNA-1273 booster vaccine from P201 Part B) against the ancestral SARS-CoV-2, and the Beta variant (the two mRNA-spike sequences contained in the mRNA-1273.211 vaccine) are based on neutralising antibody titers (ID50) using the PPSI – negative analysis set are presented in Table 23. In addition to the ancestral SARS-CoV-2 and the Beta variant antibody titers, the immunogenicity comparisons were made for the Delta and Omicron antibody titers. The geometric mean titer results are summarised below:

- Beta: The Day 29 GMR for mRNA-1273.211 50 µg booster dose versus mRNA-1273 50 µg booster dose was 1.33 (95% CI: 1.09, 1.61) and the Day 181 GMR was 2.74 (95% CI: 2.22, 3.40). The

mRNA-1273.211 50 µg boTable 23:oster dose met superiority at Day 29 and Day 181 based on GMR (lower bound of 95% CI > 1) at nominal alpha of 0.05.

- Ancestral SARS-CoV-2: The Day 29 GMR for mRNA-1273.211 50 µg booster dose versus the mRNA-1273 50 µg booster dose was 1.28 (95% CI: 1.08, 1.51) and the Day 181 GMR was 1.68 (95% CI: 1.38, 2.06). The mRNA-1273.211 50 µg booster dose met superiority at Day 29 and Day 181 based on GMR (lower bound 95% CI >1) at nominal alpha of 0.05.
- Delta: The Day 29 GMR for mRNA-1273.211 50 µg booster dose versus mRNA-1273 50 µg booster dose was 1.77 (95% CI: 1.48, 2.12) and the Day 181 GMR was 1.23 (95% CI: 1.01, 1.50). The mRNA 1273.211 50 µg booster dose met superiority at Day 29 and Day 181 based on GMR (lower bound of 95% CI > 1) at nominal alpha of 0.05.
- Omicron: The Day 29 GMR for mRNA-1273.211 50 µg booster dose versus mRNA-1273 50 µg booster dose was 2.17 (95% CI: 1.73, 2.72) and the Day 181 GMR was 2.32 (95% CI: 1.80, 2.98). The mRNA 1273.211 50 µg booster dose met superiority at Day 29 and Day 181 based on GMR (lower bound of 95% CI > 1) at nominal alpha of 0.05.

Table 23: Ancestral SARS-CoV-2, Beta, Delta, Omicron Neutralizing Antibody Titers (ID50) – (Per-Protocol Set for Immunogenicity – SARS-CoV-2 Negative) – Comparison of mRNA-1273.211 50 µg with mRNA-1273 50 µg Booster Doses

	Beta Variant		Ancestral SARS-CoV-2		Delta Variant		Omicron Variant	
	P205 Part A mRNA-1273.211 50 µg	P201 Booster mRNA-1273 50 µg	P205 Part A mRNA-1273.211 50 µg	P201 Booster mRNA-1273 50 µg	P205 Part A mRNA-1273.211 50 µg	P201 Booster mRNA-1273 50 µg	P205 Part A mRNA-1273.211 50 µg	P201 Booster mRNA-1273 50 µg
Antibody: PsVNA nAb ID_{50} titers	(N=295)	(N=149)	(N=295)	(N=149)	(N=295)	(N=149)	(N=295)	(N=149)
Pre-booster, n	295	149	295	149	294	149	294	147
Observed GMT (95% CI)	25.26 (22.67, 28.14)	37.54 (31.41, 44.86)	100.48 (89.40, 112.94)	150.22 (125.73, 179.49)	53.22 (47.45, 59.69)	50.65 (42.78, 59.96)	20.85 (18.78, 23.15)	20.87 (17.92, 24.30)
Day 29, n	295	149	295	149	294	149	294	147
GMT (95% CI)[a]	1095.25 (981.06, 1222.74)	825.63 (706.61, 964.69)	2277.98 (2073.96, 2502.07)	1782.71 (1561.27, 2035.55)	1482.99 (1335.88, 1646.30)	838.76 (724.36, 971.22)	1379.30 (1209.88, 1572.44)	636.74 (529.09, 766.29)
GMR (95% CI)[a]	1.33 (1.09, 1.61)	-	1.28 (1.08, 1.51)	-	1.77 (1.48, 2.12)	-	2.17 (1.73, 2.72)	-
Day 181, n	283	147	283	147	282	147	283	146
GMT (95% CI)[a]	343.50 (303.72, 388.50)	125.22 (105.37, 148.80)	1039.89 (926.35, 1167.34)	617.24 (525.10, 725.54)	492.87 (438.30, 554.22)	400.78 (340.43, 471.83)	308.00 (265.78, 356.93)	133.04 (108.21, 163.56)
GMR (95% CI)[a]	2.74 (2.22, 3.40)	-	1.68 (1.38, 2.06)	-	1.23 (1.01, 1.50)	-	2.32 (1.80, 2.98)	-

Abbreviations: CI = confidence interval; GMR = geometric mean ratio; GMT = geometric mean titer; ID_{50} = 50% inhibitory dilution; LLOQ = lower limit of quantification; LS = least square; MMRM = mixed model repeated measure; nAb = neutralizing antibody; PsVNA = pseudotyped virus neutralization assay; SARS-CoV-2 = severe acute respiratory syndrome-2; ULOQ = upper limit of quantification.

N = number of participants with non-missing data at the corresponding timepoint. Antibody values reported as below the LLOQ are replaced by 0.5 x LLOQ. Values greater than the ULOQ are replaced by the ULOQ if actual values are not available. The log-transformed antibody levels are analyzed using MMRM, model includes the treatment, analysis visit, treatment visit by interaction, and adjusting for age groups and pre-booster titer levels. An unstructured covariance structure is used to model the within-participant errors. The resulted LS means, difference of LS means, and 95% CI are back transformed to the original scale for presentation. Analysis is based on actual treatment group. The P201 Per-Protocol Set for Immunogenicity SARS-CoV-2 Negative Set includes participants with negative SARS-CoV-2 status and participants with missing SARS-CoV-2 status (n = 11).

[a] Estimated from MMRM model.

Source: Module 5.3.5.1 Study P205 Part A Interim Analysis Report Table 4.1, Table 4.2, Table 4.3, and Table 4.4.

Summary of Seroresponse Rate Differences to Ancestral SARS-CoV-2, Beta, Delta, and Omicron Variants

The SRR difference between the two booster vaccines (50 µg mRNA-1273.211 from Study P205 Part A versus 50 µg mRNA-1273 from Study P201 Part B) was estimated using stratified Miettinen-Nurminen method adjusted for age groups. As noted in Section 2.5.3, the SRR for the Delta variant are not included because the Delta assay is fit-for-purpose at present. The analysis was performed against ancestral, Beta,

and Omicron SARS-CoV-2 and the results are summarised in Table 20. The following summarises the results:

- Beta: The estimated SRR difference between the 50 µg mRNA-1273.211 booster dose and mRNA-1273 50 µg booster dose against the Beta variant was -1.2% (95% CI: -4.0, 1.7) at Day 29 and 14.5% (95% CI: 6.9, 22.2) at Day 181. The mRNA-1273.211 50 µg booster dose met non-inferiority at Day 29 (lower bound of 95% CI > -10% to ≤ 0), and met superiority at Day 181 (lower bound of 95% CI > 0), at nominal alpha of 0.05.
- Ancestral SARS-CoV-2: The estimated SRR difference 50 µg mRNA-1273.211 booster dose and the mRNA-1273 50 µg booster dose against the ancestral SARS-CoV-2 was 0.0% (95% CI: -2.5, 2.6) at Day 29 and 0.8% (95% CI: -2.7, 4.3) at Day 181. The mRNA-1273.211 50 µg booster dose met non inferiority at Day 29 and Day 181 based on SRR (lower bound of 95% CI > 10% to ≤ 0) at nominal alpha of 0.05.
- Omicron: The estimated SRR difference in PsVNA ID50 titers between the 50 µg mRNA-1273.211 booster dose and mRNA-1273 50 µg booster dose against the Omicron variant was 0.9% (95% CI: -2.8, 4.6) at Day 29 and 12.8% (95% CI: 4.3, 21.3) at Day 181. The mRNA-1273.211 50 µg booster dose met non inferiority at Day 29 (lower bound of 95% CI > -10% to ≤ 0), and met superiority at Day 181 (lower bound of 95% CI > 0), at nominal alpha of 0.05.

Table 24: Ancestral SARS-CoV-2, Beta and Omicron Seroresponse Rates (Per-Protocol Set for Immunogenicity – SARS-CoV-2 Negative) – Comparison of mRNA-1273.211 50 µg and mRNA-1273 50 µg Booster Doses

	Beta Variant		Ancestral SARS-CoV-2		Omicron Variant	
Antibody: PsVNA nAb ID_{50} titers	P205 Part A mRNA-1273.211 50 µg (N=295)	P201 Booster mRNA-1273 50 µg (N=149)	P205 Part A mRNA-1273.211 50 µg (N=295)	P201 Booster mRNA-1273 50 µg (N=149)	P205 Part A mRNA-1273.211 50 µg (N=295)	P201 Booster mRNA-1273 50 µg (N=149)
Day 29						
N1	295	149	295	148	294	147
Seroresponse, n (%)[a]	289 (98.0)	148 (99.3)	292 (99.0)	148 (100.0)	286 (97.3)	142 (96.6)
95% CI[b]	(95.6, 99.3)	(96.3, 100.0)	(97.1, 99.8)	(97.5, 100.0)	(94.7, 98.8)	(92.2, 98.9)
Difference (%)	-1.2	-	0.0	-	0.9	-
95% CI[c]	(-4.0, 1.7)	-	(-2.5, 2.6)	-	(-2.8, 4.6)	-
Day 181						
N1	283	147	283	146	282	146
Seroresponse, n (%)[a]	255 (90.1)	111 (75.5)	278 (98.2)	144 (98.6)	234 (83.0)	102 (69.9)
95% CI[b]	(86.0, 93.3)	(67.7, 82.2)	(95.9, 99.4)	(95.1, 99.8)	(78.1, 87.2)	(61.7, 77.2)
Difference (%)	14.5	-	0.8	-	12.8	-
95% CI[c]	(6.9, 22.2)	-	(-2.7, 4.3)	-	(4.3., 21.3)	-

Abbreviations: CI = confidence interval; ID_{50} = 50% inhibitory dilution; LLOQ = lower limit of quantification; ; nAb = neutralizing antibody; PsVNA = pseudotyped virus neutralization assay; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2.

N1 = number of participants with non-missing data at pre-vaccination baseline and the corresponding timepoint. Analysis is based on actual treatment group. The P201 Per-Protocol Set for Immunogenicity SARS-CoV-2 Negative Set includes participants with negative SARS-CoV-2 status and participants with missing SARS-CoV-2 status (n = 11).

[a] Seroresponse at a participant level is defined as a change from below the LLOQ to equal or above 4 x LLOQ, or at least a 4-fold-rise if pre-vaccination baseline is equal to or above the LLOQ. Percentages are based on N1. For mRNA-1273 P205 participants without pre-dose 1 antibody titer information and with corresponding Day 29 post-boost assessment, seroresponse is defined as ≥ 4 * LLOQ for participants with negative SARS-CoV-2 status at their pre-dose 1 of primary series, and these participants antibody titers are imputed as < LLOQ at pre-dose 1 of primary series. For participants who are without SARS-CoV-2 status information at pre-dose 1 of primary series, their pre-booster SARS-CoV-2 status is used to impute their SARS-CoV-2 status at their pre-dose 1 of primary series.

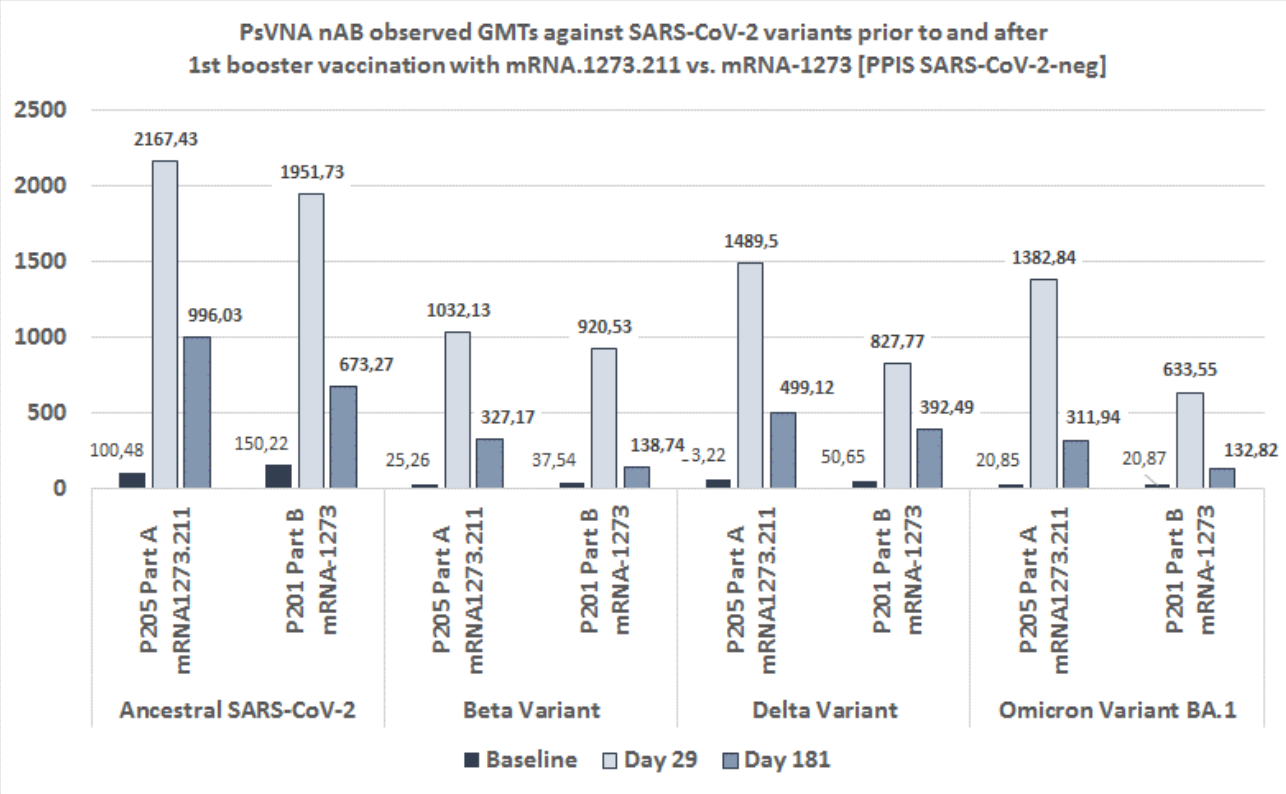
[b] 95% CI is calculated using the Clopper-Pearson method.

[c] Common risk difference and 95% CI is calculated using the stratified Miettinen-Nurminen (score) method.

Source: Module 5.3.5.1 Study P205 Part A Interim Analysis Report Table 5.1, Table 5.2, and Table 5.4.

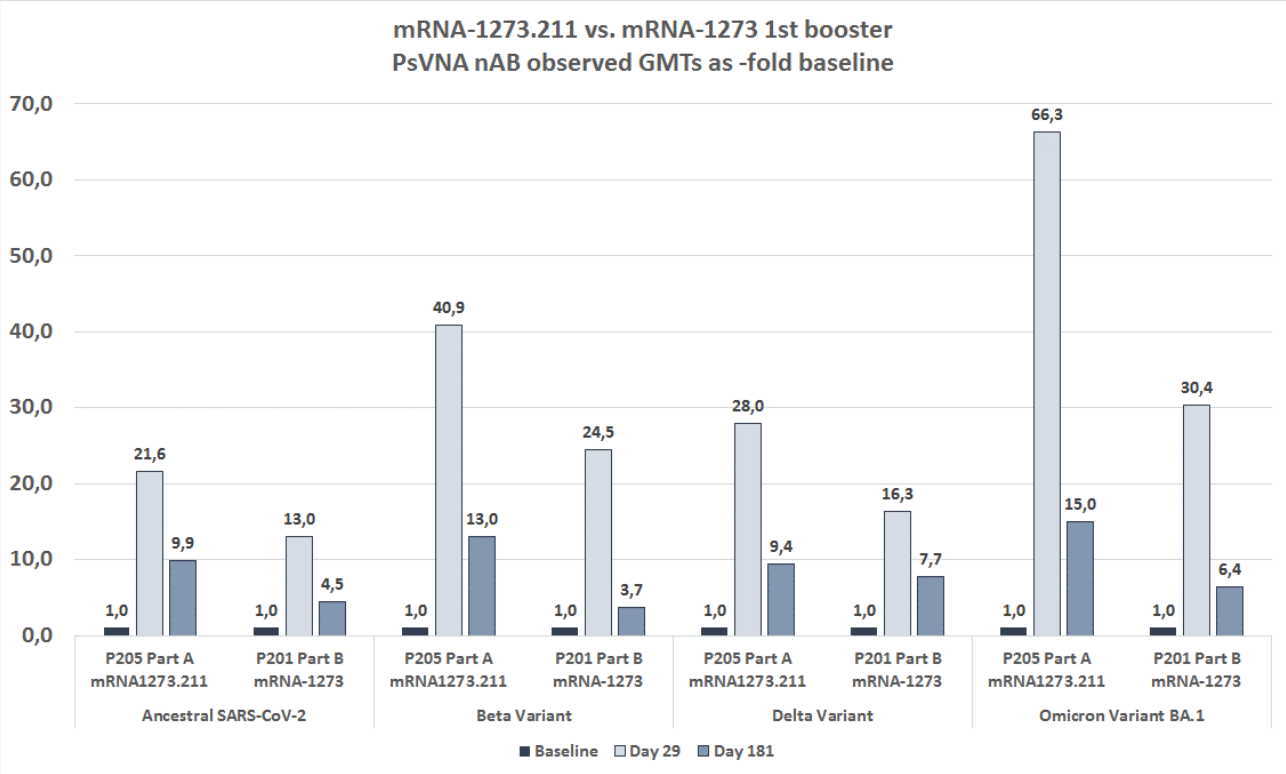
CHMP comment:

PsVNA nAB GMTs after vaccination with the bivalent vaccine mRNA-1273.211 was compared to GMTs after vaccination with mRNA-1273 as a 1st booster dose. Vaccination with mRNA-1273.211 showed superiority over vaccination with mRNA-1273 based on GMRs for all variants tested (see results as provided above). The figure below depicts the PsVNA nAB observed GMTs against ancestral SARS-CoV-2 as well as against variants Beta, Delta and Omicron BA.1.



Data source: Clinical Overview table 18

The figure shows higher nAB GMTs against ancestral SARS-CoV-2 and variants Beta, Delta and Omicron BA.1 after 1st booster vaccination with mRNA-1273.211 as compared to mRNA-1273 on Day 29 and Day 181. In order to visualise the durability of B-cell mediated immune response the figure below shows the nAB GMTs as –fold induction relative to baseline GMTs (set to 1).



Data source: Clinical Overview table 18

PsVNA nAB GMTs remain relatively higher on Day 181 after 1st booster vaccination with mRNA-1273.211 as compared to mRNA-1273.

SARS-CoV-2 Infection and Symptomatic Infection

SARS-CoV-2 Incidence Rates After the mRNA-1273.214 and mRNA-1273 Booster Vaccines

Summary of symptomatic (per primary and per secondary case definition) and asymptomatic SARS-CoV-2 infections are provided. Infections were counted starting 14 days after the booster doses (mRNA-1273.214 50 µg, mRNA-1273 50 µg) through the follow-up time of this interim analysis (Table 21).

The P205 study was not designed to evaluate booster vaccine effectiveness and occurrence of infections after the booster doses reflects the epidemiological environment in the US where Omicron proportion of isolates predominated between March through April 2022 with the majority of the isolates being BA1.1 in early March transitioning to BA.2 in early April and subsequently identifying BA2.12.1 comprising 50% of isolates by end of April.

In the mRNA-1273.214 50 µg booster dose group, with a median of 43 days of follow-up duration, 11 participants (3.2%) had SARS-CoV-2 infection starting at least 14 days after the 50 µg booster dose. Among the 11 participants with SARS-CoV-2 infection, 4 participants (1.2%) met the primary case definition of COVID-19 and 5 participants (1.5%) met the secondary case definition of COVID-19. The remaining 6 participants (1.8%) had an asymptomatic infection. The exposure adjusted incidence rate for

SARS-CoV-2 infection was 5.4 per 1000 person weeks, 1.9 per 1000 person-weeks for the primary case definition of COVID- 19, 2.4 per 1000 person-weeks for the secondary case definition of COVID-19, and 2.9 per 1000 person-weeks for asymptomatic SARS-CoV 2 infection. Among participants in the mRNA-1273.214 50 µg group who met the primary and/or secondary case definition of COVID-19, onset day ranged from Day 7 to Day 36. No participants with COVID-19 had an emergency room visit or hospitalisation due to the COVID-19 event.

In the mRNA 1273 50 µg booster dose group, with a median of 57 days of follow-up duration, 5 participants (1.9%) had SARS-CoV-2 infection starting at least 14 days after the 50 µg booster dose. Among the 5 participants with SARS-CoV-2 infection, 1 participant (0.4%) met both the primary case definition of COVID-19 and the secondary case definition of COVID-19 36 days after the booster dose. The remaining 4 participants had an asymptomatic infection (Listing 16.2.6.3.1.2.8). The exposure adjusted incidence rate for SARS-CoV-2 infection was 2.3 per 1000 person-weeks, 0.5 per 1000 person-weeks for both primary and secondary case definition of COVID-19, and 1.8 per 1000 person-weeks for asymptomatic SARS-CoV-2 infection. No participants with COVID-19 had an emergency room visit or hospitalisation due to the COVID-19 event.

Table 25: Summary of COVID-19 Infections (Second Booster Dose: mRNA-1273.214 50 µg, mRNA-1273 50 µg Comparistion) – Pre-Protocol Efficacy Set

	P205 Part G mRNA-1273.214 50 µg (N=339) n (%)	P205 Part F mRNA-1273 50 µg (N=266) n (%)
Primary case definition of COVID-19 (per Study P301) starting 14 days after injection, n[a]	4 (1.2)	1 (0.4)
Secondary case definition of COVID-19 (CDC criteria) starting 14 days after injection, n[b]	5 (1.5)	1 (0.4)
SARS-CoV-2 infection starting 14 days after injection, n	11 (3.2)	5 (1.9)
Asymptomatic SARS-CoV-2 infection starting 14 days after injection, n	6 (1.8)	4 (1.5)

Abbreviations: CDC = Centers for Disease Control and Prevention; COVID-19 = coronavirus disease 2019; RT-PCR = reverse transcriptase polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

a Primary case definition per P301 Study is positive post-baseline RT-PCR results and at least 2 of the following systemic symptoms: fever (≥ 38°C/≥ 100.4°F), chills, muscle and/or body aches (not related to exercise), headache, sore throat, new loss of taste/smell; OR at least 1 of the following respiratory signs/symptoms: cough, shortness of breath and/or difficulty breathing, OR clinical or radiographical evidence of pneumonia.

b Secondary case definition of COVID-19 is positive post-baseline RT-PCR result and at least 1 of the following systemic or respiratory symptoms: fever (≥ 38°C/≥ 100.4°F), chills, cough, shortness of breath and/or difficulty breathing, fatigue, muscle and/or body aches (not related to exercise), headache, new loss of taste/smell, sore throat, congestion, runny nose, nausea, vomiting, or diarrhea.

Source: Module 5.3.5.1 Table 14.2.4.1.1.2.8.

SARS-CoV-2 Infections After the mRNA-1273.211 Booster Vaccine

Similarly, SARS-CoV-2 infections, symptomatic or verified by testing, after the mRNA- 1273.211 50 µg booster dose are shown in Table 22. With the median follow-up time of 245 days, in the mRNA-1273.211 50 µg booster dose group, 37 participants (12.5%) had SARS-CoV-2 infection starting at least 14 days after the 50 µg booster dose. Among the 37 participants with SARS-CoV-2 infection, 22 participants (7.4%) met the primary case definition of COVID-19 and 26 participants (8.8%) met the secondary case definition of COVID-19. Among participants in the mRNA-1273.211 50 µg group who met the primary and/or secondary case definition of COVID-19, onset day ranged from Day 201 to Day 246, with the exception of 1 participant who had Day 119 onset.

Table 26: Summary of COVID-19 Infections – Part A (Per-Protocol Efficacy Set)

	P205 Part A mRNA-1273.211 50 µg (N=296) n (%)
Primary case definition of COVID-19 (per Study P301) starting 14 days after injection, n[a]	22 (7.4)
Secondary case definition of COVID-19 (CDC criteria) starting 14 days after injection, n[b]	26 (8.8)
SARS-CoV-2 infection starting 14 days after injection, n	37 (12.5)
Asymptomatic SARS-CoV-2 infection starting 14 days after injection, n	11 (3.7)

Abbreviations: CDC = Centers for Disease Control and Prevention; COVID-19 = coronavirus disease 2019; RT-PCR = reverse transcriptase polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

This interim analysis includes Part A participants immunogenicity data up to Day 181 visit. The data cutoff date for safety and SARS-CoV-2 infection is 02 Feb 2022.

[a] Primary case definition per P301 Study is positive post-baseline RT-PCR results and at least 2 of the following systemic symptoms: fever (≥ 38°C/≥ 100.4°F), chills, muscle and/or body aches (not related to exercise), headache, sore throat, new loss of taste/smell; OR at least 1 of the following respiratory signs/symptoms: cough, shortness of breath and/or difficulty breathing, OR clinical or radiographical evidence of pneumonia.

[b] Secondary case definition of COVID-19 is positive post-baseline RT-PCR result and at least 1 of the following systemic or respiratory symptoms: fever (≥ 38°C/≥ 100.4°F), chills, cough, shortness of breath and/or difficulty breathing, fatigue, muscle and/or body aches (not related to exercise), headache, new loss of taste/smell, sore throat, congestion, runny nose, nausea, vomiting, or diarrhea.

Source: Module 5.3.5.1 Study P205A Interim Analysis Report Table 14.2.4.1.1.1.2., Table 14.2.4.2.1.2, Table 14.2.4.2.2.2, Table 14.2.4.2.3.2, Table 14.2.4.2.4.2.

CHMP comment:

Incidence rates after vaccination with mRNA-1273.214 or mRNA-1273 need to be considered descriptive as study P205 was not designed to assess efficacy. In addition, P205 Part F and Part G have not been enrolled at the same time and as a consequence the viral landscape of SARS-CoV-2 might have been different according to prevalent strains. SARS-CoV-2 infections occurred in both treatment arms with a higher occurrence after vaccination with mRNA-1273.214. However, none of the infected participants had an ER visit or hospitalisation, indicating protection from severe disease after both, vaccination with mRNA-1273.214 and mRNA-1273.

Vaccination with mRNA-1273.214 and mRNA-1273 elicits an increase in nAB GMTs against ancestral SARS-CoV-2 and Omicron variants BA.1 and BA.4, BA.5. It was noted before that nAB GMTs against variants Omicron BA.1 and Omicron BA.4, BA.5 are overall lower as compared to nAB GMTs against ancestral SARS-CoV-2. It is of value to assess the durability of these nAB GMTs in a Day 91 analysis. However, not only antibody mediated immunity but also T-cell mediated immunity was intended to be assessed as an exploratory objective as "to characterise the cellular immune response of mRNA-1273.214 as a booster against SARS-CoV-2 and other variants by T-cell (and B-cell) response after the mRNA-1273.214 booster". The T-cell response has not been characterised in this interim analysis but results on T-cell mediated immunity are expected for Day 91 analysis.

A further exploratory objective was to evaluate the genetic and/or phenotypic relationships of isolated SARS-CoV-2 strains to the vaccine sequence by characterising "the SARS-CoV-2 genomic sequence of viral isolates and compare with the vaccine sequence, and to characterise the immune responses to vaccine breakthrough isolates". These analyses are also expected for Day 91 interim analysis. (**REC**)

5.3. Discussion

Study design and methods

Study P205 serves as pivotal study for the bivalent vaccine mRNA-1273.214. The study is an open-label, multi-cohort study. At the time of assessment, it consisted of 7 parts (A to G), whereof 2 parts were further structured in subparts (Part A.1 and A.2; Part F Cohort 1 and Cohort 2). Part D of the study was not further disclosed. The study was planned to enrol subjects to investigate the effect of different booster vaccine candidates (mRNA.1273.211 [bivalent original/Beta] or mRNA-1273.214 [bivalent original/Omicron] or mRNA-1273 [monovalent original]) either as first or second booster. For each part/cohort it was planned to enrol from 300 to 584 subjects depending on the objectives.

The study was not blinded and no protective measures such as firewalls or independent monitoring committees were established to reduce possible bias, to avoid data-driven decision making, and to enhance the conduct of study. While data-driven decision making is well placed in an exploratory trial it might be a possible issue in a confirmatory trial. Hence the study design is not considered optimal but acceptable in the current situation with the need for quick adaptions, often guided by information from outside the trial.

To establish immunogenicity (and efficacy) in the current type 2 variation, only subjects vaccinated with the original vaccine mRNA-1273 as second booster in Part F, Cohort 2 and subjects vaccinated with the bivalent booster candidate mRNA-1273.214 were in Part G were of relevance. No type 1 error control over the different parts and cohorts within parts was in place. Again, this is not considered optimal but acceptable for a pragmatic study conduct in the given situation with the prime aim to implement an optimised adapted vaccine in response to the challenges posed by the evolving SARS-CoV-2 variant strains.

Immunogenicity was measured with two different assays (PsVNA for neutralising antibodies and MSD multiplex for binding antibodies). It is noted that for the cohorts of interest, it was not pre-specified which assay was to be considered primary. Only for cohort A.1 it was stated that "pseudotyped virus neutralising antibody will be used as the basis to assess non-inferiority in immune response". Furthermore, for PsVNA two different measures (ID50 and ID80) were presented. It was not pre-specified which of the two was considered for the primary immunogenicity analyses. The lack of pre-specification leaves room for post-hoc choices and might in general increase the type 1 error and bias. Post hoc, there was apparently no need to choose the endpoints of interest as all endpoints were well aligned. Furthermore, it is noted that the chosen primary assay PsVNA50 was also used in previous applications. Nonetheless, better pre-specification is strongly advised to avoid issues in less clear-cut situations.

Multiplicity within Part G was controlled over 4 hypotheses (three of which were non-inferiority hypotheses and one superiority hypothesis) both at Day 29 and Day 91, respectively. The chosen hypotheses and the multiplicity control within Part G were well justified and are supported. It was noted during the assessment that the final choice of primary endpoints and the multiplicity adjustment (NI-testing for SRR at Day 29 and testing of the same hypotheses at the Day 91 visit under type 1 error control were added) were made very late with Amendment 6 (17 Mar 2022). Given that the study was an open label study and given that the data cut-off (27 Apr 2022) was only shortly after these changes, this might have been problematic. The MAH clarified that the rationale for the inclusion of Day 91 as part of the multiple testing approach was triggered by external data of the bivalent vaccine candidate (mRNA-1273.211 targeted against Beta-variant and ancestral strain), which showed enhanced nAb persistence against various strains (Beta, Omicron, Delta and the ancestral virus). Furthermore, only 29 participants in Part F, Cohort 2 had reached the 29 Day visit by the time of the change and no immunogenicity data was available for any of the two cohorts. Overall, the primary analysis models, statistical methods and study design for immunogenicity are endorsed.

For supportive efficacy data, the study design is considered problematic as subjects vaccinated with mRNA-1273 in Part F, Cohort 2 were enrolled prior to subjects in Part G. Given the dynamic nature of the pandemic (regarding incidence rates as well as circulating variant strains) this results in difficulties in interpreting the derived VE estimates. As these data are only considered supportive, this is not deemed a major issue. Nevertheless, concurrently enrolled or even randomised cohorts would be preferred.

Results

The PsVNAs against SARS-CoV-2 Delta variant and Omicron variants were not validated at the time of analysis but were considered to be qualified "fit-for-purpose". In section 2.5.3 Overview of Clinical Pharmacology in the Clinical Overview the MAH states that the BA.4, BA.5 PsVNA "was performed in a manner consistent with the BA.1 pseudovirus neutralisation assay". Though providing results from non-validated assays for the purpose of a MAA is basically not acceptable, results from the PsVNA against Omicron variants can be accepted for this procedure, given the short timelines for generation of serological results against Omicron variants BA.4, BA.5, in addition to prior experience with the validated PsVNA against D614G. However, the MAH is requested to provide the PsVNA validation reports for Omicron variants BA.1 and BA.4, BA.5 once available. Validation of these assays is expected to be completed prior to further analyses for Day 91 interim analysis.

Key results such as comparison of age groups, race and sex are presented only in raw format in accessory tables.

mRNA-1273.214 compared with mRNA-1273 as a (second) booster

(Second) booster vaccination with bivalent mRNA-1273.214 50 µg met the pre-specified criterion for non-inferiority against the ancestral SARS-CoV-2 strain and elicited superior neutralising antibody responses against Omicron variants BA.1 and BA.4, BA.5 compared to booster vaccination with mRNA-1273.

Booster vaccination with mRNA-1273.214 (25 µg Original + 25 µg Omicron) efficiently elicits even higher nAB GMTs against the ancestral SARS-CoV-2 as compared to booster vaccination with mRNA-1273 (50 µg Original).

Neutralising antibody GMTs after (second) booster vaccination with both, mRNA-1273.214 or mRNA-1273, are lower against BA.1 and again lower against BA.4, BA.5 as compared to nAB GMTs against ancestral SARS-CoV-2.

Sub-group analysis for age

There is a tendency of higher nAB GMTs in the elderly population ≥65 years of age as compared to nAB GMTs in adults ≥18 to <65 years of age. This is rather unexpected and difficult to interpret but might be at least partly explainable by the fact that also the baseline titres are somewhat higher in elderly. Most importantly, these results confirm that also in the elderly population a very good immune response increase is induced by the 2nd booster dose.

Sub-group analysis for race

Vaccination with mRNA-1273.214 and mRNA-1273 increases the nAB GMTs against ancestral SARS-CoV-2 and variant Omicron BA.1 in all sub-groups irrespective of race as compared to baseline GMTs. In all sub-groups in the PPIS-negative nAB GMTs after vaccination with mRNA-1273.214 were higher as compared to GMTs after vaccination with mRNA-1273. In PPIS-negative the pattern of nAB GMTs is overall comparable throughout all sub-groups for race. The PPIS-negative excludes the influence of prior SARS-CoV-2 infection on the nAB GMTs which may confound results in the PPIS-all sub-group analysis for race.

Sub-group analysis for sex

Vaccination with mRNA-1273.214 or mRNA-1273 elicits an increase in nAB GMTs in both sub-groups, male and female. In female the pattern of nAB GMTs after vaccination with mRNA-1273.214 or mRNA-1273 is as expected, with higher nAB GMTs after vaccination with mRNA-1273.214. Vaccination with mRNA-1273 results in higher nAB GMTs on Day 29 in male compared to nABs after vaccination with mRNA-1273.214. Results from the PPIS-negative are shown which excludes an influence on prior SARS-CoV-2 infection. The numbers of the sub-groups are not obviously too small (N=145 vs. N=126). The CIs are largely overlapping for nAB GMTs on Day 29 in male (GM Level 6144.836; 95% CI 5081.693; 7430.401 vs. GM Level 6616.689; 95% CI 5618.470; 7792.260), indicating a comparable capacity in eliciting nAB GMTs by any of the vaccines. While this concerns the question on non-inferiority of mRNA-1273.214 against ancestral SARS-CoV-2 in male population this is not considered an issue. Nonetheless, it would have been expected to be discussed by the MAH.

Geometric Mean Titer (GMT) and Geometric Mean Fold Rise (GMFR) comparison

nAB GMTs and corresponding GMFRs are consistently higher after booster vaccination with mRNA-1273.214 as compared to vaccination with mRNA-1273 against the ancestral SARS-CoV-2 strain and Omicron variants BA.1 and BA.4, BA.5. Values for GMFRs depend on the baseline values and therefore the nominal reduction of GMFRs from PPIS-neg > PPIS-all > PPIS-pos can be expected.

Seroresponse rates (SRRs)

The primary definition of seroresponse is defined as ≥ 4 × LLOQ for those with pre-dose 1 of primary series baseline < LLOQ; ≥ 4-foldrise for those with pre-dose 1 of primary series baseline ≥ LLOQ. For Part F Cohort 2 and Part G, SRRs are based on the primary definition when comparing with the mRNA-1273 primary series. Therefore, seroresponse is considered for those subjects who reach a GMT of ≥ 4 x LLOQ or a ≥ 4-foldrise from primary series baseline GMT value in this interim analysis. This definition is not appropriate for the characterisation of a booster vaccination and resulting SRR values cannot be considered clinically meaningful. This does not impact the assessment as superiority of mRNA-1273.214 based on neutralising antibody responses is shown.

Neutralising / binding ABs against SARS-CoV-2 variants

Neutralising antibodies against variants Beta and Delta have been analysed in samples from <50 subjects per group. The results are controversial in particular for variant Delta. It is understood that due to the nature of the PsVNA there are capacity constraints and analysis of currently circulating VOCs such as Omicron BA.2.75 shall be prioritised. In particular as SARS-CoV-2 Omicron variants are currently the predominantly circulating variants this explanation and ranking is endorsed, and immunogenicity results against variants Beta and Delta do not impact the assessment.

It is noted that vaccination with both mRNA.1273 and mRNA-1273.214 does elicit nABs against SARS-CoV-2 variants Beta and Delta. However, the extent to which nABs against variants Beta and Delta are elicited remains unknown from this analysis but would roughly be expected to be in the same range due to overlapping CIs.

In the analysis of binding antibodies mRNA-1273.214 50 µg booster dose met superiority at Day 29, compared to mRNA-1273 50 µg, based on GMRs for all variants tested, regardless of prior SARS-CoV-2 infection.

SARS-CoV-2 infection after vaccination with mRNA-1273.214 and mRNA-1273

Incidence rates after 2nd booster vaccination with mRNA-1273.214 or mRNA-1273 need to be considered descriptive as study P205 was not designed to assess efficacy. In addition, P205 Part F and Part G have not been enrolled in parallel and as a consequence the viral landscape of SARS-CoV-2 variants might have been different with regards to prevalent strains.

SARS-CoV-2 infections occurred in both treatment arms with a higher incidence after vaccination with mRNA-1273.214. However, none of the infected participants had an ER visit or hospitalisation, indicating protection from severe disease after vaccination with both, mRNA-1273.214 or mRNA-1273.

Out of 11 cases in mRNA-1273.214 vaccinated participants 5 have sequencing data available that demonstrate variant Omicron BA.2 infection, in line with the prevalence of Omicron BA.2 at that time. No sequencing data are presently available for cases in the mRNA-1273 vaccinated group (according to MAH responses to FDA RFI, 04-Aug-2022).

First booster vaccination in comparison of mRNA-1273.211 (original/Beta) with mRNA-1273

PsVNA nAB GMTs after 1st booster dose vaccination with the bivalent vaccine mRNA-1273.211 was compared to GMTs after vaccination with mRNA-1273. Vaccination with mRNA-1273.211 showed superiority over vaccination with mRNA-1273 based on GMRs for all variants tested (Beta, Delta and Omicron BA.1).

Also, the PsVNA nAB GMTs remain relatively higher on Day 181 after 1st booster vaccination with mRNA-1273.211 as compared to mRNA-1273, indicating not only a broadening of the immune response after 1st booster vaccination with bivalent vaccine mRNA-1273.211 but also associated increase in the B-cell mediated durability of the immune response.

In summary, provided results indicate that the bivalent Omicron BA.1-containing vaccine mRNA-1273.214 50 µg elicits superior neutralising antibody responses against Omicron BA.1 and BA.4, BA.5, compared to mRNA-1273 50 µg, regardless of prior SARS-CoV-2 infection.

The MAH is seeking approval for the bivalent mRNA-1273.214 (original / Omicron) as a 1st or 2nd booster immunisation to prevent from COVID-19 caused by SARS-CoV-2 in adolescents and adults aged 12 years of age and older. Study P205 has enrolled adults ≥18 years of age and older. The MAH has received authorisation earlier for the use of original mRNA-1273 50 µg as a 1st booster vaccination in adolescent ≥12 to 17 years of age and in adults ≥18 years of age and older. Authorisation was granted based on immunobridging between adolescents ≥12 to 17 years of age compared to young adults ≥18 to 25 years of age. As there is no obvious scientific reason to assume that basic immunogenicity characteristics would be significantly different for the immunobridging approach applied for the approval of the mRNA-1273 booster dose in adolescent this is considered valid also for mRNA-1273.214.

Notably, bivalent mRNA-1273.211 50 µg (original / Beta) was compared to mRNA-1273 as a 1st booster dose after the primary vaccination series, indicating efficient increase in neutralising antibodies against ancestral SARS-CoV-2 and variant Beta, and a broadening of the immune response to other variants.

Vaccination with mRNA-1273.214 and mRNA-1273 elicits an increase in nAB GMTs against ancestral SARS-CoV-2 and Omicron variants BA.1 and BA.4, BA.5. It was noted earlier that nAB GMTs against variants Omicron BA.1 and Omicron BA.4, BA.5 are overall lower as compared to nAB GMTs against ancestral SARS-CoV-2. Results from P205 Part A (bivalent original / Beta variant vaccine mRNA-1273.211) can be considered supportive in terms of durability of the immune response until day 181. Nevertheless, the upcoming interim analysis on Day 91 for study P205 Parts F and G is considered essential to specifically address durability of the response after vaccination with mRNA-1273.214.

Taken together, the indication for bivalent mRNA-1273.214 50 µg (original / Omicron) as a 1st or 2nd booster dose after the primary vaccination series is deemed acceptable based on the totality of serological evidence evaluating different vaccination regimens.

However, not only antibody mediated immunity but also T-cell mediated immunity was intended to be investigated as an exploratory objective "to characterise the cellular immune response of mRNA-1273.214 as a booster against SARS-CoV-2 and other variants by T-cell (and B-cell) response after the

mRNA-1273.214 booster". The T-cell response has not been characterised in this interim analysis but results on T-cell mediated immunity are expected for Day 91 analysis.

A further exploratory objective was to evaluate the genetic and/or phenotypic relationships of isolated SARS-CoV-2 strains to the vaccine sequence by characterising "the SARS-CoV-2 genomic sequence of viral isolates and compare with the vaccine sequence, and to characterise the immune responses to vaccine breakthrough isolates". These analyses are also expected for Day 91 interim analysis.

6. Clinical Safety aspects

Introduction

In response to the continued emergence of SARS-CoV-2 variants and the associated morbidity and mortality, the MAH has developed a modified, variant-matched bivalent COVID-19 mRNA vaccine that contain equal amounts of two mRNAs that encode for the Spike protein of the ancestral SARS-CoV-2 (Wuhan-Hu-1) and the antigenically divergent variant Omicron BA.1, in combination named mRNA-1273.214.

Study mRNA-1273-P205 is an ongoing open label Phase 2/3 study with multiple, sequentially enrolled cohorts to evaluate the immunogenicity and safety of variant-modified booster candidate vaccines.

This current procedure summarises the safety and immunogenicity data of mRNA-1273.214 50 µg given as a second booster dose at least 3 months after a first booster dose of mRNA-1273 50 µg (Study mRNA-1273-P205 Part G) and also includes supportive data from the mRNA-1273.211 booster vaccine (a Beta-containing bivalent formulation given as first booster dose in Study mRNA-1273-P205 Part A). This submission includes the Day 29 interim results analysing the safety and immunogenicity of the mRNA-1273.214 50 µg booster vaccine as well as supportive safety and immunogenicity data of the Day 181 interim analysis results mRNA-1273.211 50 µg booster vaccine.

MAH intends with this submission to seek authorisation for the mRNA-1273.214 Omicron-containing booster vaccine and to show its ability to elicit superior and broader antibody responses, compared to the current booster vaccine, and therefore it is likely to confer enhanced protection against COVID-19.

The MAH defined the safety set Analysis: The Safety Set consists of all participants who receive IP and will be used for all analyses of safety except for the solicited ARs. Participants will be included in the study arm corresponding to the dose of IP that they actually received.

The data snapshot for P205 Part G (mRNA-1273.214 50) and P205 Part F (mRNA-1273 50 µg) is 27 April 2022. The data snapshot for P205 Part A (mRNA-1273.211) is 02 Feb 2022.

Study population

1. mRNA-1273.214 50 µg (P205 Part G) and mRNA-1273 50 µg (P205 Part F)

Participant Disposition duration of follow-up

At the time of data snapshot (27 Apr 2022), 437 participants received the booster dose in the mRNA-1273.214 50 µg booster dose group. Of these 197 participants, (45.1%) enrolled from study P301, where

they had received the primary series and the first booster dose of mRNA-1273 and other 240 participants (54.9%) had received the primary series and the first booster dose under the EUA in the United States. The median follow-up time was 43 days. Of the 437 participants who received the mRNA-1273.214 50 µg booster dose, 2 of them discontinued from the study (withdrawal of consent by participant).

In the mRNA-1273 50 µg booster dose group, 377 participants received the booster dose. Of these, 264 participants (70.0%) enrolled from study P301 where they had received the primary series and the first booster dose of mRNA-1273 and 113 participants (30.0%) had received the primary series and the first booster dose under the EUA in the United States. The median follow-up time was 57 days (range 51 to 66 days). No participant discontinued the study by the data cut-off date. Table 27 summarises Participants Disposition in P205 Part G and Part F.

Table 27: Participant Disposition – 2nd Booster Dose: mRNA-1273.214; mRNA-1273 (Full Analysis Set), source Table 4, Clinical Overview

	P205 Part G	P205 Part F
	mRNA-1273.214 50 µg (N=437) n (%)	mRNA-1273 50 µg (N=377) n (%)
Number of participants		
Received injection	437	377
Completed study[a]	0	0
Discontinued from study	2 (0.5)	0
Reason for study discontinuation		
Withdrawal of consent by participant	2 (0.5)	0
Other	2 (0.5)	0

Percentages are based on the number of participants in the Full Analysis Set

[a] Study completion is defined as a participant who completed 12 months of follow-up after the injection.

Source: Module 5.3.5.1 Table 14.1.1.1.8.

Demography

The demographic and baseline characteristics were similar between the two groups.

In the mRNA-1273.214 50 µg booster dose group from the participants enrolled from 18 Feb 2021 to 8 Mar 2022 more were females (59.0%), most were White (87.2%), the median age was 60.0 years and (39.8%) participants were ≥ 65 years of age. The median time between doses 2 of the primary series to the first booster dose was 245 days and the median time between the first booster dose to the mRNA-1273.214 50 µg booster dose was 136 days. At baseline, 22% participants of mRNA-1273.214 booster group dose had evidence of prior SARS-CoV-2 infection.

In the mRNA-1273 50 µg booster dose group from the participants enrolled from 8-23 March 2022, 50.7% participants were female, most were White (85.4%), the median age was 60.0 years and 39.8% participants were ≥ 65 years of age. The median time between dose 2 of the primary series and the first booster dose was 242 days and the median time between the first booster dose and the mRNA-1273 booster dose was 134 days. At baseline, 26.8% participants had evidence of prior SARS-CoV-2 infection. Demographics and baseline characteristics in P205 Part G and Part F are described in Table 28.

Table 28: Participant Demographics and Baseline Characteristics – 2nd Booster Dose: mRNA-1273.214, mRNA-1273 (Safety Set), source Table 6, Clinical Overview

	P205 Part G	P205 Part F
	mRNA-1273.214 50 µg (N=437)	mRNA-1273 50 µg (N=377)
Age (years), n	437	377
Mean (SD)	57.3 (14.60)	57.5 (15.31)
Median (mix, max)	60.0 (20, 88)	60.0 (20, 96)
Age subgroups		
≥ 18 and < 65 years	263 (60.2)	227 (60.2)
Mean (SD)	48.2 (11.26)	47.9 (11.49)
Median (mix, max)	49.0 (20, 64)	48.0 (20, 64)
≥ 65 years	174 (39.8)	150 (39.8)
Mean (SD)	71.1 (5.01)	72.1 (6.03)
Median (mix, max)	70.0 (65, 88)	70.0 (65, 96)
Gender, n (%)		
Male	179 (41.0)	186 (49.3)
Female	258 (59.0)	191 (50.7)

Race, n (%)		
White	381 (87.2)	322 (85.4)
Black or African American	31 (7.1)	29 (7.7)
Asian	14 (3.2)	16 (4.2)
American Indian or Alaska Native	0	1 (0.3)
Native Hawaiian or Other Pacific Islander	0	1 (0.3)
Multiracial	7 (1.6)	2 (0.5)
Other	3 (0.7)	2 (0.5)
Not reported	1 (0.2)	3 (0.8)
Unknown	0	1 (0.3)
Ethnicity, n (%)		
Hispanic or Latino	46 (10.5)	37 (9.8)
Not Hispanic or Latino	390 (89.2)	340 (90.2)
Not reported	1 (0.2)	0
Body mass index (kg/m^2), n	437	377
Mean (SD)	30.23 (7.074)	30.84 (7.525)
Median (mix, max)	28.97 (17.8, 71.8)	29.41 (18.4, 61.8)
Pre-booster RT-PCR results, n (%)		
Negative	434 (99.3)	367 (97.3)
Positive	2 (0.5)	2 (0.5)
Missing	1 (0.2)	8 (2.1)
Pre-booster Elecsys Anti-SARS-CoV-2 Results, n (%)		
Negative	341 (78.0)	276 (73.2)
Positive	95 (21.7)	100 (26.5)
Missing	1 (0.2)	1 (0.3)
Pre-booster SARS-CoV-2 Status, n (%) [a]		
Negative	340 (77.8)	267 (70.8)
Positive	96 (22.0)	101 (26.8)
Missing	1 (0.2)	9 (2.4)
Time between dose 2 of primary series to 1st booster dose (days), n	435	374
Mean (SD)	263.3 (61.33)	258.0 (56.89)
Median (mix, max)	245.0 (143, 457)	242.0 (170, 438)
Time between the 1st booster dose to 2nd booster dose (days), n	435	374
Mean (SD)	136.6 (34.92)	133.6 (21.47)
Median (mix, max)	136.0 (88, 408)	134.0 (90, 310)

Abbreviations: COVID-19 = coronavirus disease 2019; max = maximum; min = minimum; RT-PCR = reverse transcription polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus – 2; SD = standard deviation.

Percentages are based on the number of participants in the Safety Set.

a Pre-booster/baseline SARS-CoV-2 status: positive if there is immunologic or virologic evidence of prior COVID-19, defined as positive RT-PCR test or positive Elecsys result at Day 1. Negative is defined as negative RT-PCR test and negative Elecsys results at Day 1.

Source: Module 5.3.5.1 Table 14.1.3.1.8.

2. mRNA-1273.211 50 µg (P205 Part A) and mRNA-1273 50 µg (P201 part B)

Participant Disposition duration of follow-up

At the time of data snapshot, in the P205 Part A mRNA-1273.211 50 µg booster dose group, 300 participants received the booster dose. These participants were enrolled from the study P301 with a median follow up of 264.0 days, after the second dose of the primary series. The median follow-up time from mRNA-1273.211 50 µg booster dose injection was 245.0 days and 10 participants (3.3%) discontinued from the study.

In the mRNA-1273 50 µg booster dose group (P201 part B), 171 participants received the booster dose and they were enrolled from 28 January 2021 until 02 April 2021. The median duration of follow-up after the booster dose was 174.5 days and for 3 participants was < 56 days. Of the 171 participants in total, 5 participants (2.9%) discontinued from the study. Table 29 summarises Participants Disposition in P205 Part A.

Table 29: Participant Disposition for Study mRNA-1273 P205 Part A (50 µg mRNA-1273.211 booster group) and Study mRNA-1273 P201 Part B (50 µg mRNA-1273 booster group), Source Table 7 Clinical Overview

Number of participants	P205 Part A mRNA-1273.211 50 µg booster (N=300) n(%) Full Analysis Set	P201 Part B mRNA-1273 50 µg booster (N= 200 Part A, 171 Part B) n (% Part A [% Part B][a] Safety Set
Information specific to Study P201		
Received first primary injection (P201, Part A)	NA	200 (100)
Received second primary injection (P201, Part A)	NA	198 (99.0)
Discontinued study participation in P201 Part A	NA	15 (7.5)
Consented to P201 Part B	NA	185 (92.5)
Agreed to receive booster in P201 Part B	NA	171 (85.5)
Information that applies to both Studies P205 Part A and P201 Part B		
Received booster injection	300 (100)	171 (85.5) [100]
Completed study[b]	0	176 (88.0) [102.9]
Discontinued from study	10 (3.3)	5 (2.5) [2.9]
Reason for discontinuation of study		
Adverse event	0	0
COVID-19 infection	0	0
Other	0	0
Death	1 (0.3)	0
Lost to follow-up	2 (0.7)	3 (1.5) [1.75]
Physician decision	1 (0.3)	1 (0.5) [0.6]
Pregnancy	0	0
Protocol deviation	3 (1.0)	0
SAR/reactogenicity event	0	_[c]
Study terminated by sponsor	0	0
Withdrawal of consent by participant	3 (1.0)	4 (2.0) [2.3]-
Other	3 (1.0)	4 (2.0) [2.3]
Other	0	1 (0.5) [0.6]

Abbreviations: COVID-19 = coronavirus disease 2019.

Percentages are based on the number of participants in the Full Analysis Set for Study P205 and Safety Set for Study P201. For Study P205, the Full Analysis Set was defined as all participants who received investigational product. For Study P201, the Safety Set was defined as all participants who were randomized in Part A and received any study injection during Part B.

[a] For Study P201: (% Part A) uses the denominator of n=200 from Part A to calculate percentage and [% Part B] uses the denominator of n=171 from Part B to calculate percentage.

[b] Study Completion in P205 is defined as a participant who completed 12 months of follow-up after the injection. Study completion in P201 is defined as participants who received mRNA-1273 in Study P201 Part A and who completed Part B (open-label phase) regardless of receiving the booster injection.

[c] "SAR/reactogenicity event" was not listed as a reason for discontinuation of study for Study P201 Part B.

This interim analysis includes Part A participants immunogenicity data up to Day 181 visit. The data cutoff date for safety and SARS-CoV-2 infection is 02 Feb 2022.

Source: Module 5.3.5.1 Study P205 Part A Interim Analysis Report, Table 14.1.1.1 and Study P201 CSR Addendum Part B Table 14.1.1.1 and Table 14.1.1.1.1.2.

Demography

In the P205 Part A 50 µg mRNA-1273.211 booster dose group, 167/300 participants (55.7%) were female, the median age was 51.0 years and 20.7% participants were ≥ 65 years of age. The median duration from the second dose of the mRNA-1273 primary series to the mRNA-1273.211 50 µg booster dose was 264.0 days in both PP Immunogenicity and Safety Set. At baseline, 1.3% participants in this group had evidence of prior SARS-CoV-2 infection. In the P201 Part B 50 µg mRNA-1273 booster dose group, 104/171 participants (60.8%) were female, the median age was 55.0 years and 52.0% participants were ≥ 55 years of age. The median duration from the second dose of the primary series to the mRNA-1273 50 µg booster dose was 219.0 days.

***CHMP* comments:**

P205 Part G and P205 Part F: The safety sets include data respectively: 437 participants from the mRNA-1273.214 50 µg booster dose group (P205 Part G) and 377 participants from the mRNA-1273 50 µg booster dose group (P205 Part F). The demographic and baseline characteristics were similar between the 2 groups. There were more females than males, respectively 59% females in part G and 50.7% in Part F. More than 80% of study subjects were White across the groups.
The median age was 60.0 years, respectively 60.2% participants were ≥ 18 and < 65 years and 39.8% participants were ≥ 65 years of age for both Part G and Part F. The majority of the participants were SARS-CoV-2 seronegative at baseline, respectively 77.8% in Part G and 70.8% in Part F.
In the mRNA-1273.214 50 µg the median follow-up time was 43 days, and in the mRNA-1273 50 µg booster group 57 days. The data snapshot applying for this procedure is 27 April 2022.
Information was asked on the 2 participants who discontinued the study in the mRNA- 1273.214 50 µg booster dose group, grouped as 'other' at Table 1 and for both the discontinuation reasons did not include adverse events. One subject withdrew from study due to not wanting to have blood drawn and the other subject was unable to attend scheduled visits due to work schedule.
It has been noticed that in the MAH's position the following recruitment windows have been mentioned: In the mRNA-1273.214 50 µg booster dose group from the participants enrolled from 18 Feb 2021 to 8 Mar 2022. In the mRNA-1273 50 µg booster dose group from the participants enrolled from 8-23 March 2022.
The data cut-off for both Part G and Part F is 27 April 2022. Considering that the median follow-up is considerably longer in the mRNA-1273 group (57 days), compared to the mRNA-1273.214 group (43 days), it seems that the recruitment windows were mixed up in the MAH's position (i.e., the participants who received mRNA-1273.214 were recruited during the later time window).

P205 Part A and P201 Part B: The other safety analysis sets include 300 participants from the mRNA-1273.211 50 µg booster dose group (P205 Part A) and 171 participants from the mRNA-1273 50 µg booster dose group (P201 Part B). More females than males were enrolled in both parts.
In the P205 part A, the median age was 51.0 years, and 20.7% were ≥ 65 years of age. The majority of the participants were SARS-CoV-2 seronegative at baseline, with 98.7% participants.
For part A, the median follow-up duration was 264.0 days, after the second dose of the primary series and 245.0 days for the booster dose injection. For part B, the median duration of follow-up after the booster dose was 174.5 days. The safety data cut-off is 02 Feb 2022.

Adverse events

The safety assessments for study P205 parts G, F and A were done by following the solicited local and systemic ARs during the 7-day follow-up period after vaccination. The unsolicited AEs were evaluated during the 28-day follow-up period after vaccination. The assessment included any AE reported by the participant that is not specified as a solicited AR in the protocol or is specified as a solicited AR but starts outside the protocol-defined period for reporting solicited ARs (ie, 7 days after vaccination). All the SAEs, MAAEs, AEs leading to withdrawal and AESIs were collected throughout the study. For the mRNA-1273.214 50 µg booster dose group (Part G) and the mRNA-1273 50 µg booster dose group (Part F), solicited AR data and an overview of unsolicited AE data were also summarised by subgroups based on

pre-booster baseline SARS-CoV-2 status (positive or negative) to assess whether there were reactogenicity or safety concerns for individuals with prior infection.

Solicited adverse reactions

The MAH defined the Solicited Safety Set Analysis as following:

The Solicited Safety Set consists of all participants who receive IP and contribute any solicited AR data.

It will be used for the analyses of solicited ARs. Participants will be included in the study arm corresponding to the dose of IP that they actually received.

Solicited local ARs assessed included injection site pain, injection site erythema (redness), injection site swelling/induration (hardness), and axillary (underarm) swelling or tenderness ipsilateral to the side of the injection. Solicited systemic ARs assessed were headache, fatigue, myalgia (muscle aches all over the body), arthralgia (joint aches in several joints), nausea/vomiting, chills, and fever (oral temperature). Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (DHHS 2007) was used for the severity grading of solicited ARs occurred automatically based on participant entry into the eDiary. Any solicited AR that was ongoing beyond Day 7 was to be reported in the eDiary until it resolved. The ARs captured in the eDiary within 7 days after injection for mRNA-1273.214 given as a second booster (Part G) were compared with mRNA-1273 given as a second booster (Part F).

1. Solicited Adverse Reactions in mRNA-127.214 50 µg (P205 Part G) and mRNA-1273 50 µg (P205 Part F)

Solicited local ARs

In the mRNA-1273.214 50 µg booster dose group (Part G), most participants (79.4%) had at least one solicited local AR. The most common solicited local AR after booster dose was *pain* in 77.3% participants, followed by *axillary swelling or tenderness* (17.4%). The majority of solicited local ARs were Grade 1 in (66.6%) participants and (3.4%) participants had a Grade 3 local AR, the most commonly reported was erythema (2.1%). There were no Grade 4 local ARs. Local ARs were transient; the median duration was 2.0 days (range 1 to 10 days). There was noticed a higher incidence of any erythema (redness) reported in 6.9% participants in the mRNA-1273.214 50 µg booster dose group (Part G) compared to the mRNA-1273 50 µg booster dose group (Part F).

In the mRNA-1273 50 µg booster dose group (Part F), most participants (79.5%) had at least 1 solicited local AR. *Pain* was the most common solicited local AR after the booster dose in 76.6% participants, followed by *axillary swelling or tenderness* (15.4%). The majority of solicited local ARs were Grade 1 (68.1%) and (13.4%) Grade 3 local AR, the most commonly reported was swelling (1.4%) participants. No Grade 4 solicited local ARs were reported. Local ARs were transient; the median duration was 2.0 days (range 1 to 22 days).

Solicited systemic ARs

In the mRNA-1273.214 50 µg booster dose group (Part G), most participants (70.3%) had at least one solicited systemic AR. The most common systemic AR after the booster dose was fatigue (54.9%), followed by headache (43.9%), myalgia (39.6%), and arthralgia (31.1%). The majority of solicited systemic ARs were Grade 1 (38.2%) followed by Grade 2 (26.5%). Twenty-four participants (5.5%) had a Grade 3 systemic AR and the most commonly reported was fatigue (3.4%). No Grade 4 solicited systemic ARs were reported. The median duration of systemic ARs was 2.0 days (range 1 to 21 days).

In the mRNA-1273 50 µg booster dose group (Part F), most participants (66.1%) had at least one solicited systemic AR. The most common systemic AR after the booster dose was fatigue (51.4%) participants, followed by headache (41.1%), myalgia (38.6%), and arthralgia (31.7%). The majority of solicited systemic ARs were Grade 1 with 35.3% of participants, followed by Grade 2 (26.2%). Sixteen participants (4.6%) had a Grade 3 systemic AR and the most commonly reported was myalgia (3.7%). No Grade 4 solicited systemic ARs were reported. The median duration of systemic ARs was 2.0 days (range 1 to 13 days).

The summary for local and systemic reactions in both Part G and Part F is provided in Table 30, highlighted are the differences noted among the two groups and the Grade 3 events:

Table 30: Summary of Participants with Solicited Adverse Reactions within 7 Days After the Injection by Grade – 2nd Booster Dose: mRNA-1273.214, mRNA-1273 (Solicited Safety Set), source table 23 clinical overview

	P205 Part G	P205 Part F
	mRNA-1273.214	mRNA-1273
Solicited Adverse Reaction	50 µg	50 µg
Category	(N=437)	(N=351)
Grade	n (%)	n (%)
Solicited adverse reactions - N1	437	351
Any solicited adverse reactions	380 (87.0)	301 (85.8)
95% CI	83.4, 90.0	81.7, 89.2
Grade 1	220 (50.3)	184 (52.4)
Grade 2	125 (28.6)	89 (25.4)
Grade 3	35 (8.0)	28 (8.0)
Grade 4	0	0
Solicited local adverse reactions - N1	437	351
Any solicited local adverse reactions	347 (79.4)	279 (79.5)
95% CI	75.3, 83.1	74.9, 83.6
Grade 1	291 (66.6)	239 (68.1)
Grade 2	41 (9.4)	28 (8.0)
Grade 3	15 (3.4)	12 (3.4)
Grade 4	0	0
Pain - N1	437	351
Any	338 (77.3)	269 (76.6)
Grade 1	303 (69.3)	241 (68.7)
Grade 2	31 (7.1)	24 (6.8)
Grade 3	4 (0.9)	4 (1.1)
Grade 4	0	0

Solicited Adverse Reaction Category Grade	mRNA-1273.214 50 µg (N=437) n (%)	mRNA-1273 50 µg (N=351) n (%)
Erythema (redness)[a] - N1	437	351
Any	30 (6.9)	13 (3.7)
Grade 1	15 (3.4)	5 (1.4)
Grade 2	6 (1.4)	6 (1.7)
Grade 3	9 (2.1)	2 (0.6)
Grade 4	0	0
Swelling (hardness)- N1	437	351
Any	30 (6.9)	23 (6.6)
Grade 1	17 (3.9)	13 (3.7)
Grade 2	8 (1.8)	5 (1.4)
Grade 3	5 (1.1)	5 (1.4)
Grade 4	0	0
Axillary swelling or tenderness - N1	437	351
Any	76 (17.4)	54 (15.4)
Grade 1	71 (16.2)	46 (13.1)
Grade 2	4 (0.9)	4 (1.1)
Grade 3	1 (0.2)	4 (1.1)
Grade 4	0	0
Solicited systemic adverse reactions - N1	437	351
Any solicited systemic adverse reactions	307 (70.3)	232 (66.1)
95% CI	65.7, 74.5	60.9, 71.0
Grade 1	167 (38.2)	124 (35.3)
Grade 2	116 (26.5)	92 (26.2)
Grade 3	24 (5.5)	16 (4.6)
Grade 4	0	0
Fever[b] - N1	436	351
Any	19 (4.4)	12 (3.4)
Grade 1	14 (3.2)	9 (2.6)
Grade 2	4 (0.9)	3 (0.9)
Grade 3	1 (0.2)	0
Grade 4	0	0
Headache - N1	437	350
Any	192 (43.9)	144 (41.1)
Grade 1	150 (34.3)	112 (32.0)
Grade 2	37 (8.5)	30 (8.6)
Grade 3	5 (1.1)	2 (0.6)
Grade 4	0	0
Fatigue - N1	437	350
Any	240 (54.9)	180 (51.4)
Grade 1	125 (28.6)	95 (27.1)
Grade 2	100 (22.9)	74 (21.1)
Grade 3	15 (3.4)	11 (3.1)
Grade 4	0	0
Myalgia - N1	437	350
Any	173 (39.6)	135 (38.6)
Grade 1	101 (23.1)	68 (19.4)
Grade 2	62 (14.2)	54 (15.4)
Grade 3	10 (2.3)	13 (3.7)
Arthralgia - N1	437	350
Any	136 (31.1)	111 (31.7)

Solicited Adverse Reaction Category Grade	mRNA-1273.214 50 µg (N=437) n (%)	mRNA-1273 50 µg (N=351) n (%)
Grade 1	93 (21.3)	70 (20.0)
Grade 2	39 (8.9)	38 (10.9)
Grade 3	4 (0.9)	3 (0.9)
Grade 4	0	0
Nausea/vomiting - N1	437	350
Any	45 (10.3)	35 (10.0)
Grade 1	39 (8.9)	27 (7.7)
Grade 2	5 (1.1)	8 (2.3)
Grade 3	1 (0.2)	0
Grade 4	0	0
Chills - N1	437	350
Any	104 (23.8)	74 (21.1)
Grade 1	65 (14.9)	46 (13.1)
Grade 2	38 (8.7)	27 (7.7)
Grade 3	1 (0.2)	1 (0.3)
Grade 4	0	0

Abbreviations: CI = confidence interval; SARS-CoV-2 = severe acute respiratory infection coronavirus-2.

N1 = number of exposed participants who submitted any data for the event. Any = Grade 1 or higher. Percentages are based on the number of exposed participants who submitted any data for the event (N1). The 95% CI is calculated using the Clopper-Pearson method.

[a] Toxicity grade for erythema (redness) is defined as: Grade 1 = 25 – 50 mm; Grade 2 = 51 – 100 mm; Grade 3 = greater than 100 mm.

[b] Toxicity grade for fever is defined as: Grade 1 = 38 – 38.4 °C; Grade 2 = 38.5 – 38.9 °C; Grade 3 = 39 – 40 °C; Grade 4 = greater than 40 °C.

Source: Module 5.3.5.1 Table 14.3.1.1.1.8.

CHMP comment:

Safety was evaluated by collecting solicited local and systemic adverse events for 7 days after each injections using an e-diary and monitoring unsolicited AEs for 28 days after each injection. AE of special interest, SAE and AE leading to discontinuation were collected throughout the study.
The data snapshot applying for this procedure is 27 April 2022.
As the duration of follow up is limited to 43 days in the mRNA-1273.214 50 µg booster dose group, the MAH has been required to provide further safety analyses providing information on SAEs, AESIs and MAAEs with a later cut-off. In the response provided MAH has not performed further safety analyses beyond the data-cut timepoint in the Day 29 interim analysis of study P205 part G (43 days of follow-up time), but MAH is planning a day 91 interim analysis and the MAH will have report of the day 91 interim analysis by 31 December 2022. It is concluded that the reactogenicity of mRNA-1273.214 50 µg as booster dose is covered sufficiently with a short term follow up of 43 days. However, the MAH is requested to provide an interim CSR, including a comprehensive safety analysis with a later cut-off for the mRNA-1273.214 50 µg booster dose group, once the data of the Day 91 interim analysis are available.

Solicited Adverse Events

The overall incidence of solicited local reactions was comparable between the mRNA-1273.214 50 µg booster dose group (Part G) and the mRNA-1273 50 µg booster dose group (Part F), accordingly 79.4% vs. 79.5%.
However, regarding the local reactions, it has been observed slightly higher reactogenicity in the mRNA-1273.214 50 µg booster dose group (Part G) vs. the mRNA-1273 50 µg booster dose group (Part F). The most common local ARs for both groups: were "any pain" with 77.3% vs. 76.6%, followed by "any axillary swelling or tenderness" with 17.4% vs 15.4%.

The highest differences were noticed regarding any erythema (redness) reported in 30/437 participants (6.9%) in the mRNA-1273.214 50 µg booster dose group (Part G) and in 13/ 351 participants (3.7%) in the mRNA-1273 50 µg booster dose group (Part F). While any swelling (hardness) was comparable between the two groups with 6.9 % vs 6.6%.

The majority of solicited local ARs were mild- to- moderate (Grade 1-2) for both groups.

Regarding the Grade 3 local ARs, same frequency has been observed in the two groups with (3.4%), however "erythema" was the most common Grade 3 local AR for P205 Part G with (2.1%) versus (0.6%) for P205 Part F. MAH has provided information on the Grade 3 erythema events (n=9) in the mRNA-1273.214 50 and they did have a duration from 1-5 days and reported as resolved.

No grade 4 events were reported in both Groups. After the booster injection, the median duration of solicited local ARs was 2 days.

The incidence of solicited systemic reactions was slightly higher in the mRNA-1273.214 50 µg booster dose group (Part G) compared to the mRNA-1273 50 µg booster dose group (Part F), accordingly 70.3% and 66.1%. The most common systemic AR after the booster dose for both groups accordingly were: fatigue (54.9% vs 51.4%), followed by headache (43.9% vs 41.1%), myalgia (39.6% vs 38.6%), and arthralgia (31.1% vs 31.7%). The majority of the systemic ARs in both groups were mild-to- moderate (Grade 1 -2) and they were comparable between the two groups. Grade 3 events were in higher frequency in the mRNA-1273.214 50 µg booster dose (5.5%) compared to (4.6%) in the mRNA-1273 50 µg booster dose group, the most common Grade 3 event reported in the Part G was fatigue (3.4 % vs 3.1%) while in Part F was myalgia (3.7% vs 2.3%) compared to the Part G. No Grade 4 events were reported in both groups. The median duration of systemic ARs for both groups was 2.0 days.

The MAH has been requested to provide information on the duration of the various grade 3 AE local and systemic in the mRNA-1273.214 50 µg booster dose group (Part G). MAH response: In total there were reported 58 Grade 3 local and systemic solicited ARs in 35 participants, none of them were considered SAEs. The mean duration was 1.6 days, and the median duration was 1 day, with the duration ranging from 1.0 to 14.0 days.

Table 31: Summary of Duration (Days) on Grade 3 Local and Systemic Solicited Adverse Reaction

Table 2: Summary of Duration (Days) on Grade 3 Local and Systemic Solicited Adverse Reaction

N	Mean	Median	Lower Quartile	Upper Quartile	Minimum	Maximum
58	1.6	1.0	1.0	1.0	1.0	14.0

The most frequently reported were: fatigue (n=16; duration range: 1 to 14 day), followed by myalgia (n=10; duration: all 1 day), erythema (n=9; duration range: 1 to 5 days), headache (n=6; duration: all 1 day), swelling (n=5; duration range 1 to 4 days), and pain (n=4; duration: all 1 day) and arthralgia (n=4; duration: all 1 day). The remaining grade 3 / severe solicited ARs of axillary swelling or tenderness, fever, nausea/vomiting and chills were each reported once, and the duration was 1 day for all events.

Table 32: Summary of Case Counts of Grade 3 Local and Systemic Solicited Adverse Reactions by Duration

Duration	Pain	Erythema	Swelling	Axillary Swelling/ Tenderness	Fever	Headache	Fatigue	Myalgia	Arthralgia	Nausea / Vomiting	Chills	Total
1 day	4	5	3	1	1	6	12	10	4	1	1	48
2 days		1					3					4
3 days		1										1
4 days		1	2									3
5 days		1										1
14 days							1					1
Total	4	9	5	1	1	6	16	10	4	1	1	58

One solicited AR of grade 3 / severe fatigue occurred in a 40-49 year old female and had a duration of 14 days; this event was not medically attended and resolved without treatment. The majority of the remaining 15 grade 3 / severe solicited ARs of fatigue had a duration of 1 day (n=12) followed by 2 days (n=3).

MAH has been asked to provide an analysis regarding the status of solicited ARs the mRNA-1273.214 50 µg booster dose group (Part G) that persisted beyond 7 days after vaccination. The results provided show that of the 28 solicited ARs in 21 participants occurred within 7 days of vaccination and persisted beyond Day 7 post vaccination. None of the events were serious and they all resolved and only one event was medically attended (event of fatigue from Study Day 2 to Study Day 13). The most frequently reported solicited AR that persisted beyond Day 7 were fatigue (n=9) followed by arthralgia (n=6), headache (n=5), injection site pain (n=3) and myalgia (n=2). The remaining events of injection site swelling/induration, injection site erythema and axillary swelling or tenderness were reported once each. No safety concerns have been identified.

Table 33: Summary of Duration (Days) for Solicited ARs Persisted Beyond 7 Days after Vacccination

Vaccination Group	n	Mean	Median	Lower Quartile	Upper Quartile	Minimum	Maximum
mRNA-1273.214 50 ug	28	8.6	9.0	7.5	9.0	2.0	14.0

*Included all SARs occurred within 7 days and lasted beyond Day 7 post vaccination, all SARs with an end date post 7 days of vaccination are included.

Solicited Adverse Reactions by Pre-booster SARS-CoV-2 Status

Solicited Safety Set in the mRNA-1273 50 µg booster dose group (Part G) based on the pre-booster baseline SARS-CoV-2 status included 96/437 participants (22.0%) with a positive pre-booster SARS-CoV-2 status and 340/437 participants (77.8%) with a negative pre-booster SARS-CoV-2 status (1 participant had missing status). In summary, the frequency of solicited local ARs was comparable among participants with a positive versus participants with a negative pre-booster SARS-CoV-2 status (77.1% versus 80.0%). The frequency of solicited systemic ARs was also comparable among the participants in both groups, accordingly with 65.6% versus 71.8%. The frequency of Grade 3 solicited local ARs was similar among these two groups respectively, 1/96 participants (1.0%) with positive pre-booster SARS-CoV-2

status and 14/340 participants (4.1%) with negative pre-booster SARS-CoV-2 status. This similarity applied also for the Grade 3 systemic ARs between the 2 groups, accordingly (5.2%) vs (5.6%) participants.

In the mRNA-1273 50 µg booster dose group (Part F) there were included 92/351 participants (26.2%) participants with a positive pre-booster SARS-CoV-2 status and 250/351 participants (71.2%) with a negative status (9 participants had missing status). The frequency of solicited local ARs were similar in the two groups, with 79.3% among the participants with positive status and 80.0% among participants with a negative pre-booster SARS-CoV-2 status. The frequency of solicited systemic ARs was comparable between the 2 groups respectively, 62.0% vs. 68.4%. Grade 3 local ARs had a similar frequency among participants of the 2 groups, respectively (3.3%) and (3.6%). Grade 3 systemic ARs were similar in participants with positive pre-booster SARS-CoV-2 status (1/92 [1.1%]) and participants with negative pre-booster SARS-CoV-2 status (15/250 [6.0%]). Table 34 summaries the solicited ARs according to Pre-booster SARS-CoV-2 Status after 2nd-Booster Dose in P205 Part G and Part F.

Table 34: Summary of Participants with Solicited Adverse Reactions Within 7 Days After the Injection by Grade and Pre-booster SARS-CoV-2 Status – 2nd. Booster Dose: mRNA-1273.214; mRNA-1273 (Solicited Safety Set)

	P205 Part G mRNA-1273.214 50 µg		P205 Part F mRNA-1273 50 µg	
	Pre-booster SARS-CoV-2 Status		Pre-booster SARS-CoV-2 Status	
Solicited Adverse Reaction Category Grade	Negative (N=340) n (%)	Positive (N=96) n (%)	Negative (N=250) n (%)	Positive (N=92) n (%)
Solicited adverse reactions - N1	340	96	250	92
Any solicited adverse reactions	299 (87.9)	80 (83.3)	217 (86.8)	77 (83.7)
95% CI	84.0, 91.2	74.4, 90.2	82.0, 90.7	74.5, 90.6
Grade 1	168 (49.4)	51 (53.1)	129 (51.6)	50 (54.3)
Grade 2	102 (30.0)	23 (24.0)	64 (25.6)	23 (25.0)
Grade 3	29 (8.5)	6 (6.3)	24 (9.6)	4 (4.3)
Grade 4	0	0	0	0
Solicited local adverse reactions - N1	340	96	250	92
Any solicited local adverse reactions	272 (80.0)	74 (77.1)	200 (80.0)	73 (79.3)
95% CI	75.3, 84.1	67.4, 85.0	74.5, 84.8	69.6, 87.1
Grade 1	224 (65.9)	66 (68.8)	171 (68.4)	63 (68.5)
Grade 2	34 (10.0)	7 (7.3)	20 (8.0)	7 (7.6)
Grade 3	14 (4.1)	1 (1.0)	9 (3.6)	3 (3.3)
Grade 4	0	0	0	0
Pain - N1	340	96	250	92
Any	265 (77.9)	72 (75.0)	193 (77.2)	71 (77.2)
Grade 1	236 (69.4)	66 (68.8)	173 (69.2)	64 (69.6)
Grade 2	25 (7.4)	6 (6.3)	17 (6.8)	6 (6.5)
Grade 3	4 (1.2)	0	3 (1.2)	1 (1.1)
Grade 4	0	0	0	0

	P205 Part G mRNA-1273.214 50 µg		P205 Part F mRNA-1273 50 µg	
	Pre-booster SARS-CoV-2 Status		Pre-booster SARS-CoV-2 Status	
Solicited Adverse Reaction Category Grade	Negative (N=340) n (%)	Positive (N=96) n (%)	Negative (N=250) n (%)	Positive (N=92) n (%)
Erythema (redness)[a] - N1	340	96	250	92
Any	27 (7.9)	3 (3.1)	10 (4.0)	3 (3.3)
Grade 1	13 (3.8)	2 (2.1)	3 (1.2)	2 (2.2)
Grade 2	6 (1.8)	0	6 (2.4)	0
Grade 3	8 (2.4)	1 (1.0)	1 (0.4)	1 (1.1)
Grade 4	0	0	0	0
Swelling (hardness)- N1	340	96	250	92
Any	26 (7.6)	4 (4.2)	19 (7.6)	4 (4.3)
Grade 1	14 (4.1)	3 (3.1)	11 (4.4)	2 (2.2)
Grade 2	7 (2.1)	1 (1.0)	3 (1.2)	2 (2.2)
Grade 3	5 (1.5)	0	5 (2.0)	0
Grade 4	0	0	0	0
Axillary swelling or tenderness - N1	340	96	250	92
Any	58 (17.1)	18 (18.8)	35 (14.0)	18 (19.6)
Grade 1	54 (15.9)	17 (17.7)	29 (11.6)	16 (17.4)
Grade 2	3 (0.9)	1 (1.0)	3 (1.2)	1 (1.1)
Grade 3	1 (0.3)	0	3 (1.2)	1 (1.1)
Grade 4	0	0	0	0
Solicited systemic adverse reactions - N1	340	96	250	92
Any solicited systemic adverse reactions	244 (71.8)	63 (65.6)	171 (68.4)	57 (62.0)
95% CI	66.7, 76.5	55.2, 75.0	62.2, 74.1	51.2, 71.9
Grade 1	130 (38.2)	37 (38.5)	89 (35.6)	32 (34.8)
Grade 2	95 (27.9)	21 (21.9)	67 (26.8)	24 (26.1)
Grade 3	19 (5.6)	5 (5.2)	15 (6.0)	1 (1.1)
Grade 4	0	0	0	0
Fever[b] - N1	339	96	250	92
Any	16 (4.7)	3 (3.1)	10 (4.0)	2 (2.2)
Grade 1	11 (3.2)	3 (3.1)	8 (3.2)	1 (1.1)
Grade 2	4 (1.2)	0	2 (0.8)	1 (1.1)
Grade 3	1 (0.3)	0	0	0
Grade 4	0	0	0	0
Headache - N1	340	96	250	92
Any	154 (45.3)	38 (39.6)	106 (42.4)	37 (40.2)
Grade 1	120 (35.3)	30 (31.3)	82 (32.8)	29 (31.5)
Grade 2	29 (8.5)	8 (8.3)	22 (8.8)	8 (8.7)
Grade 3	5 (1.5)	0	2 (0.8)	0
Grade 4	0	0	0	0
Fatigue - N1	340	96	250	92
Any	194 (57.1)	46 (47.9)	134 (53.6)	42 (45.7)
Grade 1	100 (29.4)	25 (26.0)	71 (28.4)	21 (22.8)
Grade 2	83 (24.4)	17 (17.7)	53 (21.2)	20 (21.7)
Grade 3	11 (3.2)	4 (4.2)	10 (4.0)	1 (1.1)
Grade 4	0	0	0	0

	P205 Part G mRNA-1273.214 50 µg		P205 Part F mRNA-1273 50 µg	
	Pre-booster SARS-CoV-2 Status		Pre-booster SARS-CoV-2 Status	
Solicited Adverse Reaction Category Grade	Negative (N=340) n (%)	Positive (N=96) n (%)	Negative (N=250) n (%)	Positive (N=92) n (%)
Myalgia - N1	340	96	250	92
Any	137 (40.3)	36 (37.5)	93 (37.2)	40 (43.5)
Grade 1	77 (22.6)	24 (25.0)	44 (17.6)	23 (25.0)
Grade 2	50 (14.7)	12 (12.5)	36 (14.4)	17 (18.5)
Grade 3	10 (2.9)	0	13 (5.2)	0
Grade 4	0	0	0	0
Arthralgia - N1	340	96	250	92
Any	110 (32.4)	26 (27.1)	80 (32.0)	29 (31.5)
Grade 1	70 (20.6)	23 (24.0)	50 (20.0)	19 (20.7)
Grade 2	36 (10.6)	3 (3.1)	27 (10.8)	10 (10.9)
Grade 3	4 (1.2)	0	3 (1.2)	0
Grade 4	0	0	0	0
Nausea/vomiting - N1	340	96	250	92
Any	36 (10.6)	9 (9.4)	25 (10.0)	10 (10.9)
Grade 1	31 (9.1)	8 (8.3)	18 (7.2)	9 (9.8)
Grade 2	5 (1.5)	0	7 (2.8)	1 (1.1)
Grade 3	0	1 (1.0)	0	0
Grade 4	0	0	0	0
Chills - N1	340	96	250	92
Any	86 (25.3)	18 (18.8)	58 (23.2)	15 (16.3)
Grade 1	54 (15.9)	11 (11.5)	39 (15.6)	7 (7.6)
Grade 2	31 (9.1)	7 (7.3)	18 (7.2)	8 (8.7)
Grade 3	1 (0.3)	0	1 (0.4)	0
Grade 4	0	0	0	0

Abbreviations: CI = confidence interval; SARS-CoV-2 = severe acute respiratory infection coronavirus-2.

N1 = number of exposed participants who submitted any data for the event. Any = Grade 1 or higher. Percentages are based on the number of exposed participants who submitted any data for the event (N1). The 95% CI is calculated using the Clopper-Pearson method.

a Toxicity grade for erythema (redness) is defined as: Grade 1 = 25 – 50 mm; Grade 2 = 51 – 100 mm; Grade 3 = greater than 100 mm.

b Toxicity grade for fever is defined as: Grade 1 = 38 – 38.4 °C; Grade 2 = 38.5 – 38.9 °C; Grade 3 = 39 – 40 °C; Grade 4 = greater than 40 °C.

Source: Module 5.3.5.1 Table 14.3.1.1.1.8.1.

CHMP comments:

The monitoring of the safety and reactogenicity of mRNA-1273.214 50 µg summarised by subgroups based on pre-booster baseline SARS-CoV-2 status (positive or negative) for P205 Part G showed no safety concerns between participants with and without SARS-CoV-2 infection prior to the booster dose. The incidence of the solicited ARs was comparable among the two parts of the study (Part G and Part F), regardless the SARS-CoV-2 status on pre-booster baseline.
In summary, safety and reactogenicity of mRNA-1273.214 50 µg was consistent between participants with and without SARS-CoV-2 infection prior to the booster dose.

Solicited Adverse Reactions in mRNA-1273.211 50 µg (P205 Part A)

Solicited Local ARs

In the mRNA- 1273.211 50 µg booster dose group, most participants (85.2%) had at least 1 solicited local AR. Pain was the most commonly reported local AR (84.9%) followed by axillary swelling or tenderness (24.8%). The majority of solicited local ARs were Grade 1 (68.8%). Eight participants (2.7%) had a Grade 3 solicited local AR and *pain* was the most commonly reported (1.7%). The median duration of local ARs was 3.0 days (range 1 to 24 days).

Solicited Systemic ARs

In the mRNA-1273.211 50 µg booster dose group, most participants (75.8%) had at least 1 solicited systemic AR. The most commonly reported systemic ARs were *fatigue* (64.4%) followed by headache (50.7%) and myalgia (49.0%). The majority of solicited systemic ARs were Grade 1 (35.2%) and Grade 2 (32.2%). Twenty-five participants (8.4%) had a Grade 3 solicited systemic AR and the most commonly reported was *fatigue* (6.4%). There were no Grade 4 solicited systemic ARs. The median duration of systemic ARs was 2.0 days (range 1 to 39 days). Table 35 describes the solicited ARs in P205 Part A.

Table 35: Summary of Participants with Solicited Adverse Reactions within 7 Days Table 36 After the Injection by Grade - 1st Booster Dose: mRNA-1273.211 50 µg (Solicited Safety Set)

Solicited Adverse Reaction Category Grade	P205 Part A mRNA-1273.211 50 µg (N=298) n (%)
Solicited adverse reactions - N1	298
Any solicited adverse reactions	270 (90.6)
95% CI	86.7, 93.7
Grade 1	140 (47.0)
Grade 2	98 (32.9)
Grade 3	32 (10.7)
Grade 4	0
Solicited local adverse reactions - N1	298
Any solicited local adverse reactions	254 (85.2)
95% CI	80.7, 89.1
Grade 1	205 (68.8)
Grade 2	41 (13.8)
Grade 3	8 (2.7)
Grade 4	0
Pain - N1	298
Any	253 (84.9)
Grade 1	211 (70.8)
Grade 2	37 (12.4)
Grade 3	5 (1.7)
Grade 4	0

Solicited Adverse Reaction Category Grade	P205 Part A mRNA-1273.211 50 µg (N=298) n (%)
Erythema (redness)[a] - N1	298
Any	8 (2.7)
Grade 1	5 (1.7)
Grade 2	2 (0.7)
Grade 3	1 (0.3)
Grade 4	0
Swelling (hardness)- N1	298
Any	10 (3.4)
Grade 1	7 (2.3)
Grade 2	2 (0.7)
Grade 3	1 (0.3)
Grade 4	0
Axillary swelling or tenderness - N1	298
Any	74 (24.8)
Grade 1	59 (19.8)
Grade 2	13 (4.4)
Grade 3	2 (0.7)
Grade 4	0
Solicited systemic adverse reactions - N1	298
Any solicited systemic adverse reactions	226 (75.8)
95% CI	70.6, 80.6
Grade 1	105 (35.2)
Grade 2	96 (32.2)
Grade 3	25 (8.4)
Grade 4	0
Fever[b] - N1	298
Any	14 (4.7)
Grade 1	11 (3.7)
Grade 2	3 (1.0)
Grade 3	0
Grade 4	0
Headache - N1	298
Any	151 (50.7)
Grade 1	108 (36.2)
Grade 2	39 (13.1)
Grade 3	4 (1.3)
Grade 4	0
Fatigue - N1	298
Any	192 (64.4)
Grade 1	89 (29.9)
Grade 2	84 (28.2)
Grade 3	19 (6.4)
Grade 4	0
Myalgia - N1	298
Any	146 (49.0)
Grade 1	75 (25.2)
Grade 2	59 (19.8)
Grade 3	12 (4.0)
Grade 4	0

Solicited Adverse Reaction Category Grade	P205 Part A mRNA-1273.211 50 µg (N=298) n (%)
Arthralgia - N1	298
Any	104 (34.9)
Grade 1	59 (19.8)
Grade 2	37 (12.4)
Grade 3	8 (2.7)
Grade 4	0
Nausea/vomiting - N1	298
Any	35 (11.7)
Grade 1	25 (8.4)
Grade 2	10 (3.4)
Grade 3	0
Grade 4	0
Chills - N1	298
Any	63 (21.1)
Grade 1	32 (10.7)
Grade 2	30 (10.1)
Grade 3	1 (0.3)
Grade 4	0

Abbreviations: CI = confidence interval; SARS-CoV-2 = severe acute respiratory infection coronavirus-2.

N1 = number of exposed participants who submitted any data for the event. Any = Grade 1 or higher. Percentages are based on the number of exposed participants who submitted any data for the event (N1). The 95% CI is calculated using the Clopper-Pearson method. This interim analysis includes Part A participants immunogenicity data up to Day 181 visit. The data cutoff date for safety and SARS-CoV-2 infection is 02 Feb 2022.

[a] Toxicity grade for erythema (redness) is defined as: Grade 1 = 25 – 50 mm; Grade 2 = 51 – 100 mm; Grade 3 = greater than 100 mm.

[b] Toxicity grade for fever is defined as: Grade 1 = 38 – 38.4 °C; Grade 2 = 38.5 – 38.9 °C; Grade 3 = 39 – 40 °C; Grade 4 = greater than 40 °C.

Source: Module 5.3.5.1 Study P205 Part A Interim Analysis Report Table 19.

CHMP comments:

The incidence of solicited local reactions in P205 Part A was 85.2%, with the pain reported as most common (84.9%). The majority of the local solicited ARs were Grade 1(68.8%) and Grade 2 (13.8%). The Grade 3 events had a frequency of (2.7%) with *Pain* reported as most common (1.7%).
The incidence of solicited systemic reactions in participants P205 Part A was (75.8%), with fatigue (64.4%) reported as most common. The majority of the events were Grade 1-Grade 2 (35.2%-32.2%). The frequency of the Grade 3 events was (8.4%), with *fatigue* (6.4%) reported as most common.
According to the overall incidence of local and systemic adverse events it seems that the reactogenicity of the bivalent mRNA-1273.211 given as a 1st booster dose appears similar with the ancestral mRNA-1273.
Based in the Clinical Protocol in Part A.2 of Study P205, a second booster dose of 50 µg mRNA-1273.214 is to be administrated in about 300 participants who received mRNA-1273.211 50 µg as a first booster dose in Part A.1. There are no safety data available at this time.

Unsolicited Adverse Events

Unsolicited Adverse Events in mRNA-1273.214 50 µg (P205 Part G) and mRNA-1273 50 µg (P205 Part F)

The unsolicited AEs experienced within 28 days (Day 1 through Day 29) after injection for 50 µg mRNA-1273.214 given as a second booster (Part G) were comparable with 50 µg mRNA-1273 given as a second booster (Part F). The median follow-up was 43 days for Part G and median of 57 days for Part F.

(P205 Part G)

In the mRNA-1273.214 50 µg booster dose group (Part G), within 28 days after vaccination there were no fatal events and 0.5% participants had SAEs; both SAEs (prostate cancer and traumatic fracture) were considered to be unrelated to study vaccination by the investigator. 9.8% participants had at least one MAAE, of whom 2 participants (0.5%) had an MAAE that was considered by the investigator to be related to study vaccination (fatigue and dermatitis). No participants had TEAEs leading to study discontinuation.

In the mRNA.214 50 µg booster dose (Part G), the unsolicited TEAEs within 28 days, regardless of relationship to study vaccination, were reported in 18.5% participants. Of those, 5.7% participants had a TEAE that was assessed by the investigator as related to study vaccination. The most frequently reported TEAEs considered by investigators to be related to vaccination were: fatigue (2.1%), arthralgia (1.4%), and headache (1.4%), and all other treatment-related TEAEs were reported in < 1.0% of participants. One participant experienced a severe TEAE that was considered related to vaccination: a 40-49 year old female had non-serious severe fatigue that began on Day 1 and continued until Day 14; the event was not medically attended. Up to the data cut-off date (27 Apr 2022), one additional SAE occurred in the mRNA-1273.214 50 µg booster dose group (nephrolithiasis; considered unrelated to vaccination by the investigator). As of the data cut-off, there were no fatal events or study discontinuations due to AEs.

(P205 Part F)

In the mRNA-1273 50 µg group (Part F) within 28 days after vaccination, there were no fatal events and 0.3% participants had an SAE; the SAE (spinal osteoarthritis) was assessed by the investigator as unrelated to study vaccination. At least one MAAE was reported for 13.8% participants, of whom 0.5% had an MAAE that was considered by the investigator to be related to study vaccination (hypertension and urticaria). No participants had TEAEs leading to study discontinuation.
In the mRNA-1273 50 µg booster dose (Part F), the unsolicited TEAEs within 28 days, regardless of relationship to study vaccination were reported in 20.7% participants. Of those, 5.8% participants had a TEAE that was assessed by the investigator as related to study vaccination, and the most frequently TEAs reported were: fatigue (2.9%), arthralgia (1.6%), and myalgia (1.6%). Two participants experienced severe TEAEs that were considered related to vaccination and were not medically attended: a 30-39 year old male with history of chronic fatigue had non-serious severe fatigue that began on Day 2 and resolved on Day 8 and occurred concurrently with an unsolicited AE of moderate asthma (verbatim: asthma exacerbation) that was assessed by the Investigator as not related to vaccination; and a 40-49 year old male had non-serious severe myalgia that began on Day 7 and resolved on Day 8. As of the data cut-off, there were no fatal events or study discontinuations due to AEs. One additional MAAE was considered related to vaccination by the investigator occurred in the mRNA-1273 50 µg booster dose group (back pain). The summary of the unsolicited ARs for both parts is described in Table 36:

Table 37: Summary of Unsolicited TEAEs up to 28 Days After the Injection – 2nd Booster Dose: mRNA-1273.214, mRNA-1273 (Safety Set), source table 26, Clinical Overview

	P205 Part G	P205 Part F
	mRNA-1273.214 50 µg (N=437) n (%)	mRNA-1273 50 µg (N=377) n (%)
Unsolicited TEAEs regardless of relationship to study vaccination		
All	81 (18.5)	78 (20.7)
Serious	2 (0.5)	1 (0.3)
Fatal	0	0
Medically-attended	43 (9.8)	52 (13.8)
Leading to study discontinuation	0	0
Grade 3 or higher	4 (0.9)	3 (0.8)
At least 1 non-serious event [a]	79 (18.1)	78 (20.7)
Grade 3 or higher	3 (0.7)	2 (0.5)
Unsolicited TEAEs related to study vaccination		
All	25 (5.7)	22 (5.8)
Serious	0	0
Fatal	0	0
Medically-attended	2 (0.5)	2 (0.5)
Leading study discontinuation	0	0
Grade 3 or higher	1 (0.2)	2 (0.5)
At least 1 non-serious event [a]	25 (5.7)	22 (5.8)
Grade 3 or higher	1 (0.2)	2 (0.5)

Abbreviation: TEAE = treatment-emergent adverse event.

A TEAE is defined as any event not present before exposure to study vaccination or any event already present that worsens in intensity or frequency after exposure. Percentages are based on the number of participants in the Safety Set.

[a] Participants with at least one non-serious TEAE regardless of reporting any SAE or not.

Source: Module 5.3.5.1 Table 14.3.1.7.1.8.

CHMP comments:

The incidence of unsolicited TEAs regardless of relationship with study vaccination within to 28 days were lower in the mRNA-1273.214 50 µg booster dose group (Part G) compared to mRNA-1273 50 µg group (Part F), accordingly 18.5% vs. 20.7%. There were 2 SAEs in the first group and 1 SAEs in the second group (which are going to be analysed in the SAEs section).

The same applies for the unsolicited TEAS related to the study vaccination, slightly lower in the mRNA-1273.214 50 µg booster dose group (Part G) compared to mRNA-1273 50 µg group (Part F), respectively 5.7% vs. 5.8%. Regarding the medically- attended events related to the study IP the incidence was the same between the two groups with 0.5%. The most two commons TEAS related to the study vaccination for both 2 groups were respectively: fatigue (2.1% vs 2.9) and arthralgia (1.4 vs 1.6 %). No fatal cases and no discontinuation of the study was due to an AE.

Unsolicited Adverse Events by Pre-booster SARS

In the safety Set there were included in the mRNA-1273 50 µg booster dose group (Part G) 22.0% participants with a positive pre-booster SARS-CoV-2 status and 77.8% participants with a negative pre-booster SARS-CoV-2 status (1 participant with missing status). The incidence of all unsolicited TEAEs was comparable in participants with positive pre-booster SARS-CoV-2 status (13.5%) and participants with negative pre-booster SARS-CoV-2 status (20.0%). The incidence of SAEs, MAAEs, and Grade 3 or higher TEAEs was similar between the two groups. In the mRNA-1273 50 µg booster dose group (Part F), 26.8% participants had positive pre-booster SARS-CoV-2 status and 70.8% participants had a negative pre-booster SARS-CoV-2 status (9 participants with missing status). The incidence of all unsolicited TEAES was comparable between the two groups respectively 16.8% in the positive group and 22.1% in the negative pre-booster SARS-CoV-2 status. There were no safety concerns or differences identified in SAEs, MAAEs, and severe TEAEs based on pre-booster status.

Unsolicited Adverse Events mRNA-1273.211 50 µg (P205 Part A)

In the mRNA-1273.211 50 µg booster dose group (Part A) within 28 days after vaccination, there were no fatal events and no SAEs were reported. At least one MAAE was reported for 7.0%, participants, of whom one participant (0.3%) had an MAAE that was assessed by the investigator as related to the study vaccination (arthralgia). No participants had TEAEs leading to study discontinuation.
Unsolicited TEAEs within 28 days after the mRNA-1273.211 50 µg booster dose (Part A), in total were reported in 21.0% participants. Of those, 9.0% participants had a TEAE that was assessed by the investigator as related to study vaccination. The most commonly reported unsolicited TEAEs within the 28 days were fatigue (3.3%); arthralgia (1.7%); myalgia and injection site lymphadenopathy (1.3%); and rhinovirus infection and headache (1.0%). One participant (0.3%) had a severe TEAE of fatigue that was considered by the investigator to be related to vaccination. Table 37 describes a summary of the unsolicited TEAEs in P205 Part A.

Table 38: Summary of Unsolicited TEAEs up to 28 Days After the Injection – Study P205 Part A (Safety Set), source Table 27, clinical overview

	P205 Part A mRNA-1273.211 50 µg (N=300) n (%)
Unsolicited TEAEs regardless of relationship to study vaccination	
All	63 (21.0)
Serious	0
Fatal	0
Medically-attended	21 (7.0)
Leading to study discontinuation	0
Grade 3 or higher	1 (0.3)
Unsolicited TEAEs related to study vaccination	
All	27 (9.0)
Serious	0
Fatal	0
Medically-attended	1 (0.3)
Leading study discontinuation	0
Grade 3 or higher	1 (0.3)

Abbreviation: SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; TEAE = treatment-emergent adverse event.
A TEAE is defined as any event not present before exposure to study vaccination or any event already present that worsens in intensity or frequency after exposure. Percentages are based on the number of participants in the Safety Set. This interim analysis includes Part A participants immunogenicity data up to Day 181 visit. The data cutoff date for safety and SARS-CoV-2 infection is 02 Feb 2022.
Source: Module 5.3.5.1 Study P205 Part A Interim Analysis Report, Table 20.

The summary of unsolicited TEAEs up to the data cut-off date (02 Feb 2022) included a total of 1.3 % participants in the mRNA-1273.211 50 µg booster dose group had an SAE, 51.0% participants had an MAAE, 1.3% participants had a Grade 3 or higher TEAE. One participant (0.3%) in the mRNA-1273 50 µg booster dose group had a fatal TEAE that was assessed by the investigator as not related to the study vaccination (myocardial infarction). The median follow-up time after the mRNA-1273.211 booster dose was 245 days.

Deaths

No deaths were reported in either the 50 µg mRNA-1273.214 booster dose group (P205 Part G) or the 50 µg mRNA-1273 booster dose group (P205 Part F).

One death was reported in the 50 µg mRNA-1273.211 booster dose group (Part A) 159 days after vaccination. A ≥60 year old male with a medical history of type 2 diabetes mellitus, hypertension, hyperlipidaemia, and severe obesity had a fatal myocardial infarction 159 days after receiving the mRNA-1273.211 50 µg booster dose. The investigator assessed the event as not related to mRNA-1273.211 and the MAH agreed with this assessment.

CHMP comments:

The narrative on the fatal myocardial infarction happened 159 days after receiving the mRNA-1273.211 in a ≥60 year male, was randomly assigned to receive a booster dose of 50 µg mRNA-1273.211 in Part

A of the study. The participant received the booster injection in the left arm on Study Day 1. The participant's pre-booster SARS-CoV-2 status was negative. The medical history included: gastroesophageal reflux disease, hyperlipidaemia, hypertension, severe obesity, and Type 2 diabetes with the ongoing concomitant medications at the time of the event included: aspirin, omeprazole, simvastatin, hydrochlorothiazide, lisinopril, metoprolol, Ozempic, Invokana, metformin, pioglitazone, and Januvia. 158 days after the booster dose, the participant experienced a Grade 5 serious adverse event (SAE) of myocardial infarction, meeting the SAE criteria of death. 158 days after the booster dose of the participant fell and became unresponsive. Cardiopulmonary resuscitation was performed until EMS arrived. Upon arrival to the hospital, the participant was intubated but the event of myocardial infarction was fatal and the participant died on the same day. The participant never had a stable rhythm and the blood sugar was 182. The cause of death was myocardial infarction and an autopsy was not performed. The investigator assessed the event of myocardial infarction to be not related to the IP. It is agreed with the investigator judgement that the event is not related to the study IP and the assessment of relatedness might have been confounded by participant's underlying cardiovascular and metabolic comorbidities.

Other Serious Adverse Events

In the mRNA-1273.214 50 µg booster dose group (Part G), 2 participants (0.5%) had one SAE each within 28 days of the booster dose and both SAEs were assessed as not related to vaccination by the Investigator. The first case was a ≥60 year old female hospitalised on Study Day 14 due to a severe traumatic pelvic fracture. The second event was a ≥60 year old male diagnosed with moderate prostate cancer on Study Day 2, with elevated prostate-specific antigen level 6 weeks prior to receipt of the booster dose and an enlarged prostate and underwent a biopsy 2 days prior to receiving the booster dose, confirming the diagnosis of prostate cancer. At the time of the data cut-off, both events were ongoing. There was an SAE beyond 28 days after the booster dose, a ≥60 male was hospitalised due to severe nephrolithiasis on Study Day 44 and the event resolved on Study Day 55. The Investigator assessed the event as not related to vaccination.

In the mRNA-1273 50 µg booster dose group (Part F), 1 participant had an SAE within 28 days of the booster dose. A ≥60 year old female with a medical history of osteoarthritis had severe spinal osteoarthritis (verbatim: worsening lumbar osteoarthritis) on Study Day 9. The participant was hospitalised on Study Day 44 and underwent lumbar fusion, and the event resolved on Study Day 49. The Investigator assessed the event as not related to vaccination. No SAE were reported beyond 28 days from the booster, as of the data cut-off date (27 April 2022). A summary is provided in the table below for P205 Part G and Part F.

Table 39: Participant Incidence of Serious TEAEs by System Organ Class and Preferred Term up to the Data Cut-off Date – 2nd Booster Dose: mRNA-1273, source Table 28, Clinical Overview

System Organ Class Preferred Term	P205 Part G mRNA-1273.214 50 µg (N=437) n (%)	P205 Part F mRNA-1273 50 µg (N=377) n (%)
Number of participants reporting unsolicited AEs	3 (0.7)	1 (0.3)
Number of unsolicited AEs	3	1
Neoplasms benign, malignant and unspecified (incl. cysts and polyps)	1 (0.2)	0
Prostate cancer	1 (0.2)	0
Musculoskeletal and connective tissue disorders	0	1 (0.3)
Spinal osteoarthritis	0	1 (0.3)
Renal and urinary disorders	1 (0.2)	0
Nephrolithiasis	1 (0.2)	0
Injury, poisoning and procedural complications	1 (0.2)	0
Traumatic fracture	1 (0.2)	0

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities; TEAE = treatment-emergent adverse event.

A TEAE is defined as any event not present before exposure to study vaccination or any event already present that worsens in intensity or frequency after exposure. Percentages are based on the number of participants in the Safety Set. MedDRA Version 23.0.

Source: Module 5.3.5.1 Table 14.3.1.12.2.8.

In the mRNA-1273.211 50 µg booster dose group (Part A), there were no SAEs up to 28 days after the booster dose. Four participants (1.3%) had 5 SAEs after Day 28 and through the data cut-off date; One participant had SAEs of dehydration and hypotension concurrently, and the remaining SAEs from 3 different participants included myocardial infarction (fatal event), peripheral arterial occlusive disease, and cholelithiasis. None of the SAEs were considered treatment related by the Investigator or MAH. The median follow-up time after the mRNA 1273.211 booster dose was 245 days. A summary is provided in the table below for P205 Part A.

Table 40: Participant Incidence of Serious TEAEs by System Organ Class and Preferred Term up to the Data Cut-off Date – Part A (Safety Set), source Table 29 Clinical Overview

System Organ Class Preferred Term	P205 Part A mRNA-1273.211 50 µg (N=300) n (%)
Number of participants reporting serious TEAEs	4 (1.3)
Number of serious TEAEs	5
Metabolism and nutrition disorders	1 (0.3)
Dehydration	1 (0.3)
Cardiac disorders	1 (0.3)
Myocardial infarction	1 (0.3)
Vascular disorders	2 (0.7)
Hypotension	1 (0.3)
Peripheral arterial occlusive disease	1 (0.3)
Hepatobiliary disorders	1 (0.3)
Cholelithiasis	1 (0.3)

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; TEAE = treatment-emergent adverse event.

A TEAE is defined as any event not present before exposure to study vaccination or any event already present that worsens in intensity or frequency after exposure. Percentages are based on the number of participants in the Safety Set. MedDRA Version 23.0. This interim analysis includes Part A participants immunogenicity data up to Day 181 visit. The data cutoff date for safety and SARS-CoV-2 infection is 02 Feb 2022.

Source: Module 5.3.5.1 Study P205 Part A Interim Analysis Report Table 21.

CHMP comments:

As of the data cut-off date (27 April 2022) the most SAEs were reported until D28 after booster vaccination and can be considered as not related to the study IP.

In the mRNA-1273.214 50 µg booster dose group (Part G), there were 3 SAEs in total, 2 SAEs reported within 28 days and 1 SAE reported beyond 28 days; a severe traumatic pelvic fracture on a ≥60 year old female hospitalised on D14 and the second SAE was a prostate cancer on D2. It is agreed upon with the investigator assessment for both cases not to be related with the Study IP.

A Grade 3/SAE was reported beyond 28 days (43 days after the booster dose), on a ≥60 year old male as nephrolithiasis. The participant had ongoing medical conditions (including hypertension, hypothyroidism, water retention) and concomitant medications reported included lisinopril, levothyroxine, gabapentin, vitamin B complex, and Lasix). The participant developed kidney stones and was hospitalised, treatment in hospital included tamsulosin (Flomax), ibuprofen, hydrocodone acetaminophen (opioid/analgesic), Dilaudid (hydromorphone), and oxybutynin. Participant underwent right percutaneous nephrostomy tube placement; left ureteroscopy and an attempted cystoscopy with left ureteral stent. Patient was discharged five days after hospital admission and had a worsening nephrolithiasis six days after and being discharged again.

Updated information was requested and provided from MAH: The event resolved nine days after the event onset on Study Day 55. Since the data cut-off, and as of 27 Jul 2022, the site confirmed the event was considered ongoing as the kidney stone had not passed. The site will continue to monitor this event, which was assessed as not related to vaccination by the investigator and it is agreed upon.

In the mRNA-1273 50 µg booster dose group (Part F) the SAE of spinal osteoarthritis on D9 after the booster dose is reported on a ≥60 year old female with previous medical conditions and concomitant medications. It is agreed upon with the investigator assessment not to be related with the study IP.

Medically-Attended ARs and Vaccine Related ARs

P205 Part G

In the mRNA-1273.214 50 µg booster dose group (Part G) up to the data cut-off were reported in total 74 MAAEs, in 13.3% participants and from them 52 MAAEs (9.8% participants) were reported within 28 days after the booster dose. In summary, the most commonly reported MAAEs (for more than 1 participant), were: COVID-19 (1.8%), upper respiratory tract infection (1.4%) and coronavirus infection (non-COVID; 0.9%). 2 MAAE were also SAEs (traumatic fracture and prostate cancer) reported within 28 days and one SAE (nephrolithiasis) beyond 28 days. There have been 2 non-serious MAAEs in 2 participants assessed as related to vaccination by the Investigator as follows: An event of moderate fatigue in a ≥60 year old female with a relevant medical history of chronic obstructive pulmonary disease and iron deficiency. The event had a self-limiting reactogenicity with a duration from D2 to D13 of the study. The other event was a mild dermatitis (verbatim: unspecified dermatitis, with the duration from D7 to day D19 of the study and the treatment included oral and topical diphenhydramine hydrochloride. One MAAE was also reported as a SAE and it is described in the respective section.

P205 Part F

In the mRNA-1273 50 µg booster dose group (Part F) up to the data cut-off were reported in total 104 MAAEs, in 22.5% participants and from them 56 MAAEs (13.8% participants) were reported within 28 days after the booster dose. In total, the most commonly reported were: upper respiratory tract infection (4.0%), followed by coronavirus infection (2.4%). Two non-serious MAAEs in 2 participants within 28 days were assessed as related to vaccination by the Investigator: one event of mild hypertension (verbatim: high blood pressure without diagnosis of hypertension) in a 50-59 year old female, with no relevant medical history had mild hypertension starting on Study Day 13. The event worsened as a moderate MAAE of hypertension, no longer assessed as related to Study IP on D30 and the treatment included amlodipine. The other event was a mild urticarial (verbatim: generalised urticaria), reported in a 30-39 year old female with no relevant medical history, with a duration from D18- D22 and treatment oral diphenhydramine hydrochloride. One of the MAAEs with onset beyond 28 days from the booster dose was assessed as related to vaccination by the Investigator was an event of mild back pain (verbatim: worsening of chronic back pain) reported in a 50-59 year old female with a medical history of chronic back pain started on Study Day 33. At the time of the data cut-off, the event was ongoing.

P205 Part A

In the mRNA-1273.211 50 µg booster dose group (Part A) up to the data cut-off were reported in total 268 MAAEs in 51.0% participants and from them 22 MAAEs (7.0% participants) were within 28 days after the booster dose. The most commonly reported MAAEs were COVID-19 (12.3%); rhinovirus infection

(3.0%), viral infection (2.7%), urinary tract infection (2.3%), hypertension (2.3%), sinusitis (2.0%), and anxiety (2.0%). One participant had an MAAE that was assessed by the investigator as related to the study IP, a grade 2 event of arthralgia started from D3-D16 and also a reactivation beyond 28 Days. The median follow-up time after the mRNA-1273.211 booster dose was 245 days.

CHMP comments: The follow up period for P205 Part G study and P205 Part F study as described above was 43 days. The total number of MAAE reported for Part G was 74 and mostly of them were manifested within 28 days (52 out of 74).
The number of MAAE for P205 Part F was 104 and 56 of them were reported within 28 days. It is observed that for the same follow up period the number of MAAEs was lower in the Omicron bivalent booster dose group compared to the original Spikevax dose group. The cases have been assessed and described in the respective sections throughout the text and it is agreed with the investigator judgement on the ARs study related vaccine. Additional information has been provided from MAH regarding the nephrolithiasis event. The follow up period was longer for the P205 Part A with a median of 245 days, however the number of MAAEs reported within 28 days was relatively low with 22 out of 268 in total.
The comparison of unsolicited AEs and the Study IP related ARs in the mRNA-1273 and mRNA-1273.214 groups did not raise any new safety concerns.

Comparison with Study 201 Part B, a 50 µg first booster of mRNA-1273 after mRNA-1273 primary series

In study 201 Part B, a 50 µg first booster dose of mRNA-1273 after mRNA-1273 primary series was administered in 330 participants The most common solicited local AR after the 50 µg boost dose was pain (86.3%). Any erythema was reported in 5.5% participants. Most solicited local ARs were grade 1 to grade 2 in severity. Pain was the most commonly reported grade 3 local AR in P201 Part B and no grade 4 solicited local ARs were reported. The most common systemic ARs were: headache (57.4%), fatigue, myalgia (muscle aches all over the body), arthralgia (aching in several joints), nausea/vomiting, fever, and chills. No grade 4 solicited systemic ARs were reported.

CHMP comments:

Reactogenicity results of mRNA-1273.214 50 µg as a second booster were comparable with the results for the mRNA-1273, given as a 50 µg first booster dose after mRNA-1273 primary series, in 330 participants enrolled in study 201 part B.

Discontinuation From IP or Study Participation

No participants in either the mRNA-1273.214 group or the mRNA-1273 group discontinued due to a Treatment related adverse event (TEAE). In Study P205 Part A, no participants who received mRNA-1273.211 50 µg discontinued due to a TEAE.

Adverse Events of Special Interest

Analysis of AESIs has been performed using investigator assessment of events at the time of reporting, based on programmed summaries of reported AEs based on SMQs, and based on PTs consistent with the CDC working case definitions for myocarditis and pericarditis (CMQ).A priority list of AESIs relevant to the development of COVID-19 vaccines was created by the Brighton Collaboration (Law 2020) and was included in the Study P205 protocol (Appendix 4, Section 10.4).

Investigator-Assessed AESIs in mRNA-1273.214 50 µg (P205 Part G) and mRNA-1273 50 µg (P205 Part F)

Up to the data cut-off date, no participants in the mRNA-1273.214 50 µg booster dose group (Part G) had an investigator-assessed AESI. In the mRNA-1273 50 µg booster dose group (Part F), 1/377 participants (0.3%) had an investigator-assessed AESI. There was reported a moderate non-serious irregular heart rate (verbatim: unspecified irregular heartbeat) on a ≥60 year old male, on Day 17 after the mRNA1273 50 µg booster dose. Medical history included hypertension, Type 2 diabetes mellitus, hyperlipidaemia, osteoarthritis, prostate cancer, and seasonal allergy. The event had not resolved and no diagnosis has been made available and the assessed the event as not related to study vaccination. Information has been asked regarding the event of the unspecified irregular heartbeat.
In the mRNA-1273.211 50 µg (P205 Part A), there were no investigator-assessed AESIs up to 28 days after the mRNA-1273.211 50 µg booster dose but one participant (0.3%) had an investigator-assessed AESI of fatal acute myocardial infarction on Study Day 159, considered by the investigator to be unrelated to vaccination.

CHMP comments:

The event of the unspecified irregular heartbeat has not been resolved and no final diagnoses have been made available until the data cut-off. Upon request information has been obtained on the event of the 'unspecified irregular heartbeat' on Day 17 after the mRNA1273 50 µg booster dose. The site updated the event term from irregular heart rate to supraventricular tachycardia following diagnosis by a cardiologist. The participant had commenced treatment with Pradaxa around three months earlier (reported as ongoing) and at the time when this application was submitted for assessment the participant was awaiting treatment with intravenous adenosine for cardioversion followed by cardiac ablation. The event of supraventricular tachycardia was reported as non-serious and moderate in intensity, and the Investigator assessed the event as not related to vaccination. It is agreed upon, however close monitoring is recommended until all the procedures have been performed. The AESI of fatal acute myocardial infarction is described in the section of deaths.

Events of clinical interest based on the narrow and the narrow and broad hypersensitivity standard Medical Dictionary for Regulatory Activities queries (SMQs) of Hypersensitivity

The SMQ analyses (narrow and broad scope) of AEs within 28 days of the booster in all three parts was done for the list of diagnoses: ischemic heart disease, cardiac failure, cardiomyopathy, embolic and thrombotic events, CNS vascular disorders, hearing and vestibular disorders, convulsions, demyelination,

hematopoietic cytopenias, peripheral neuropathy, thrombophlebitis, or vasculitis. Table 40 provides a summary of these cases.

Table 41: Subject Incidence of Select Unsolicited Adverse Event up to 28 Days after the Injection by SMQ (Narrow Scope) — 2nd Booster. Dose: mRNA-1273.214, mRNA-1273, source Table 14.3.1.22.2.1.8

SMQ Subordinate SMQ Preferred Term	Part G mRNA-1273.214 50 ug (N=437) n (%)	Part F Cohort 2 mRNA-1273 50 ug (N=377) n (%)
Ischaemic Heart Disease	0	0
Myocardial Infarction	0	0
Other Ischaemic Heart Disease	0	0
Cardiac Arrhythmia	0	1 (0.3)
Arrhythmia Related Investigations, Signs and Symptoms	0	0
Cardiac Arrythmia Terms (Including Bradycardia and Tachycardia)	0	1 (0.3)
Heart rate irregular	0	1 (0.3)
Congenital and Neonatal Arrhythmias	0	0
Cardiac Failure	0	0
Cardiomyopathy	0	0
Embolic and Thrombotic Events	0	0
Central Nervous System Vascular Disorders	0	0
Hypersensitivity	4 (0.9)	5 (1.3)
Dermatitis	1 (0.2)	0
Dermatitis contact	1 (0.2)	2 (0.5)
Eczema	0	1 (0.3)
Hypersensitivity (Cont.)		
Rash macular	1 (0.2)	0
Urticaria	1 (0.2)	2 (0.5)
Angioedema	1 (0.2)	2 (0.5)
Urticaria	1 (0.2)	2 (0.5)
Arthritis	2 (0.5)	1 (0.3)
Osteoarthritis	1 (0.2)	0
Rheumatoid arthritis	1 (0.2)	0
Spinal osteoarthritis	0	1 (0.3)
Hearing and Vestibular Disorders	0	0
Convulsions	0	0
Demyelinating Disease of Central Nervous System	0	0
Haematopoietic Cytopenias	0	0
Peripheral Neuropathy	0	0
Thrombophlebitis	0	0
Vasculitis	0	0

Study P205 Part G

In the mRNA-1273.214 50 µg booster dose group (Part G) within 28 days after vaccination, no events were captured in the SMQs (narrow and broad scope) for the above mentioned diagnoses. Cases with PTs identified by SMQs were medically reviewed and addressed as follows:

Cardiac arrhythmia SMQ: Moderate Tachycardia event, on a 50-59 year old female with a medical history of obesity, start on D7 and resolved the same day and assessed as unrelated to the study IP and of unknown aetiology.

Hypersensitivity SMQ: Nonserious mild Dermatitis, on a 50-59 year old female, with a duration from D7-D19 and was assessed by the investigator as related to vaccination. A nonserious event of mild urticaria (also reported under angioedema SMQ) on a ≥60 year old male, with a duration from D2 to Day 34, not medically attended and assessed by the investigator as related to vaccination. A case of contact dermatitis (Day7) and a case of macular rash on study Day 5, both events assessed as unrelated to the study IP by the investigator.
Arthritis SMQ: A moderate Rheumatoid arthritis on a ≥60 year old female, on Study Day 27 and on the same day the participant underwent excision of rheumatoid nodules of her hand. The medical history included bilateral osteoarthritis of the hands, myocardial infarction, and hypothyroidism. The event was ongoing as of the data cut-off and was assessed by the investigator as unrelated to vaccination.
No new events were reported captured in SMQs beyond 28 days up to the cut-off data.

Study P205 Part F

In the mRNA-1273 50 µg group (Part F) within 28 days after vaccination, no events were captured for the SMQs (narrow and broad scope). Cases of interest following medical review and considered as unrelated to the study IP were: Irregular heart rate; peripheral oedema; urticarial event on a 50-59 year old female; asthma; contact dermatitis (2 participants); eczema and muscular weakness. One event of nonserious MAAE of mild urticarial, on a 30-39 year old female has been assessed as related to vaccination by the investigator. Cases considered of interest beyond 28 days were: cardiac failure, arthritis, anaemia and hypoesthesia. They were all assessed as unrelated to the study IP by the investigator, and it is agreed upon.

Study P205 Part A

In the mRNA-1273.211 50 µg booster dose group (Part A) within 28 days after vaccination, no events were captured for the SMQs (narrow and broad scope) for the list of diagnoses mentioned above. Some events of clinical interest were: Supraventricular tachycardia (1 participant) and Dyspnoea (1 participant) and they were assessed as not related to the study vaccine and it is agreed upon. There were not reported events of clinical interest beyond 28 days up to the data cut-off (median of 245 days).

CHMP comment:
As of the data cut-off no cases of anaphylaxis or severe hypersensitivity reaction were reported. For the listed diagnoses under the SMQ analyses (narrow and broad scope) of AEs there were no imbalances found between the parts of the Study 205.
The cases identified by SMQs for the Study P205 Part G for each of the diagnoses mentioned above were observed each in 1/437= 0.2 % participants and did not identify any new safety concerns. It is agreed upon the cases assessed as non-related to the study IP. Regarding the events assessed as Study IP related they include: One event of mild dermatitis (verbatim: unspecified dermatitis [both palms and (L) pinky toe]) on a 50-59 year old female, with the duration from on Day 7- Day 19 and treatment included oral and topical diphenhydramine hydrochloride. The event of mild urticaria on a

≥60 year old male, with the duration from Day 2- Day 34, not medically attended, was considered by the investigator to be related to vaccination, and it is agreed upon.
Upon request the full narrative and causality assessment has been provided for the event of 'Tachycardia' captured in the Cardiac Arrhythmia SMQ Assessment in a 50-59 year old female participant who received mRNA-1273.214 (Part G). The participant, a 50-59 year old female, received a second booster dose of 50 ug mRNA-1273.214, medical history include chronic diseases and concomitant medications. On Study Day 5, the participant developed non-serious grade 2 / moderate symptomatic COVID-19 characterised by chills, cough, fatigue headache, sore throat, congestion and a runny nose. On the same day, treatment for the COVID-19 infection included oral dextromethorphan 10 mg; paracetamol 325 mg; phenylephrine 5 mg. Two days later, on Study Day 7, the participant experienced non-serious grade 2 / moderate tachycardia (reported verbatim: tachycardia [unknown aetiology]) that was self-limiting and resolved the same day. On the same day, treatment for the COVID-19 infection ended and on Study Day 37, the symptomatic COVID-19 infection was considered fully resolved. The Investigator assessed the event of tachycardia as not related to vaccination and it is agreed upon, most likely it was confounded by the concurrent symptomatic COVID-19 infection and treatment with phenylephrine.
In the Study P205 Part F the cases identified by SMQs for the above mentioned diagnoses were observed each in 1/377 = 0.3% participants with the exception of urticarial which has been observed in 2 participants (0.5%). One of the urticarial events (verbatim: generalised urticaria) was assessed as related to the study vaccination, an event of mild urticaria on a 30-39 year old female with no relevant medical history, with the onset on Study Day 18 and the event resolved 4 days later (Study Day 22). Treatment included oral diphenhydramine hydrochloride and the event resolved 4 days later (Study Day 22).
No events assessed related to the study vaccination have been reported in the Study P205 Part A for the list of diagnoses mentioned above. There were 2 reported events of clinical interest: Supraventricular tachycardia on a 50-59 year old (Study Day 9) (verbatim: worsening of SVT non-sustained). Treatment included metoprolol and was resolved 3 days later. The event was an MAAE and was reported as resolved with sequelae 3 days later. This event was also captured in the CMQ of myocarditis and pericarditis. It is agreed with the investigator assessment for the event as not related to vaccination. The other event of dyspnoea on a 50-59 year old male (Study Day 8), with an unknown aetiology was self-resolved 2 days later, no medical treatment was provided and it was assessed as not related to the study IP.
The follow up duration of 43 days for both *parts G* and F is considered relatively short however no safety concern have been raised and the results reassure the acceptable reactogenicity profile of mRNA-1273.214, when administered 3 months following a booster dose of mRNA-1273 after completion of a primary series. This is adequately addressed in the Risk Section of the CTP.

Myocarditis and Pericarditis Events Enhanced Assessment Using CMQ

The CMQ analysis did not identify any cases fulfilling the CDC working case definition for probable or confirmed cases of acute myocarditis or acute pericarditis in P205 Parts G, F, and A.

No additional events of potential myocarditis or pericarditis were identified with the CMQ that had not already been captured in the SMQ analyses in the mRNA-1273.214 50 µg (Part G); mRNA-1273 50 µg (Part F) booster dose groups or in the mRNA-1273.211 50 µg (Part A) booster dose group.

Pregnancy

No pregnancies were reported in the mRNA-1273.214 50 µg booster dose group (P205 Part G) or in the mRNA-1273 50 µg booster dose group (P205 Part F) up to the data cut-off.
Also, no pregnancies were reported in the mRNA-1273.211 50 µg booster dose group (P205 Part A) up to the data cut-off.

Post-marketing experience

Safety Data for Dose 3 and Dose 4

Cumulatively as of 15 Apr 2022, a total of 1,091,443,760 doses of mRNA-1273 had been delivered to 88 countries, and an estimated total of 633,071,724 doses of mRNA-1273 had been administered, including an estimated 126,556,471 doses administered that were Dose 3 or a subsequent dose. Up to the data cut-off, the MAH has received 531,696 cases including 2,1014,965 events reported after any dose. The MAH reported to having received 42,226 cases (124,242 events) reported after Dose 3 or Dose 4 of mRNA-1273. There were 41,625 cases (122,901 events) reported after Dose 3 and 607 cases (1,341 events) after Dose 4. Of the cumulatively reported Dose 3 and Dose 4 cases, 11,926 cases were medically confirmed, 15,551 cases were serious, and 361 cases had fatal outcomes. The majority of cases were reported in females (64.4%) compared to males (31.0%, 13,089) with the mean age of 49.2 years (SD: 16.2; median: 48.0 years).

CHMP comment: The MAH has explained that the post-marketing global safety database does not fully distinguish between a third 100-µg dose, indicated for immunocompromised individuals in some settings, and a 50-µg booster dose for immunocompetent individuals. Dose 4 may refer to the first 50 µg booster dose for immunocompromised individuals or may refer to the second booster for immunocompetent individuals.

Myocarditis and Pericarditis in Individuals Receiving a Third Dose

Cumulatively as of 15 Apr 2022, there were reported 431 events of myocarditis and pericarditis, respectively 283 events of myocarditis and 148 events of pericarditis following a third dose of mRNA-1273. The cases involved 272 males (65.9%) and 140 females (33.9%), with a mean age of 42.9 years and a median age of 40 years. The time to onset from vaccination was less than 7 days for 69.1% of the events. A summary of the observed events of Myocarditis after any dose are given in Table 41:

Table 42: Observed Versus Expected Analyses of Myocarditis, Cases Occurring within 7 Days of a Known Dose, Cumulative Through April 2022, source table 30, Clinical Overview*

	Observed vs Expected (95% CI)		
	Dose 1	**Dose 2**	**Dose 3**
All	0.75 (0.65, 0.85)	2.51 (2.23, 2.83)	0.97 (0.8, 1.17)
By age			
<12 years	NA	NA	NA
12-17 years	0.55 (0.26, 1.15)	2.75 (1.55, 4.87)	0.22 (0.05, 1.03)
18-24 years	1.82 (1.32, 2.5)	8.79 (6.51, 11.88)	1.43 (0.87, 2.36)
25-39 years	1.4 (1.09, 1.78)	3.9 (3.09, 4.93)	1.69 (1.19, 2.4)
40-49 years	0.45 (0.3, 0.67)	1.47 (1.06, 2.02)	0.88 (0.54, 1.43)
50-64 years	0.3 (0.21, 0.44)	0.47 (0.32, 0.67)	0.59 (0.38, 0.93)
65-74 years	0.11 (0.05, 0.24)	0.28 (0.16, 0.49)	0.51 (0.28, 0.92)
75+ years	0.12 (0.05, 0.3)	0.12 (0.04, 0.33)	0.32 (0.13, 0.79)
By gender			
Male	0.85 (0.72, 1)	3.47 (3.01, 4.01)	1.07 (0.84, 1.35)
Female	0.59 (0.46, 0.75)	0.96 (0.76, 1.22)	0.84 (0.6, 1.17)
By age and gender			
Male			
<12 years	NA	NA	NA
12-17 years	0.72 (0.31, 1.71)	4.01 (2.00, 8.01)	0.18 (0.02, 1.5)
18-24 years	2.43 (1.65, 3.57)	12.84 (8.83, 18.68)	1.88 (1.03, 3.41)
25-39 years	1.64 (1.22, 2.22)	5.54 (4.16, 7.37)	2 (1.3, 3.08)
40-49 years	0.43 (0.26, 0.72)	1.71 (1.16, 2.54)	0.82 (0.44, 1.53)
50-64 years	0.19 (0.1, 0.34)	0.5 (0.32, 0.78)	0.6 (0.34, 1.06)
65-74 years	0.11 (0.05, 0.29)	0.26 (0.12, 0.53)	0.51 (0.24, 1.09)
75+ years	0.11 (0.03, 0.38)	0.05 (0.01, 0.35)	0.17 (0.04, 0.75)
Female			
<12 years	NA	NA	NA
12-17 years	0.28 (0.06, 1.36)	0.7 (0.2, 2.53)	0.31 (0.03, 2.96)
18-24 years	0.86 (0.46, 1.62)	2.21 (1.24, 3.93)	0.74 (0.28, 1.98)
25-39 years	1 (0.64, 1.55)	1.27 (0.8, 2.03)	1.25 (0.67, 2.33)
40-49 years	0.51 (0.27, 0.96)	1.12 (0.63, 1.99)	1.04 (0.48, 2.26)
50-64 years	0.5 (0.29, 0.86)	0.44 (0.23, 0.82)	0.56 (0.26, 1.21)
65-74 years	0.12 (0.04, 0.4)	0.35 (0.15, 0.83)	0.53 (0.2, 1.44)
75+ years	0.13 (0.03, 0.58)	0.25 (0.07, 0.88)	0.59 (0.17, 2.04)

*Reference rates from Boehmer et al 2021. Because age by sex stratified estimates of the reference rate were not available in the source material, estimates are obtained by multiplying the age specific rate estimate by the ratio of the sex-specific stratum-specific rate to the overall rate.

CHMP comments:

The events of myocarditis and pericarditis occurring in a time period within 7 days after any dose are more frequent in males compare to females. The myocarditis rates are higher after dose 2 compared to dose 1 and they continue to be lower following dose 3 compared with dose 2 for almost all the age strata. The age group at the highest risk belong to age strata of 12 to 39 years of age; the highest observed vs expected ratio was reported in the age group 18-24 years old and accordingly the ratio is 1.82% post Dose 1; 8.79% post Dose 2 and 1.43% post Dose 3. However, it is agreed with MAH that the post-marketing data for dose 3 are limited and estimates may change as the demographic characteristics of dose 3 recipients change over time.

The reporting rates for myocarditis are given for a time frame of 7 days post any dose. This is considered relatively short and analyses of myocarditis cases should take an extended time frame of at least 30 days into account. However, the MAH in previous responses has explained that the 7 days post dose analysis is based on all the evaluations conducted by health authorities, research organisations,

as well as information from the MAH's safety database that have identified the 0-7 days as the greatest risk interval to experienced vaccine related myocarditis or pericarditis events. Analysis conducted by the Centers for Disease Control and Prevention (CDC) utilising the Vaccine Safety Datalink (VSD) as of 15 January 2022, showed an increased risk for myocarditis and pericarditis observed after both dose 1 and dose 2 of Spikevax in the 0–7-day risk interval, with a greater risk following dose 2 (ACIP 2022). The MAH has reported the number of myocarditis and pericarditis following dose 3 but there was no information on these events following dose 4. Upon request data were provided and cumulative as of 18 July 2022, there have been 7 reports of myocarditis after receiving a dose 4 of Spikevax, 5 males and 2 females. All the reports were considered serious (SAEs). Median age for these reports was 75 years old (min: 40/ max: 81), with a time onset from 1 day to 102 days. All cases are from the European Economic Area. WHO-UMC causality assessment has been used and two cases were considered unlikely related to the vaccine due to associated comorbidities that provide a more plausible explanation for the occurrence of the events. The other 5 cases were considered assessable due to the lack on important information including any laboratory or diagnostic results. A brief summary of the cases is described: A pericarditis event reported in a >70 year old man with other co morbidities and on current medications. A myocarditis events in a >70 year old female, with no information on previous medical history or on going medications, considered as recovered with sequelae. A pericarditis event in a >80 year old male with co-morbidities and current medications; a pericarditis in a >70 year old male; a pericarditis in a 40-49 year old female and a myocarditis event in a 40-49 year old male. The information is submitted from MAH as an Excel format with the above respective cases. The cases were assessable at the time of data submission however, close monitoring and follow up information is recommended.

Laboratory findings

No scheduled laboratory assessments for safety were implemented in the study.

Safety in special populations

No data were available regarding the use of mRNA-1273.214 50 in pregnant women and there were no pregnancies reported in Study P205 in Part G, Part F and Part A.

Regarding the Pre-booster SARS- CoV-2 Status, it was observed that reactogenicity for mRNA-1273.214 50 µg given as a second booster (Part G) was overall similar to that observed with the mRNA-1273 booster vaccination 50 µg given as a second booster, regardless of prior SARS-CoV-2 infection before the booster dose.

The mRNA-1273.214 has been studied in individuals $\geqslant$ 18 years of age.

Safety related to drug-drug interactions and other interactions

Drug-drug interactions were not evaluated in this trial.

6.1. Discussion on clinical safety

Safety data base and follow-up

Study mRNA-1273-P205 is an ongoing open label Phase 2/3 study with multiple, sequentially-enrolled cohorts to evaluate the immunogenicity and safety of variant-modified booster candidate vaccines. The part included in this submission are P205 Part G, Part F and Part A.
This submission intends to seek authorisation for the mRNA-1273.214 Omicron-containing bivalent vaccine and to show its ability to elicit superior and broader antibody responses, compared to the current monovalent booster vaccine mRNA-1273, and therefore it is likely to confer enhanced protection against COVID-19.
The data snapshot for P205 Part G (mRNA-1273.214 50) and P205 Part F (mRNA-1273 50 µg) is 27 April 2022. The data snapshot for P205 Part A (mRNA-1273.211) is 02 Feb 2022.
The safety data base comprises safety and immunogenicity data from subjects summarised below:
The 50 µg mRNA-1273.214 (P205 Part G) given as a second booster dose in 437 subjects, administered with a median of 136 days after a first booster dose of mRNA-1273 50 µg and a median follow-up duration of 43 days. (P205 Part G)
Comparative data for the 50 µg mRNA-1273 given as a second booster in 377 subjects, administered with a median of 134 days after a first booster dose of mRNA-1273 50 µg and a median follow-up duration of 57 days. (P205 Part F)
Supplemented data from the bivalent 50 µg mRNA-1273.211 vaccine given as a first booster in 300 subjects, administered with a median of 264 days after the second dose of the primary series and with a median follow-up duration of 245 days. (P205 Part A)
The safety assessments for study P205 parts G, F and A consisted in the observation of the solicited local and systemic adverse events during the 7-day follow-up period after vaccination. The unsolicited adverse events were monitored during the 28-day follow-up period after vaccination. This has included any AE reported by the participant that is not specified as a solicited AR in the protocol or is specified as a solicited AR but starts outside the protocol-defined period for reporting solicited ARs (ie, 7 days after vaccination). The SAEs, MAAEs, AEs leading to withdrawal and AESIs were collected throughout the study. The solicited adverse events and an overview of unsolicited AE data were summarised by subgroups based on pre-booster baseline SARS-CoV-2 status (positive or negative) for the mRNA-1273.214 50 µg booster dose group (Part G) and the mRNA-1273 50 µg booster dose group (Part F).

Demography and baseline characteristics

Demography and baseline characteristics were balanced between the Part G and Part F of the P205 study. This has also applied for the P205 Part A. There were more females than males, respectively 59% females in part G and 50.7% in Part F. More than 80% of study subjects were White across the groups. The median age was 60.0 years, respectively 60.2% participants were ≥ 18 and < 65 years and 39.8% participants were ≥ 65 years of age for both Part G and Part F. The majority of the participants were SARS-CoV-2 seronegative at baseline, respectively 77.8% in Part G and 70.8% in Part F.
In the P205 Part A there were enrolled more females (55.7%), the median age was 51.0 years, and 20.7% were ≥ 65 years of age. The majority of the participants were SARS-CoV-2 seronegative at baseline, with 98.7% participants.

Local and systemic reactogenicity

The incidence of solicited local reactions is comparable between the mRNA-1273.214 50 µg booster dose group (Part G) and the mRNA-1273 50 µg booster dose group (Part F), accordingly 79.4% vs. 79.5%.

However, if comparison is done between each solicited local reaction, it has been observed slightly higher reactogenicity in the mRNA-1273.214 50 µg booster dose group (Part G) vs. the mRNA-1273 50 µg booster dose group (Part F). The most common local ARs for both groups: were "any pain" with 77.3% vs. 76.6%, followed by "any axillary swelling or tenderness" with 17.4% vs 15.4%. The highest differences were noticed regarding any erythema (redness) reported in 30/437 participants (6.9%) in the mRNA-1273.214 50 µg booster dose group (Part G) and in 13/ 351 participants (3.7%) in the mRNA-1273 50 µg booster dose group (Part F).
The majority of solicited local ARs were mild- to- moderate (Grade 1-2) for both groups.

Regarding the Grade 3 local ARs, same frequency has been observed in the two groups with (3.4%), however "erythema" was the most common Grade 3 local AR for P205 Part G with 2.1% versus 0.6% for P205 Part F. MAH has provided information on the Grade 3 erythema events (n=9) in the mRNA-1273.214 50 and they did have a duration from 1-5 days. No grade 4 events were reported in both Groups. After the booster injection, the median duration of solicited local ARs was 2 days. The slightly higher rate of erythema after booster vaccination with mRNA-1273.214 is deemed acceptable and does not trigger concerns about the reactogenicity profile of the bivalent vaccine.

The incidence of solicited systemic reactions was slightly higher in the mRNA-1273.214 50 µg booster dose group (Part G) compared to the mRNA-1273 50 µg booster dose group (Part F), accordingly 70.3% and 66.1%. The most common systemic AR after the booster dose for both groups accordingly were: fatigue (54.9% vs 51.4%), followed by headache (43.9% vs 41.1%), myalgia (39.6% vs 38.6%), and arthralgia (31.1% vs 31.7%). The majority of the systemic ARs in both groups were mild-to- moderate (Grade 1 -2) and they were comparable between the two groups. Grade 3 events were in higher frequency in the mRNA-1273.214 50 µg booster dose (5.5%) compared to (4.6%) in the mRNA-1273 50 µg booster dose group, the most common Grade 3 event reported in the Part G was fatigue (*3.4* % vs 3.1%) while in Part F was myalgia (*3.7*% vs 2.3%).

Regarding grade 3 AE local and systemic in the mRNA-1273.214 50 µg booster dose group (Part G): there were 58 Grade 3 local and systemic solicited ARs reported in 35 participants, none of them were considered SAEs. The most frequently reported were: fatigue (n=16; duration range: 1 to 14 day), followed by myalgia (n=10; duration: all 1 day), erythema (n=9; duration range: 1 to 5 days), headache (n=6; duration: all 1 day), swelling (n=5; duration range 1 to 4 days), and pain (n=4; duration: all 1 day) and arthralgia (n=4; duration: all 1 day). The remaining grade 3 / severe solicited ARs of axillary swelling or tenderness, fever, nausea/vomiting and chills were each reported once, and the duration was 1 day for all events.

The 28 solicited ARs in 21 participants occurred within 7 days of vaccination and persisted beyond Day 7 post vaccination. None of the events were serious and they all resolved and only one event was medically attended (event of fatigue from Study Day 2 to Study Day 13). The most frequently reported solicited AR that persisted beyond Day 7 were fatigue (n=9) followed by arthralgia (n=6), headache (n=5), injection site pain (n=3) and myalgia (n=2). The remaining events of injection site swelling/induration, injection site erythema and axillary swelling or tenderness were reported once each. No safety concerns have been identified. It is concluded that the reactogenicity between groups G (mRNA-1273.214) and F (mRNA-1273) is comparable. There seems to be a trend towards slightly higher incidences for systemic ADRs or

regarding the local ADR of erythema. However, it is these differences are not clinically relevant and do not trigger safety concerns.

Unsolicited AEs

Unsolicited adverse events regardless of the relationship to vaccination up to 28 days after the second booster doses were reported in 18.5% of participants in the mRNA-1273.214 and 20.7% in the m RNA-1273 groups. It is observed that it was slightly lower in the mRNA-1273.214 booster group dose. The incidences of AEs considered related to the study vaccination by the investigator were comparable between the two booster groups, respectively 5.7% in the mRNA-1273.214 group and 5.8% in the mRNA-1273 group. In the mRNA-1273.214 group, 2 (0.5%) participants each experienced two serious adverse events (SAEs, prostate cancer and traumatic fracture) and 1 (0.3%) participant in the mRNA-1273 group reported an SAE of spinal osteoarthritis within 28 days of the booster dose; none were assessed by the investigator to be related to study vaccination. Medically-attended adverse events (MAAEs) were 9.8% in the mRNA-1273.214 and 13.8% in the mRNA-1273 groups up to 28 days after vaccination. Regarding some of the unsolicited events additional information has been required and provided adequately from MAH.

It is concluded that the reactogenicity of mRNA-1273.214 50 µg as booster dose is covered sufficiently with a short term follow up of more than a month (6 weeks). However, the MAH is requested to provide an interim CSR, including a comprehensive safety analysis with a later cut-off for the mRNA-1273.214 50 µg booster dose group, once the data of the Day 91 interim analysis are available.

Serious Adverse Events

In the mRNA-1273.214 50 µg booster dose group (Part G), there were 3 SAEs reported in total, 2 events within 28 days and one event beyond 28 days as following: one severe traumatic pelvic fracture on a ≥60 year old female hospitalised on D14, and the second SAE was a prostate cancer. For both these events is agreed upon with the investigator assessment not to be related with the Study IP.

A Grade 3/SAE was reported beyond 28 days on a ≥60 year old male as nephrolithiasis. The participant had ongoing medical conditions and concomitant medications and developed kidney stones and was hospitalised, treatment in hospital included tamsulosin (Flomax), ibuprofen, hydrocodone acetaminophen (opioid/analgesic), Dilaudid (hydromorphone), and oxybutynin. Participant underwent right percutaneous nephrostomy tube placement; left ureteroscopy and an attempted cystoscopy with left ureteral stent. Patient was discharged five days after hospital admission and had a worsening nephrolithiasis five days later and being discharged again. The event resolved nine days after the event onset on Study Day 55. Since the data cut-off, and as of 27 Jul 2022, the site confirmed the event was considered ongoing as the kidney stone had not passed. The site will continue to monitor this event, which was assessed as not related to vaccination by the investigator ad it is agreed upon.

In the mRNA-1273 50 µg booster dose group (Part F), 1 participant had an SAE within 28 days of the booster dose, an event of lumbar osteoarthritis on Study Day 9. It is agreed upon that the investigator

judgement that the event was not related to vaccination. No SAE were reported beyond 28 days in part F of the study.
In the mRNA-1273.211 50 µg booster dose group (Part A), there were no SAEs up to 28 days while 5 SAEs have been reported in 4 participants after Day 28 and through the data cut-off date. The events included 1 event of dehydration and hypotension concurrently, one event of myocardial infarction (fatal event), 1 SAE as peripheral arterial occlusive disease, and 1 SAE of cholelithiasis. None of the SAEs were considered treatment related by the Investigator or MAH and it is agreed upon.

Deaths

No deaths were reported in either the 50 µg mRNA-1273.214 booster dose group (P205 Part G) or the 50 µg mRNA-1273 booster dose group (P205 Part F).
One death was reported in the 50 µg mRNA-1273.211 booster dose group (Part A) 159 days after vaccination. A ≥60 year old male with a medical history of type 2 diabetes mellitus, hypertension, hyperlipidaemia, and severe obesity had a fatal myocardial infarction 159 days after receiving the mRNA-1273.211 50 µg booster dose. The investigator assessed the event as not related to mRNA-1273.211. It is agreed with the investigator judgement that the event is not related to the study IP and the assessment of relatedness might have been confounded by participant's underlying cardiovascular and metabolic comorbidities.

Medically-Attended ARs and Vaccine Related ARs

In the P205 Part G study the number of MAAEs within 28 days was 52 out of a total of 74 and the most common reported were: COVID-19 (1.1%) and upper respiratory tract infection (1.1%) followed by coronavirus infection (non-COVID-19/0.7%), procedural pain (0.7%), and fatigue (0.5%). The Two non-serious MAAEs in 2 participants assessed as related to the Study IP by the Investigator were an event of moderate fatigue on Study Day 2- Day 13 in a ≥60 year old female with a relevant medical history of chronic obstructive pulmonary disease and iron deficiency. The other event was a mild dermatitis on Day Study 7- 19. The Treatment included oral and topical diphenhydramine hydrochloride. There was event of nephrolithiasis beyond 28 days is described in the sections of serious adverse events.

In P205 Part F, the number of MAAEs reported within and beyond 28 days was almost comparable, respectively 56 and 48, with a total number of 104 MAAEs. The most common reported were: Upper respiratory tract infection (2.4%), followed by coronavirus infection (2.1%), urinary tract infection (0.8%), hypertension (0.8%), rhinovirus infection (0.5%), and urticaria (0.5%). Two non-serious MAAEs in 2 participants within 28 days were assessed as related to Study IP and were one event of mild hypertension and the other an event of generalised urticaria, with a duration from D18- D22 and treatment oral diphenhydramine hydrochloride. One MAAEs with onset beyond 28 days, was an event of worsening of chronic back pain, at the time of the data cut-off, the event was ongoing.

In the mRNA-1273.211 50 µg booster dose group (Part A) up to the data cut-off were reported in total 268 MAAEs and from them 22 MAAEs were within 28 days after the booster dose. The most commonly reported MAAEs were COVID-19 (12.3%); rhinovirus infection (3.0%), viral infection (2.7%), urinary tract infection (2.3%), hypertension (2.3%), sinusitis (2.0%), and anxiety (2.0%). One participant had

an MAAE that was assessed by the investigator as related to the study IP, a grade 2 event of arthralgia started from D3-D16 and also a reactivation beyond 28 Days.

Adverse Events of Special Interest

Up to the data cut-off date, no participants in the mRNA-1273.214 50 µg booster dose group (Part G) had an investigator-assessed AESI.

In the mRNA-1273 50 µg booster dose group (Part F), 1/377 participants (0.3%) had an investigator-assessed AESI. There was reported a moderate non-serious irregular heart rate (verbatim: unspecified irregular heartbeat) on a ≥60 year old male, on Day 17 after the mRNA1273 50 µg booster dose, with a relevant medical history including hypertension, Type 2 diabetes mellitus, hyperlipidaemia, osteoarthritis, prostate cancer, and seasonal allergy. Upon request information has been submitted by the MAH and the event term was updated to supraventricular tachycardia following diagnosis by a cardiologist. At the time when this application was submitted for assessment the participant was awaiting treatment with intravenous adenosine for cardioversion followed by cardiac ablation. The event of supraventricular tachycardia was reported as non-serious and moderate in intensity, and the Investigator assessed the event as not related to vaccination. It is agreed upon, however close monitoring is recommended until all the procedures have been performed.

In the mRNA-1273.211 50 µg (P205 Part A), there were no investigator-assessed AESIs up to 28 days after the mRNA-1273.211 50 µg booster dose but one participant (0.3%) had an investigator-assessed AESI of fatal acute myocardial infarction on Study Day 159, considered by the investigator to be unrelated to vaccination and described accordingly in the section of SAEs and deaths.

SMQ Hypersensitivity, Narrow and Broad Scope:

In Study 205 Part G, regarding the events assessed as Study IP related they include one event of mild dermatitis, with the duration from on Day 7- Day 19 and treatment included oral and topical diphenhydramine hydrochloride. Another event of mild urticarial, from Day 2- Day 34, not medically attended, was considered by the investigator to be related to vaccination, and it is agreed upon. Hypersensitivity is already included in section 4.8 of the SmPC.

Cardiac Arrhythmia SMQ: Upon request, information has been provided for the event of 'Tachycardia' captured in the Cardiac Arrhythmia SMQ Assessment in a 50-59 year old female participant who received mRNA-1273.214 (Part G). The participant, a 50-59 year old female, received a second booster dose of 50 ug mRNA-1273.214, medical history includes chronic diseases and concomitant medications. On Study Day 5, the participant developed non-serious grade 2 / moderate symptomatic COVID-19 characterised by chills, cough, fatigue headache, sore throat, congestion and a runny nose. On the same day, treatment for the COVID-19 infection included oral dextromethorphan 10 mg; paracetamol 325 mg; phenylephrine 5 mg. Two days later, on Study Day 7, the participant experienced non-serious grade 2 / moderate tachycardia (reported verbatim: tachycardia [unknown aetiology]) that was self-limiting and resolved the same day. On the same day, treatment for the COVID-19 infection ended and on Study Day 37, the symptomatic COVID-19 infection was considered fully resolved. The Investigator assessed the event of tachycardia as not related to vaccination and it is agreed upon, most likely it was confounded by the concurrent symptomatic COVID-19 infection and treatment with phenylephrine.

Upon request information has been provided on the 'unspecified irregular heartbeat' on Day 17 after the mRNA1273 50 µg booster dose. The site updated the event term from irregular heart rate to supraventricular tachycardia following diagnosis by a cardiologist. The participant had commenced treatment with Pradaxa approximately three months earlier (reported as ongoing) and at the time when this application was submitted for assessment the participant was awaiting treatment with intravenous adenosine for cardioversion followed by cardiac ablation. The event of supraventricular tachycardia was reported as non-serious and moderate in intensity, and the Investigator assessed the event as not related to vaccination. It is agreed upon, however close monitoring is recommended until all the procedures have been performed.
No imbalance with regard to the SMQ Cardiomyopathy (Narrow and Broad Scope) has been identified in the two parts G and F, and the additional information has been adequately submitted from MAH.
However, as the duration of follow up has been limited to 43 days in the mRNA-1273.214 50 µg booster dose group, MAH has been asked for a further safety analysis providing information on SAEs, AESIs and MAAEs with a later cut-off. MAH has not performed further safety analyses beyond the data-cut timepoint in the Day 29 interim analysis of study P205 part G (43 days of follow-up time), but MAH is planning a day 91 interim analysis and the MAH will have report of the day 91 interim analysis by 31 December 2022.
Therefore, it is concluded that the reactogenicity of mRNA-1273.214 50 µg as booster dose is covered sufficiently with a short term follow up of 43 days.

Evaluation of myo-and pericarditis

The CMQ analysis did not identify any cases fulfilling the CDC working case definition for probable or confirmed cases of acute myocarditis or acute pericarditis in P205 Parts G, F, and A.
No additional events of potential myocarditis or pericarditis were identified with the CMQ that had not already been captured in the SMQ analyses in the mRNA-1273.214 50 µg (Part G); mRNA-1273 50 µg (Part F) booster dose groups or in the mRNA-1273.211 50 µg (Part A) booster dose group.

The Myocarditis and Pericarditis in Individuals Receiving a Third Dose/ Post-marketing experience

Cumulatively as of 15 Apr 2022, there were reported 283 events of myocarditis and 148 events of pericarditis following a third dose of mRNA-1273. The cases involved 272 males (65.9%) and 140 females (33.9%), with a mean age of 42.9 years and a median age of 40 years. The time to onset from vaccination was less than 7 days for 69.1% of the events.
Upon request, data were provided on the number of myocarditis and pericarditis following dose 4.
Cumulative as of 18 July 2022, there have been 7 reports of myocarditis after receiving a dose 4 of Spikevax, 5 males and 2 females. All the reports were considered serious (SAEs). Median age for these reports was 75 years old (min: 40/ max: 81), with a time onset from 1 day to 102 days. All cases are from the European Economic Area. WHO-UMC causality assessment has been used and two cases were considered unlikely related to the vaccine due to associated comorbidities that provide a more plausible explanation for the occurrence of the events. The other 5 cases were considered not assessable due to the lack on important information including any laboratory or diagnostic results. Close monitoring is

recommended, and MAH had implemented an enhanced surveillance on the events of myocarditis and pericarditis after vaccination with mRNA based COVID-19 vaccines.

Discontinuations

No participants in either the mRNA-1273.214 group or the mRNA-1273 group discontinued due to a Treatment related adverse event (TEAE). Regarding the 2 participants who discontinued the study in the mRNA- 1273.214 50 µg booster dose group, for both the discontinuation reasons did not include adverse events. This applied also in Study P205 Part A, where no participants who received mRNA-1273.211 50 µg discontinued due to a TEAE.

6.2. Conclusions on clinical safety

In conclusion, based on the evaluation of all safety-related data reveals that the clinical safety profile of mRNA-1273.214 50 µg when administered as second booster dose is comparable to that of the currently approved monovalent booster vaccine mRNA-1273 50 µg (Wuhan-Hu-1). Slightly higher incidences of local and systemic adverse events were observed, when comparing mRNA-1273.214 50 µg administered as second booster to mRNA-1273 50 µg given as booster dose. All these adverse events fully resolved after several days. However, these minor differences are not clinically relevant and are not considered to have a significant impact on the safety profile of mRNA-1273.214 when compared to that of mRNA-1273.

The safety set included 437 participants in Study P205 in Part G and 377 participants in Part F.

Safety and reactogenicity of mRNA-1273.214 50 µg was consistent between participants with and without SARS-CoV-2 infection prior to the booster dose.

Data from the long-term (up to Day 181) safety follow up of the Beta-containing bivalent vaccine (mRNA-1273.211) has also been evaluated as an estimate for mRNA-1273.214 bivalent vaccine safety. No new safety signals or concerns have been observed to date with this bivalent vaccine. No new safety signals were identified during trial P205 in the Part G, Part F and Part A until the respective data cut-off.

The safety short term follow-up was up to 43 days for the Study P205 Part G and Part F, with a cut-off date April 27, 2022. The MAH is planning a day 91 interim analysis and the MAH will have report of the day 91 interim analysis by 31 December 2022.
It is concluded that the reactogenicity of mRNA-1273.214 50 µg as booster dose is covered sufficiently with a short term follow up of more than a month and that no concerns are emerging from the available data set regarding the safety profile of this bivalent vaccine.

6.3. Clinical recommendations

The MAH provided a commitment to comply with the following clinical recommendations:

Immunogenicity

The MAH should provide the PsVNA validation reports for Omicron variants BA.1 and BA.4/BA.5 once available. Validation of these assays is expected to be completed prior to further analyses for Day 91 interim analysis.

The MAH should provide results on T-cell mediated immunity, as well as analyses of the genetic and/or phenotypic relationships of isolated SARS-CoV-2 strains to the vaccine sequence by characterising "the SARS-CoV-2 genomic sequence of viral isolates and compare with the vaccine sequence, and to characterise the immune responses to vaccine breakthrough isolates" for the Day 91 interim analysis.

Safety

The MAH should provide an interim CSR, including a comprehensive safety analysis with a later cut-off for the mRNA-1273.214 50 µg booster dose group, once the data of the Day 91 interim analysis are available.

7. Risk management plan

The MAH submitted an updated RMP version 4.2 with this application. The (main) proposed RMP changes were the following:

Summary of significant changes in this RMP:

Compared to the previously approved Spikevax European Union (EU) RMP version 4.0, this RMP version 4.2 has been updated:

- To add Spikevax bivalent Original/Omicron BA.1 as a new medicinal product to the RMP
- To add the INN elasomeran/imelasomeran
- To update the epidemiology of the Omicron variant
- To add clinical study exposure for - mRNA-1273-P205 relating to Spikevax bivalent
- To update clinical study exposure for mRNA-1273-P201 (Part B) as 50 µg Spikevax booster vaccine in this study is used as a comparator for 50 µg mRNA-1273.211 booster vaccine in mRNA-1273-P205 (P205 Part A)
- To add myocarditis or pericarditis as exclusion criteria in pivotal clinical studies in Section SIV.1
- To include mRNA-1273-P205 as a Category 2 study to the Pharmacovigilance Plan to further characterise long-term safety of Spikevax bivalent and to update other relevant sections of the RMP
- To remove study mRNA-1273-P902 and to add mRNA-1273-P919 as a Category 3 study to the Pharmacovigilance Plan as per the outcome of MEA 005.3. Enrolment of study mRNA-1273-P902 has been insufficient to meet study objectives, and further challenges are expected given limited use of Spikevax in individuals <30 years of age in some European countries. Data concerning safety in pregnancy will be obtained based on the ongoing mRNA-1273-P905 study in Europe and the new secondary database study in the US (mRNA-1273-P919).
- To add the study protocols for mRNA-1273-P205 and mRNA-1273-P919, and to remove the study protocol for mRNA-1273-P902 in Annex 2
- To add study protocol details for mRNA-1273-P205 and mRNA-1273-P919, to remove study protocol details for mRNA-1273-P902, and to update study protocol details for mRNA-1273-P203 in Annex 3
- To update the pharmacotherapeutic group and MAH address in line with the approved SmPC

7.1. Overall conclusion on the RMP

The changes to the RMP are acceptable.

8. Update to the Product Information

As a result of this group of variation, the SmPC, Labelling and the Package Leaflet have been updated (see Attachment 1).

8.1.1. Labelling exemptions

The following exemptions from labelling requirements have been granted on the basis of Article 63.3 of Directive 2001/83/EC. In addition, the derogations granted should be seen in the context of the flexibilities described in the Questions and Answers on labelling flexibilities for COVID-19 vaccines (EMA/689080/2020 rev.1, from 16 December 2020) document which aims at facilitating the preparedness work of COVID-19 vaccine developers and the associated logistics of early printing packaging activities. The ultimate goal is to facilitate the large scale and rapid deployment of COVID-19 vaccines for EU citizens within the existing legal framework.

Labelling exemptions

Outer and immediate labelling (from start of supply to end of October 2022).

All EU Member States have agreed to grant a temporary exemption for the use of the 120-200 initial PPQ batches with a "temporary labelling". The exemption is granted until end of October 2022. These exemptions are justified on the necessity to label batches ahead of time.

Outer carton

- Invented name qualifier: "Spikevax 0 (Zero) / O (Omicron)" (initially proposed), instead of "Spikevax bivalent Original/Omicron BA.1" (agreed during evaluation).
- INN/common name: "COVID-19 mRNA Vaccine (nucleoside modified)" (common name initially proposed), instead of common name "COVID-19 mRNA Vaccine (nucleoside modified)" and INN "elasomeran/imelasomeran" (during evaluation).
- Strength: "0.1 mg/mL' (initially proposed)", instead of "(50 micrograms/50 micrograms)/mL" (agreed during evaluation).
- Statement of the active substance: "One dose (0.5 mL) contains 25 micrograms of elasomeran and 25 micrograms of imelasomeran" (agreed during evaluation).

Vial label

- Invented name qualifier: "Spikevax 0 / O"' (initially proposed), instead of "Spikevax bivalent Original/Omicron BA.1" (agreed during evaluation).
- INN or common name: "COVID-19 mRNA Vaccine (nucleoside modified)" (common name initially proposed), instead of INN "elasomeran/imelasomeran" (agreed during evaluation).
- Strength: "0.1 mg/mL' (initially proposed)", instead of "(50 mcg/50 mcg)/mL" (agreed during evaluation).

9. Overall conclusion and impact on the benefit-risk balance

The MAH has developed a modified bivalent vaccine mRNA-1273.214 (Spikevax bivalent Original/Omicron BA.1) which contains 25 µg each of mRNA encoding the ancestral SARS-CoV-2 spike sequence and the Omicron BA.1 spike sequence in response to the continued emergence of SARS-CoV-2 variants. With the present variation procedure, the MAH is seeking approval for mRNA-1273.214 to be used as 1st or 2nd booster vaccine in adolescents and adults ≥12 years of age and older at least 3 months following a primary series or previous booster dose with Spikevax or another authorised mRNA based or adenoviral

vectored COVID-19 vaccine. In support of this claim, the MAH has submitted data from clinical trials addressing the immunogenicity, safety and reactogenicity.

Vaccine efficacy is inferred from immunological endpoints, mostly titres of neutralising antibodies. No efficacy/effectiveness studies have been conducted in support of the present variation procedure. Results from study P205 Part F and Part G are presented for comparison of neutralising antibody titres after 2nd booster vaccinations with either bivalent mRNA-1273.214 or monovalent mRNA-1273. Neutralising antibody titres against the ancestral (Wuhan-Hu-1) strain and SARS-CoV-2 variants Omicron BA.1 and BA.4, BA.5 are compared, as well as the geometric mean fold rise and geometric mean ratio. Statistical analysis of the results on Day 29 supported superiority in eliciting neutralising antibody responses after 2nd booster vaccination with the bivalent mRNA-1273.214 vs. original mRNA-1273. ELISA results on binding antibodies against ancestral SARS-CoV-2 S-protein and SARS-CoV-2 variants supports the broadening of the immune response after vaccination with mRNA.1273.214.

The MAH presented supporting immunogenicity data on 1st booster vaccination from study P205. In study P205 Part A another bivalent vaccine mRNA-1273.211 (Original + Beta) 50 µg was used as a 1st booster vaccination. A booster-to-booster comparison of neutralising antibody results after 1st booster vaccination with mRNA-1273.211 vs. mRNA-1273 from study P201 Part B is presented. Statistical analysis of the results supported superiority in eliciting neutralising antibody responses after 1st booster vaccination with the bivalent mRNA-1273.211 vs. original mRNA-1273 on Day 29 and Day 181.

Incidence rates of COVID-19 after 2nd booster vaccination with mRNA-1273.214 or mRNA-1273 need to be considered descriptive as study P205 was not designed to assess efficacy. In addition, P205 Part F and Part G have not been enrolled in parallel and as a consequence the viral landscape of SARS-CoV-2 variants might have been different with regards to prevalent strains.

SARS-CoV-2 infections occurred in both treatment arms with a higher incidence after vaccination with mRNA-1273.214. However, none of the infected participants had an ER visit or hospitalisation. Out of 11 cases in mRNA-1273.214 vaccinated participants 5 have sequencing data available that demonstrate variant Omicron BA.2 infection, in line with the prevalence of Omicron BA.2 at that time. No sequencing data are presently available for cases in the mRNA-1273 vaccinated group.

The MAH is seeking approval for the bivalent mRNA-1273.214 (Original + Omicron) as a 1st or 2nd booster immunisation to prevent from COVID-19 caused by SARS-CoV-2 in adolescents and adults aged 12 years of age and older. Study P205 has enrolled adults ≥18 years of age and older.

The MAH has previously received authorisation for the use of the original mRNA-1273 50 µg as a 1st booster vaccination in adolescent ≥12 to 17 years of age and in adults ≥18 years of age and older. Authorisation was granted based on immunobridging between adolescents ≥12 to 17 years of age compared to young adults ≥18 to 25 years of age. As there is no obvious scientific reason to assume that basic immunogenicity characteristics would be significantly different for the immunobridging approach applied for the approval of the mRNA-1273 booster dose in adolescents this is considered valid also for mRNA-1273.214.

Bivalent mRNA-1273.211 50 µg (Original + Beta) was compared to mRNA-1273 as a 1st booster dose after the primary vaccination series, indicating efficient increase in neutralising antibodies against ancestral SARS-CoV-2 and variant Beta, and a broadening of the immune response to other variants.

The safety set included 437 participants in Study P205 in Part G and 377 participants in Part F.

The evaluation of all safety-related data reveals that the clinical safety profile of mRNA-1273.214 50 µg when administered as second booster dose is comparable to that of the currently approved monovalent booster vaccine mRNA-1273 50 µg (Wuhan-Hu-1). Slightly higher incidences of local and systemic adverse events were observed, when comparing mRNA-1273.214 50 µg administered as second booster

to mRNA-1273 50 µg given as booster dose. All these adverse events fully resolved after several days. However, these minor differences are not clinically relevant and are not considered to have a significant impact on the safety profile of mRNA-1273.214 when compared to that of mRNA-1273.

Data from the long-term (up to Day 181) safety follow up of a Beta-containing bivalent vaccine (mRNA-1273.211) has also been evaluated as a supportive for mRNA-1273.214 bivalent vaccine safety. No new safety signals or concerns have been observed to date with this bivalent vaccine.

Taken together, the indication for bivalent mRNA-1273.214 50 µg (Spikevax bivalent Original/Omicron BA.1) as a 1st or 2nd booster dose after the primary vaccination series is deemed acceptable based on the totality of the serological evidence evaluating different vaccination regimens.

Based on review of the immunogenicity and safety data of mRNA-1273.214, the benefit-risk profile of mRNA-1273.214 50 µg as regards the clinical aspects is considered favourable, independently of the information requested via recommendations.

The MAH will continue to closely monitor and characterise the safety profile of modified vaccines in clinical studies and via post-authorisation pharmacovigilance activities.

The MAH has provided a commitment to comply with a number of quality and clinical recommendations (see sections 4.3 and 7.3).

The benefit-risk balance is considered positive in the applied indication.

10. Recommendations

Outcome

Based on the review of the submitted data, this application regarding the following changes:

Variations requested		**Type**	**Annexes affected**
B.I.a.6.a	B.I.a.6.a - Changes to the active substance of a vaccine against human coronavirus - Replacement or addition of a serotype, strain, antigen or coding sequence or combination of serotypes, strains, antigens or coding sequences for a human coronavirus vaccine	Type II	I, II, IIIA, IIIB and A

B.I.a.6.a (Type II): Addition of a new strain (Omicron BA.1) resulting in two new Spikevax bivalent Original/Omicron BA.1 (50 micrograms elasomeran / 50 micrograms imelasomeran)/mL 0.1 mg/mL dispersion for injection presentations. The Annex A, the SmPC, the labelling and the Package Leaflet and Labelling are updated accordingly. A revised RMP version 4.2 has been approved. The variation also includes a number of quality scopes.

The information between these lines is considered commercially confidential and may not be disclosed to third parties in accordance with the ''HMA/EMA guidance on the identification of commercially confidential information and personal data'.

Variations requested		Type	Annexes affected
B.II.b.3.c	B.II.b.3.c - Change in the manufacturing process of the finished or intermediate product - The product is a biological/immunological medicinal product and the change requires an assessment of comparability	Type II	I, IIIA and IIIB
B.II.b.1.c	B.II.b.1.c - Replacement or addition of a manufacturing site for the FP - Site where any manufacturing operation(s) take place, except batch release/control, and secondary packaging, for biol/immunol medicinal products or pharmaceutical forms manufactured by complex manufacturing processes	Type II	I, IIIA and IIIB
B.I.a.4.b	B.I.a.4.b - Change to in-process tests or limits applied during the manufacture of the AS - Addition of a new in-process test and limits	Type IA	I, IIIA and IIIB
B.I.a.2.c	B.I.a.2.c - Changes in the manufacturing process of the AS - The change refers to a [-] substance in the manufacture of a biological/immunological substance which may have a significant impact on the medicinal product and is not related to a protocol	Type II	I, IIIA and IIIB
B.I.a.2.a	B.I.a.2.a - Changes in the manufacturing process of the AS - Minor change in the manufacturing process of the AS	Type IB	I, IIIA and IIIB
B.I.d.1.b.2	B.I.d.1.b.2 - Stability of AS - Change in the storage conditions - Change in storage conditions of biological/immunological ASs, when the stability studies have not been performed in accordance with a currently approved stability protocol	Type II	I, IIIA and IIIB
B.I.a.4.a	B.I.a.4.a - Change to in-process tests or limits applied during the manufacture of the AS - Tightening of in-process limits	Type IA	I, IIIA and IIIB
B.II.b.3.a	B.II.b.3.a - Change in the manufacturing process of the finished or intermediate product - Minor change in the manufacturing process	Type IB	I, IIIA and IIIB
B.I.d.1.c	B.I.d.1.c - Stability of AS - Change in the re-test period/storage period or storage conditions - Change to an approved stability protocol	Type IA	I, IIIA and IIIB
B.II.e.5.c	B.II.e.5.c - Change in pack size of the finished product - Change in the fill weight/fill volume of sterile multidose (or single-dose, partial use) parenteral medicinal products, including biological/immunological medicinal products	Type II	I, IIIA and IIIB
B.II.d.2.d	B.II.d.2.d - Change in test procedure for the finished product - Other changes to a test procedure (including replacement or addition)	Type IB	I, IIIA and IIIB
B.II.d.1.e	B.II.d.1.e - Change in the specification parameters and/or limits of the finished product - Change outside the approved specifications limits range	Type II	I, IIIA and IIIB
B.I.b.1.f	B.I.b.1.f - Change in the specification parameters and/or limits of an AS, starting material/intermediate/reagent -	Type II	I, IIIA and IIIB

	Change outside the approved specifications limits range for the AS		
B.I.d.1.a.3	B.I.d.1.a.3 - Stability of AS - Change in the re-test period/storage period - Extension of storage period of a biological/immunological AS not in accordance with an approved stability protocol	Type II	I, IIIA and IIIB
B.II.b.3.b	B.II.b.3.b - Change in the manufacturing process of the finished or intermediate product - Substantial changes to a manufacturing process that may have a significant impact on the quality, safety and efficacy of the medicinal product	Type II	I, IIIA and IIIB

☒is recommended for approval.

Amendments to the marketing authorisation

In view of the data submitted with the group of variations, amendments to Annexes I, II, IIIA, IIIB and A and to the Risk Management Plan are recommended.

11. EPAR changes

The EPAR will be updated following Commission Decision for this variation. In particular the EPAR module 8 "*steps after the authorisation*" will be updated as follows:

Scope

Please refer to the Recommendations section above

Summary

Please refer to Scientific Discussion 'Spikevax-H-C-005791-II-0075-G'

12. Attachments

1. Product Information (changes highlighted) as adopted by the CHMP on 1 September 2022

Literature reference

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Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021a;384(5):403-16. doi: 10.1056/NEJMoa2035389.

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