FOOD AND DRUG ADMINISTRATION
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

MEMORANDUM

Review Memorandum

March 12, 2025

To:	(b) (6), OVRR, CBER, FDA
From:	(b) (6), OVRR, CBER, FDA
Through:	(b) (6) OVRR, CBER, FDA
Through:	(b) (6), OVRR, CBER, FDA
	(b) (6), OVRR, CBER, FDA
	(b) (6), OVRR, CBER, FDA
Subject:	STN 125817/0 Submitted 1-31-2024
Product Name	Nuvaxovid, Novavax COVID-19 Vaccine, Adjuvanted
Applicant	Novavax
Proposed Indication	For active immunization for the prevention of coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 in individuals 12 years of age and older.
Action Due Date	April 1st 2025
Cross-reference(s)	DMF (b) (4) Phosphatidylcholine (PC) (b) (4)

Review of the Chemistry, Manufacturing, and Control information (CMC) relevant to Matrix-M adjuvant submitted as part of the initial Biologics License Application (BLA)

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1.0 EXECUTIVE SUMMARY AND RECOMMENDATION

1.1 NOVAVAX COVID-19 NANOPARTICLE VACCINE DRUG PRODUCT DESCRIPTION

Product name: Novavax COVID-19 Vaccine, Adjuvanted
Proprietary name: Nuvaxovid
Product Type: SARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine (SARS-CoV-2 rS) with Matrix-M Adjuvant

SARS-CoV-2 rS Protein (COVID-19) Nanoparticle Vaccine is a recombinant spike (rS) protein vaccine for the prevention of COVID-19 disease caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). The vaccine is a sterile aqueous buffered suspension containing (b) (4) µg/mL of SARS-CoV-2 rS Protein co-formulated with (b) (4) µg/ml of Matrix-M adjuvant and Formulation Buffer (containing sodium phosphate heptahydrate and monohydrate, sodium chloride, and polysorbate 80). The active substance is the protein product of a recombinant SARS-CoV-2 S-gene encoding the (b) (4) The SARS-CoV-2 rS protein has been genetically modified to confer protease resistance and to preserve a stable prefusion conformation. SARS-CoV-2 rS Protein Drug Substance (DS) is produced in Sf9 insect cell line using Recombinant Baculovirus system.

Matrix-M is a saponin-based adjuvant included in the SARS-CoV-2 rS Protein Vaccine Drug Product (DP). Matrix-M adjuvant is composed of Matrix-A and Matrix-C adjuvant components, each produced from saponin materials: lyophilized Fraction-A and Fraction-C, respectively. Matrix-A and Matrix-C are mixed at (b) (4) ratio (%) to formulate Matrix-M adjuvant immediately prior to adding to rS DS during DP manufacture. Matrix-A and Matrix-C are nanoparticles suspended in phosphate buffered saline (PBS) solution at pH (b) (4). Cholesterol and phosphatidylcholine are present as excipients in the Matrix-A and Matrix-C solutions.

The final vaccine product is a sterile, preservative free suspension presented in a single dose Pre-Filled Syringe or in 5-dose or 10-dose multidose vials. A single 0.5 mL human dose of DP contains 5 µg of rS protein and 50 µg of Matrix-M adjuvant. The recommended storage condition of DP is 2 to 8°C.

The vaccine is administered intramuscularly as a series of two doses (0.5 mL each) 3 weeks apart for individuals 12 years of age and older not previously vaccinated with any COVID-19 vaccine. For

individuals previously vaccinated with any COVID-19 vaccine, a single dose (0.5 mL) is administered ≥ 2 months after receipt of the last dose of COVID-19 vaccine. An additional 0.5 mL dose may be administered to individuals with certain kinds of compromised immunity ≥ 2 months after the most recent dose of any COVID-19 vaccine.

Mode of action The saponin-based Matrix-M adjuvant facilitates activation of the cells of the innate immune system, which enhances the magnitude of the B- and T-cell immune responses to the S protein, including neutralizing antibodies, which may contribute to protection against COVID-19.

This review covers quality-related information in Module 3 of BLA 125817 related to manufacture of Matrix-M adjuvant Drug Substance and SARS-CoV-2 rS Protein DP submitted in the following BLA amendments. This review memo is complementary to the review memo prepared by (b) (6) product reviewer, (b) (6) . Complete list of abbreviations used the review memo is provided in Appendix 1.

1.2 MATRIX-A AND MATRIX-C ADJUVANT COMPONENTS

Matrix-A and Matrix-C are regularly shaped, uniform, and stable complexes with an average size of approximately (b) (4) by (b) (4) that are suspended in phosphate buffered saline (PBS) solution at pH (b) (4) Matrix-A and Matrix-C adjuvant components are each produced separately from Saponin-A and Saponin-C, which are aqueous solutions of starting materials: lyophilized Fraction-A and Fraction-C, respectively. Fraction-A and Fraction-C are isolated by preparative (b) (4) from the aqueous extract of the bark of the soap tree, *Quillaja Saponaria* Molina, enriched for saponins. Several measures are in place to control for impurities in Fraction-A/-C including control over the agricultural areas where *Quillaja saponaria* trees are grown. (b) (4) process was designed to remove contaminants such as (b) (4) from the aqueous extract. The preparative (b) (4) process separates out impurities for which the retention on the (b) (4) differs from the saponins. An improved manufacturing process for isolation of Fraction-A and Fraction-C from the bark extract, (b) (4) was introduced in 2014. In (b) (4) saponin (used in the original (b) (4) was replaced with a (b) (4) saponin (b) (4) saponin content). (b) (4) was developed at (b) (4) and was used to produce Fraction-A and Fraction-C materials for Phase-1 to Phase-3 clinical studies. During 2020/2021, the (b) (4) process was (b) (4) Comparability of lyophilized Fraction-A (b) (4) batches) and Fraction-C (b) (4) batches) starting material manufactured at (b) (4) was confirmed by comparing analytical attributes including saponin (b) (4) and by demonstrating (b) (4) of saponin (b) (4) for batches manufactured at the three sites.

The production of Matrix-A and Matrix-C particles starts with preparation of (b) (4)

(b) (6)

(b) (4)

Manufacture of Matrix-A and Matrix-C was developed at Novavax AB (Uppsala, Sweden) at (b) (4) production scale, was scaled up to (b) (4) at Novavax-AB, and was tech transferred and scaled up to (b) (4) production scale at AGC Biologics, Copenhagen, Denmark (AGC-CPH). The scaled-up process is mostly a like-for-like scale-up of the original (b) (4) process at Novavax-AB. The acceptance criteria for all In Process Controls (IPC) are identical or very similar between both sites. Additional IPC were introduced due to scaled up process. (b) (4) types of container closure are used for packaging of concentrated Matrix-A and Matrix-C (b) (4) adjuvant components: (b) (4) (AGC-CPH).

To support commercial manufacture, in-process, release, and characterization data for process performance qualification (PPQ) batches for Matrix-A and Matrix-C adjuvant components were provided from Novavax-AB and AGC-CPH manufacturing sites. PPQ data is available for Matrix-A and Matrix-C produced at both manufacturing sites for all (b) (4) production scales. Certificates of Analysis (CoAs) for the (b) (4) GMP commercial Matrix-A and Matrix-C batches from each site, each scale, and both container closure systems are included in the submission and confirm consistent process performance.

The (b) (4) Matrix-A and (b) (4) Matrix-C reference batches were manufactured at Novavax AB (b) (4) using a validated process. The comparability studies were performed for Matrix-A and Matrix-C batches manufactured Novavax-AB at (b) (4) scale and at AGC-CPH at (b) (4) scale using respective reference batches. The results of comparability studies are provided for (b) (4) batches per site per scale. The assessment was based on the analytical comparability to reference batches at release with identical acceptance criteria between all sites and on the results of characterization tests to assess changes in the quality of the product that are not captured by the release assays. The data supported the conclusion that Matrix-A and Matrix-C manufactured at NVX-AB (b) (4) and at AGC-CPH (b) (4) are deemed equal to Matrix-A and Matrix-C, respectively, made at (b) (4) scale at Novavax-AB using a validated process.

The analytical procedures developed and used for the release and stability monitoring of Matrix-A and Matrix-C include non-compendial tests for purity, identity, impurities, and properties. All analytical procedures used for release of commercial supply of Matrix-M adjuvant have been adequately qualified. The summaries of the qualification results demonstrate specificity, precision, accuracy, robustness, and reproducibility for each evaluated analytical assay, indicating that they are suitable for intended use.

Stability studies have been designed to support the use of Novavax COVID-19 vaccine. All available stability data generated using the Matrix-A and Matrix-C lots at the two manufacturing sites support the release of adjuvant for formulation of SARS-CoV-2 rS protein vaccine for the BLA. Stability studies of Matrix-A and Matrix-C are on-going and will continue to be monitored. Data will be submitted to the BLA as they become available.

Biodistribution study of Matrix-M1 adjuvant following intramuscular injection was performed in mice and the report is provided in the BLA as part of the nonclinical package. This study demonstrated a rapid distribution of Matrix-M1 adjuvant from the injection site to the draining lymph nodes, thus excluding a depot effect as central to the mechanism of action for this adjuvant. The difference in clearance

patterns for saponins and cholesterol are suggestive of at least partial disassembly of the Matrix-particles in vivo.

1.3 SARS-COV-2 rS PROTEIN DRUG PRODUCT

Initial nonclinical and clinical development of the SARS-CoV-2 rS nanoparticle vaccine, Adjuvanted were conducted by Novavax-AB using rS antigen (DS) from Wuhan-Hu-1 strain of the SARS-CoV-2. Novavax manufactured batches of DP at their subcontracted GMP-compliant manufacturing sites Emergent BioSolutions (Rockville, MD, USA) and PAR (Par Sterile Products, LLC Rochester, MI, USA). The manufactured product was used in the clinical trials conducted in Australia, South Africa, the United Kingdom, and the USA. The SARS-CoV-2 rS Wuhan DP was granted Emergency Use Authorization (EUA) for individuals aged 18 years of age and older on July 13, 2022. The manufacturing process for Wuhan rS protein DS is described in the BLA 125817-0 (January 31, 2024).

The developed manufacturing process of SARS-CoV-2 rS nanoparticle Vaccine Adjuvanted DP was tech transferred to Serum Institute of India Pvt. Ltd. (SIIPL), Pune, Maharashtra, India in November 2021 for manufacturing of DP clinical (for Indian clinical study) and commercial batches. Initial production started at (b) (4) scale in the SIIPL development facility (Building No. (b) (4) SIIPL (b) (4)), and has been transferred to the facility (b) (4) site for batch scale up to (b) (4) and subsequently transferred to (b) (4) facility of (b) (4) premises for batch size formulated at (b) (4) scale and (b) (4) scale (both for multidose presentation), and later (b) (4) batches for single dose presentation. The transition from (b) (4) DP manufacturing facilities in USA to SIIPL included changes in the manufacturing process for SARS-CoV-2 rS protein and in DP formulation that are covered in detail by the product reviewer. Only DP manufactured at SIIPL will be used for commercial supply.

Due to changes in the predominant strain of circulating SARS-CoV-2, Novavax subsequently developed two more DP that contained rS protein antigen from Omicron XBB.1.5 variant and most recently from Omicron JN.1 variant. The Omicron XBB.1.5 (2023 - 2024 formula) and Omicron JN.1 variant (2024 – 2025 Formula) of the Novavax COVD-19 vaccine were granted EUA on October 3, 2023, and August 30, 2024, respectively.

The Omicron variant rS protein antigens represent full-length spike proteins in a stable prefusion conformation and are produced in Sf9 insect cell line using the same Recombinant Baculovirus system and process that was used to manufacture Wuhan rS DS. The DP containing the XBB.1.5 rS protein DS or the JN.1 rS protein DS has the same composition as the original DP containing Wuhan rS DS (Wuhan DP, authorized under EUA), which contains rS protein at a nominal concentration of (b) (4) of Matrix-M adjuvant. To achieve nominal concentration of (b) (4) µg/ml of rS protein, the JN.1 DP is formulated with rS DS at a range of (b) (4) There are no differences between the three DPs with respect to the usage of Matrix-A during DP formulation. The small (b) (4) of Matrix-C (b) (4) µg/mL) was introduced during the formulation of XBB.1.5 DP to ensure the concentration of Matrix-C is no lower than (b) (4) µg/mL at release and throughout the shelf life (same (b) (4) is applied to JN.1 DP). There were no other changes in DP formulation related to the adjuvant. There were changes in the DP formulation scale and final container during product development. The Wuhan DP was formulated at (b) (4) scale and filled in 5-dose (5DV) and 10-dose vials (10DV), respectively. XBB.1.5 DP is formulated at (b) (4) scale and filled in 5DV only. JN.1 DP is formulated at (b) (4) scale and filled in single use Pre-Filled Syringe (PFS).

To support commercial manufacture of COVID-19 vaccine, in-process, release, and characterization data for process performance qualification (PPQ) batches for Wuhan DP 10DV and 5DV, Omicron variant XBB.1.5 DP 5DV, and Omicron variant JN.1 DP PFS were provided from SIIPL manufacturing sites. Wuhan DP PPQ campaign included (b) (4) GMP batches manufactured at each scale (b) (4) and (b) (4) batches per scale were used to validate the filling process. Similar PPQ campaign was performed for XBB1.5 DP manufactured at (b) (4) scale for 5DV presentation. For JN.1 DP, (b) (4) PPQ campaigns were performed for DP formulated at (b) (4) scale, for DP batches formulated at a target rS protein concentration of (b) (4) µg/ml and at (b) (4) µg/ml. Each PPQ campaign for JN.1 DP involved (b) (4) batches of formulated DP and (b) (4) batches to validate the filling stage. The acceptance criteria for Key Process Parameters (KPP), Key Process Attributes (KPA), IPC, and CPP for formulated DP were met in all PPQ campaigns. Results of release testing for PPQ batches (filled) are reported. All analytical attributes (including Matrix-A and Matrix-C content and particle size) met acceptance criteria.

To support bridging of SIIPL DP batches with clinical lots manufactured by Novavax that were used in the pivotal Phase 3 clinical trial, multiple comparability studies were performed during development that cover changes in the manufacturing sites, strain change, the number of doses per container, and change in container closure. A generic protocol for comparability studies was developed to assure continuity of variant SARS-CoV-2 DP manufacturing by comparing analytical data and the results of characterization assays. Analytical comparability was performed between reference clinical batches of Wuhan DP manufactured at ESBI and Par and subsequent batches manufactured at SIIPL: Wuhan DP batches manufactured at (b) (4) scale and (b) (4) scale and filled in 5DV and 10DV, respectively; XBB1.5 DP manufactured at (b) (4) scale for 5DV presentation; XBB1.5 DP and JN.1 DP manufactured at (b) (4) scale and filled in PFS. Analytical parameters tested via non-compendial methods in comparability studies included rS protein identity, content, and potency and Matrix-A and Matrix-C content. The results of analytical testing showed that all lots met lot release specifications. Regarding adjuvant-related parameters, the Matrix-A and Matrix-C content was consistent in all batches used for analytical comparability and Matrix-A and Matrix-C concentrations in DP batches remained within acceptance criteria. Thus, the important ratio of Matrix-A to Matrix C of (b) (4) remained unchanged. In addition, characterization tests that primarily assayed adjuvant-related parameters: (b) (4) confirmed that the scaled-up manufacture, change in manufacturing sites, strain change, and change in container closure did not negatively affect quality of Matrix-M1, and its physicochemical characteristics remained consistent throughout development.

The release tests for DP includes testing for Matrix-A and Matrix-C content by (b) (4) method that detects a (b) (4) are tested at SIIPL and at Novavax-AB manufacturing sites, respectively, using validated methods.

There were very few changes in the manufacture of Matrix-A and Matrix-C adjuvant components in the BLA 125817 package compared to the information included in the EUA 28237. Issues that were identified during review of EUA 28237 were adequately addressed by the sponsor and the updated information was incorporated in the BLA. The major change since EUA was related to the (b) (4)

(b) (4) Thus, the commercial SARS-CoV-2 DP is formulated using Matrix-A and Matrix-C manufactured at Novavax-AB and AGC-CPH facilities only. Additional issues were identified during the BLA review related to the validation of (b) (4) methods for release testing of DP and characterization methods for cholesterol and phosphatidylcholine content and saponin integrity index in DP. All issues were communicated to Novavax and were adequately addressed by the sponsor.

The primary focus of this review memo (which is complementary to the review memo from (b) (6)) is Matrix-M adjuvant. Therefore, the manufacturing process for Matrix-A and Matrix-C DS is reviewed in detail and is split between two sections: Matrix-A and Matrix-C DS manufactured at Novavax-AB and Matrix-A and Matrix-C manufactured at AGC-CPH. The current BLA was submitted via rolling submission in January 2024, when the predominant strain or SARS-CoV-2 was Omicron variant XBB.1.5. As agreed with CBER, in the follow up Amendment, the sponsor submitted dossier for XBB1.5 DP (Formula 2023 – 2024) and a dossier for Wuhan DP as a reference since Wuhan DP was used in Phase 3 clinical trial (both in 3.2.P) on April 1, 2024. The applicant updated DP to Formula 2024 – 2025 and added information on manufacture of JN.1 DP on October 31, 2024. Because of the schedule of submissions, the three DPs were reviewed and presented in this memo in the following order: review of Omicron XBB.1.5 DP, followed by JN.1 DP, and Wuhan DP.

1.4 CONCLUSIONS AND RECOMMENDATION

In summary, the CMC information, and data relevant to the Matrix-M1 (Matrix-M) adjuvant presented in this BLA is adequate to demonstrate that the Matrix-M1 adjuvant is manufactured under GMP by a validated process and the Matrix-M1 adjuvant meets accepted standards of purity and quality as required for an adjuvant as per 21 CFR 610.15. Overall, the Matrix-M1-relevant CMC information presented in the Quality Module of the BLA supports its use for the manufacture of Novavax COVID-19 Vaccine, Adjuvanted [Nuvaxovid] under this BLA.

2.0 INFORMATION REQUESTS (IR) SUBMITTED TO NOVAVAX RELATED TO MATRIX-M ADJUVANT, COMPANY UPDATE AND CONCLUSIONS

1. In IR1 (December 13, 2024), the firm was informed that CBER does not agree with change in the acceptance criteria for repeatability in supplementary validation for the (b) (4) method (b) (4) for JN.1 DP as described in the report QAG_27777 (Amendment 42).

Company's response (Amendment 55, Sequence 58, received December 18, 2024). Novavax agrees with CBER's feedback to align the acceptance criteria for (b) (4) method validation for all strains, since the (b) (4) method remains the same. The tighter acceptance criteria used for JN.1 DP was set to better reflect the range of the specification, completed after the original validation was performed. Novavax has now aligned the acceptance criteria for repeatability for JN.1 DP with the previously analyzed strains, (b) (4) for (b) (4). The validation results are still within the new acceptance criteria. Validation of (b) (4) method (b) (4) for JN.1 DP is valid. Updated validation report,

QAG_27777 ver. 2.0 is attached. (b) (4) was revised as requested by CBER.
Reviewer's assessment: the response is acceptable.

2. In IR2 (December 23, 2024), the firm was asked to provide a detailed information on the characterization methods (including controls and system suitability criteria) used for cholesterol, phosphatidylcholine content analysis, and Saponin Integrity Index for XBB1.5, JN.1, and Wuhan DP PPQ lots during stability studies per stability protocols provided in stability sections 3.2.P.8.1 for DP (BLA 125817 Amendment 4 SN 005 and Amendment 42 SN 044) and to indicate the validation status of these assays.

Company's response (Amendment 59, Sequence 61, received 1-5-2025). The DP dossier has been updated with detailed information on the characterization methods for Cholesterol and Phosphatidylcholine (PC) content, Saponin Integrity Index (SII) and Saponin Degradation Index (SDI). The Cholesterol and PC content method has been used for Wuhan, XBB.1.5, and JN.1 DP PPQ lots. SII has been used for the Wuhan DP PPQ lot and SDI, which is a further development of Saponin Integrity Index, has been used for XBB.1.5 and JN.1 DP PPQ lots. The (b) (4) method for quantification of PC and Cholesterol is a qualified characterization method. The analysis is performed using (b) (4) where the (b) (4) and are detected with (b) (4) covering the range of (b) (4) for cholesterol and (b) (4) for PC. For Cholesterol content, the DP is (b) (4). For PC content, the DP is (b) (4). The system suitability for the assay is described and is acceptable. Saponin Integrity Index (SII) is a qualified characterization method based on (b) (4). The method was applied to DP containing Wuhan-strain and was later replaced by the (b) (4) method for consecutive strains. The data from (b) (4) analysis (b) (4), are used to calculate the SII, which is an index between (b) (4), used to follow the degradation events of the Saponins. An index near (b) (4) indicates that the sample has not been degraded, while values below (b) (4) indicate degradation, and a value close to (b) (4) indicates completely degraded Saponins. The calculation is designed to monitor the aging of DP, which is achieved by (b) (4) on a (b) (4). Each Saponin is identified by the (b) (4) determined from its (b) (4) data, and the (b) (4) determined from its (b) (4) data. The Saponin degradation index (SDI) method is a validated method used for characterization purposes measuring the state of degradation of the constituent saponins. The index is calculated using the following equation: (b) (4) SDI is more robust and efficient method compared with SII method and the reported results from the two methods are essentially equivalent. The SII method was applied to DP containing Wuhan-strain and was later replaced by the (b) (4) method for consecutive strains. Samples are analyzed by (b) (4) method. Reviewer's comment: the (b) (4) methodology for SDI is the same as for SII. The calculations are slightly different, and the SDI provides a direct measure of saponin degradation rather than integrity and thus the SDI values are more relevant to stability of DP.

Reviewer's assessment: the response is acceptable.

3. In IR3 (December 31, 2023), the firm was asked to provide clarification on the difference between Version (V) V2 and V3 of the (b) (4) method (b) (4) where V2 (effective February 29, 2024) used for release and stability evaluation of Omicron XBB.1.5 DP (section 3.2.P.5.2 Amendment 4) and V3 of the same method (Effective June 4, 2024), which used for release and stability evaluation of Omicron JN.1 DP (section 3.2.P.5.2 Amendment 42).

Company's response (Amendment 60, Sequence 62, received 1-8-2025) Global method (b) (4) for the determination of Matrix-A and Matrix-C by (b) (4) was revised from version 2.0 to version 3.0 to include the additional (b) (4) in Section 9. The (b) (4) was introduced to the global method to better align with the guidance in (b) (4) .

Reviewer's assessment: the response is acceptable.

4. In IR4 (January 21, 2025), the firm was requested to provide information on container closure system and to provide stability update for lyophilized Fraction-A batches (b) (4) for Fraction-C batches (b) (4) that showed only (b) (4) months stability in the BLA and only initial stability (Time 0) for Fraction-A batches (b) (4) (report D_PD_01177 Revision 7).

Company's response (Amendment 65, Sequence 67, received 1-27-2025). Document D_PD_01177 has been updated to version 8.0 with stability data for the initial timepoint and up to (b) (4) months. Specifically, the stability data is provided for Fraction-A batches (b) (4) for (b) (4) months. Similarly, for Fraction-C batches (b) (4) months and for batch (b) (4) for (b) (4) months. Additional note: the batch numbers have been corrected in D_PD_01177 v8.0 to align with internal source documents: (b) (4) This document has also been updated with a new Section 2.6.1.1 in the Matrix-DS dossier detailing the information regarding container closure system for Fraction-A and Fraction-C. The final (b) (4) lyophilized Fraction-A and Fraction-C are stored in (b) (4) The container closure systems used for storage of stability samples are representative of those used for the (b) (4) material.

Reviewer's assessment: the provided stability update for up to (b) (4) months for batches of lyophilized Fraction-A and Fraction-C and update on container closure system are acceptable.

5. In IR5 (January 21, 2025) the firm was requested to provide clarification on the method validation for method (b) (4) for saponin concentration and purity (report QAG_02474), where the observed average concentration of saponin-A in Matrix-A in the intermediate precision-stability study was (b) (4) and the shelf-life acceptance criterion for saponin

is indicated as (b) (4) , suggesting that the samples used in the validation study are outside the acceptance range.

Company's response (Amendment 65, Sequence 67, received 1-27-2025) At the time of validation, the observed saponin-A concentration of (b) (4) mg/mL fell within the acceptable shelf-life range of (b) (4) . Since then, the limits have been narrowed to (b) (4) . Please note that the validated range of the method is (b) (4)

Reviewer's assessment: the response is acceptable.

3.0 FULL REVIEW

This review memo is based on review of the following submissions in BLA 125817:

Sequence	Amendment
001	0
003	2
005	4
015	15
017	16
026	24
028	26
030	28
044	42
047	45
055	53
058	55
061	59
062	60
067	65

The following sections were assigned to and were reviewed by adjuvant reviewer:
In 3.2.S Drug Substance Matrix-A and Matrix-C Novavax-AB, and in 3.2.S Drug Substance Matrix-A and Matrix-C AGC-CPH

- General Information [3.2.S.1]
  - Nomenclature
  - Structure
  - General Properties
- Manufacture [3.2.S.2]
  - Manufacturers
  - Description of Manufacturing Process and Controls
  - Controls of Materials
  - Controls of Critical Step and Intermediates

- Process Validation and/or Evaluation.
- Manufacturing Process Development

- Characterization [3.2.S.3]
  - Elucidation of Structure and Other Characteristics
  - Impurities
- Control of Drug Substance [3.2.S.4]
  - Specifications
  - Analytical Procedures
  - Batch Analyses
  - Justification of Specification
- Reference Standards or Materials [3.2.S.5]
- Container Closure System [3.2.S.6]
- Stability [3.2.S.7]
  - Stability Summary and Conclusions
  - Post-Approval Stability Protocol and Stability commitment
  - Stability Data

In 3.2.P SARS-CoV-2 XBB.1.5 Drug Product; 3.2.P SARS-CoV-2 JN.1 Drug Product; and 3.2.P SARS-CoV-2 Wuhan Drug Product that contained information on Matrix-M adjuvant

3.2.P.1 Description and Composition of the Drug Product
3.2.P.2 Pharmaceutical Development
3.2.P.3 Manufacture
3.2.P.5 Control of Drug Product
3.2.P.8 Stability

The list of reviewed reports for DSs and DPs (included in reviewed sections of BLA) is shown in Appendix 1.

3.1 MATRIX-A AND MATRIX-C DRUG SUBSTANCES (NOVAVAX AB)

3.1.1 NOMENCLATURE, GENERAL PROPERTIES, COMPOSITION OF MATRIX-A AND MATRIX-C

(b) (4)

(b) (6)

(b) (4)

(b) (4)

A(b) (4)

3.1.2 MANUFACTURE OF MATRIX-A AND MATRIX-C DS

3.1.2.1 MANUFACTURERS

Novavax AB (b) (4)

68 pages determined to be not releasable: (b)(4)

- (b) (4)

3.3 SARS-COV-2 RS XBB.1.5 DRUG PRODUCT

3.3.1 DESCRIPTION AND COMPOSITION OF DRUG PRODUCT; PHARMACEUTICAL DEVELOPMENT

3.3.1.1 DESCRIPTION AND COMPOSITION OF DP

The SARS-CoV-2 rS Vaccine with Matrix-M1 adjuvant consists of (b) (4) µg/mL of SARS-CoV-2 rS antigen DS with (b) (4) µg/mL Matrix-M1 adjuvant in formulation buffer. The formulation buffer has (b) (4) mg/mL Disodium hydrogen phosphate heptahydrate, (b) (4) mg/mL Sodium dihydrogen phosphate monohydrate, (b) (4) mg/mL Sodium chloride, and (b) (4) mg/mL Polysorbate 80 (pH (b) (4)) (Table 22).

Matrix-M1 adjuvant is composed of a mixture of Matrix-A (b) (4) and Matrix-C (b) (4), each produced from saponin materials, Fraction-A and Fraction-C, respectively. During DP manufacturing, Matrix-A and Matrix-C are added at calculated amounts targeting (b) (4) µg/mL of Matrix-A and (b) (4) µg/mL of Matrix-C. Matrix-A and Matrix-C are nanoparticles suspended in phosphate buffered saline (PBS) solutions at pH (b) (4). Cholesterol and Phosphatidylcholine are present as excipients in the Matrix solutions.

A single human dose of DP is 0.5 mL. The recommended storage conditions of DP are 2 to 8°C and the intended route of administration is intramuscular injection.

Table 22 SARS-CoV-2 rS protein (COVID-19) nanoparticle vaccine DP composition

Name of Ingredient	Quantity per Dose	Function
SARS-CoV-2 rS Omicron XBB.1.5 (nominal concentration)	5 µg	Immunogen/active ingredient
Matrix-M Adjuvant (b) (4) [1]	50 µg	Adjuvant
Cholesterol[2]	30.5 µg	Formulation agent
Phosphatidylcholine[2]	23 µg	Formulation agent
Potassium dihydrogen phosphate[2]	3.85 µg	Buffer

Name of Ingredient	Quantity per Dose	Function
Potassium chloride[2]	2.25 µg	Tonicity Agent
Disodium hydrogen phosphate dihydrate[2]	14.7 µg	Formulation Buffer agent
Sodium Chloride[2]	(b) (4)	Formulation Buffer agent
Disodium hydrogen phosphate heptahydrate[2]	2.465 mg[4]	Formulation Buffer agent
Sodium dihydrogen phosphate monohydrate[3]	0.445 mg[4]	Formulation Buffer agent
Sodium Chloride[3]	8.766 mg[4]	Isotonicity adjuster
Polysorbate 80[3]	0.050 mg[4]	Stabilizer
Sodium hydroxide	q. s.	pH Adjustment
Hydrochloric acid	q. s.	pH Adjustment
Water for Injections	q. s.	Vehicle

[1] Matrix-M consists of Matrix-A and Matrix-C components in (b) (4) (by weight) proportion.
[2] Excipients used for Matrix adjuvant: Phosphatidylcholine is of animal origin (from (b) (4) contains (b) (4) α-Tocopherol (DL-α-tocopherol) according to the specification. The (b) (4) of PC in one dose of Matrix-M will contain a maximum of 50 nanograms of α-Tocopherol.
[3] Excipients used for the DP formulation buffer: Disodium hydrogen phosphate heptahydrate referred to as Sodium phosphate dibasic heptahydrate on supplier's CoA. Sodium dihydrogen phosphate monohydrate referred to as Sodium phosphate monohydrate monobasic on supplier's CoA.
[4] Concentration of the excipients to make the DP formulation buffer. Actual amount of these excipients in the final DP may vary by (b) (4) as Matrix-M adjuvant components are formulated in PBS.

The container closure system XBB1.5 DP is filled into 5 mL Clear Tubular (b) (4) type (b) (4) siliconized Glass Vials closed with 13 mm Bromobutyl RFS uncoated siliconized Rubber Stoppers and 13 mm Aluminum Seal with blue plastic flip-off cap (reviewed in detail by product reviewer).

3.3.1.2 PHARMACEUTICAL DEVELOPMENT

3.3.1.2.1 PHARMACEUTICAL DEVELOPMENT, STABILITY OF MULTIDOSE VIALS AFTER PUNCTURE, (b) (4) FOR MATRIX-C, COMPARABILITY STUDIES

For the Phase 1/2, Part 1 study, SARS-CoV-2 rS antigen was used for bedside mixing with Matrix-M1 adjuvant prior to administration. All other clinical studies were performed using SARS-CoV-2 rS pre-mixed with Matrix-M1 adjuvant by the manufacturer. The combined formulation provides advantages over bedside mixing by avoiding the antigen/adjuvant mixing step at the clinic. During formulation development, efforts were made to identify and optimize formulation buffer to minimize changes in particle size/integrity and minimize changes in concentration/potency of the antigen. As a result, the optimal composition was selected and remained the same throughout the development to the commercial stage. The buffer remained the same (b) (4) Phosphate buffer, (b) (4) NaCl, (b) (4) PS80, pH (b) (4). The amount or ratio of two phosphate salts (mono- and di-basic) needed to achieve (b) (4) strength was optimized during development to achieve a pH much closer to the target of pH (b) (4)

Additional challenge was related to the potential incompatibility of the formulations with the vial that was used (b) (4)). Matrix-M1 adjuvant is known to have stability issues (b) (4) in some glass vials due to (b) (4) . Matrix-M1 incompatibility with the (b) (4) vials was confirmed and another glass vial from (b) (4), which demonstrated better compatibility, was chosen for single-dose-vial (SDV) filling of co-formulated clinical trial materials by Novavax.

The DP presentation evolved during clinical development. Initially, DP was presented as 10 dose vials (Wuhan strain) followed by 5-dose presentation for Wuhan strain and then 5-dose vials and Pre-filled Syringe (PFS) for Omicron XBB.1.5 strain; the Omicron JN.1 strain DP is only presented as PFS.

As part of pharmaceutical development, in-use stability studies were performed for multi-puncture of adjuvanted SARS-CoV-2 rS vaccine in multi-dose vials as described in (b) (4) to evaluate the quality of the product after multiple needle punctures and to establish a period of time during which multi-puncture can be performed for a preservative-free DP (QAG_08393 and QAG_10442). , sThese studies were conducted with the (b) (4) vial and (b) (4) stopper container closure system manufactured at (b) (4) . The chemical stability for DP (Wuhan strain) stored at 2 – 8°C or at (b) (4) for 6, (b) (4) hours after multi-puncture (b) (4) punctures) was evaluated and was compared with unpunctured control. Protein concentration, % relative potency, particle size, (b) (4), and adjuvant concentration (µg/ml) were tested. The results showed that there were no changes in tested attributes during storage of punctured vials (Table 12, section 3.2.P.2.2 in the submission). Based on the study results, 6 hour in-use hold time is recommended after the first puncture of the multi-dose vial when stored between 2-8°C or at 25°C. Similar study was performed at SIIPL for 5-dose vials of XBB1.5 DP (report QAG_26531). There were no changes in quality attributes tested including rS protein concentration % relative potency, Matrix-A/-C content, endotoxin, and sterility in DP in 5-dose vials after multipuncture and subsequent storage for 6 (b) (4) h at 2 – 8°C or 20-25°C.

(b) (4) **(Matrix-C)** An (b) (4) is applied to the Matrix C target during the DP formulation process. The concentration of (b) (4) µg/mL in the formulation is targeted to achieve the nominal Matrix C concentration of (b) (4) µg/mL at product release and throughout the shelf life. The target was established in QAG_24231. The (b) (4) does not impact the release specification.

Report QAG_24231 “Matrix C Content Target Input Analysis for SARS-CoV-2 rS Drug Product (b) (4) µg/mL with (b) (4) µg/mL Matrix-M1”.

This report describes the results of statistical analysis of (b) (4) ten-dose commercial batches and of (b) (4) single-dose vials. In the report, the Matrix-C concentration (µg/ml) in the (b) (4) and in final containers was subjected to theoretical distribution. During formulation of DP, Matrix-C is added to target (b) (4) µg/ml. However, the statistical analysis of Matrix-C concentration in batches showed the mean Matrix-C concentration at (b) (4) µg/ml. The analysis recommended to shift the target Matrix C concentration to (b) (4) µg/mL to center the target Matrix C concentration within the current DP specification of (b) (4) µg/mL to (b) (4) µg/mL. Based on the theoretical target of (b) (4) µg/mL, analysis on the (b) (4) lots shows this will yield a mean of (b) (4) µg/mL in the final DP. This change is needed to maintain the Matrix-A to Matrix-C ratio of (b) (4). **Reviewer’s conclusion from the report QAG_24231: There are no comments related to this report; change to Matrix-C target concentration at (b) (4) µg/ml during DP formulation is acceptable.**

3.3.1.2.2 MANUFACTURING PROCESS DEVELOPMENT FOR XBB.1.5 DP IN 5-D0SE VIAL PRESENTATION AND IN (b) (4) FOR XBB.1.5 CLINICAL TRIAL MATERIAL

The original process for manufacture of SARS-CoV-2 rS Protein (COVID-19) Nanoparticle Vaccine was developed by Novavax at their subcontracted GMP-compliant manufacturing sites, Emergent BioSolutions (EBSI) and Par. The manufactured product was used in the clinical phase of development in Australia, South Africa, the UK, and the USA. The developed process was tech transferred to SIIPL India for manufacturing of clinical (for Indian clinical study) and commercial batches of DP. Initial process at

1 page determined to be not releasable: (b)(4)

(b) (6)

(b) (4)

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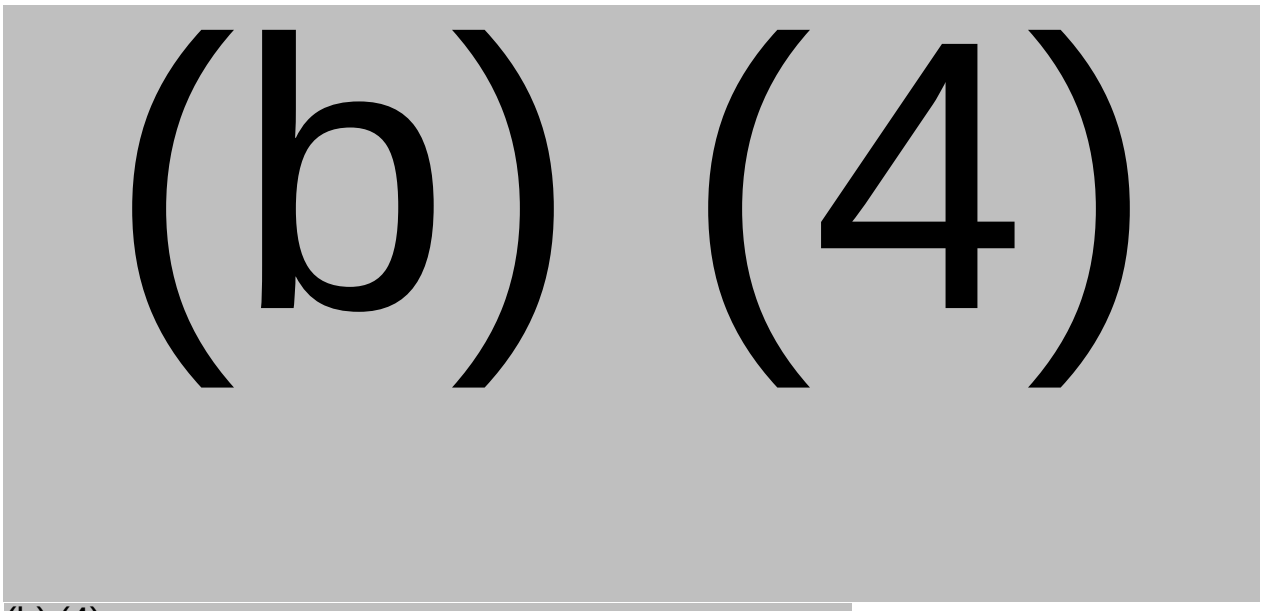
3.3.2 MANUFACTURE AND PROCESS VALIDATION

3.3.2.1 MANUFACTURERS AND BATCH FORMULA

The following facilities are involved in manufacture and release of SARS CoV2 rS Nanoparticle vaccine DP batches in the PFS presentation:

- Serum Institute of India Pvt. Ltd. (SIIPL) (b) (4)
    - DP manufacture: (b) (4)
- Novavax AB Kungsgatan 109 SE-753 18 Uppsala, Sweden
    - (b) (4)

(b) (6)



(b) (4)

3.3.2.2 DESCRIPTION OF THE MANUFACTURING PROCESS AND PROCESS CONTROLS SII 5-DOSE

(b) (4)

1 page determined to be not releasable: (b)(4)

(b) (6)

(b) (4)

EXCIPIENTS

The following excipients are used in formulation of XBB1.5 DP for FB or for pH adjustment: Disodium Hydrogen Phosphate Heptahydrate, Sodium Dihydrogen Phosphate Monohydrate, Sodium Chloride, Polysorbate 80, Sodium Hydroxide, Hydrochloric Acid, and Ware for Injections. All these excipients are tested according to the applicable compendial methods:(b) (4) . The CoAs are included in the submission.

Phosphatidylcholine (PC) used in the manufacture of Matrix-A and Matrix-C adjuvant components is produced from (b) (4)). According to the manufacturer, (b) (4) has been validated according to the guideline (b) (4) is available from the supplier (Section 3.2.S.2.3 for Matrix adjuvant in the submission). PC is not considered to be a risk for TSE/BSE as defined in EMA/410/01 Rev. 3. There are no other excipients of human and animal origin. There are no novel excipients in the formulation.

3.3.3 CONTROL OF DRUG PRODUCT XBB.1.5 5DV

3.3.3.1 SPECIFICATIONS OF DRUG PRODUCT

The testing program and specifications for control of the SARS-CoV-2 rS Protein (COVID-19) Nanoparticle Vaccine DP XBB.1.5 5DV are shown in Table 24

Table 24 Drug Product Release and Stability Specifications for XBB.1.5 5DV

Test Method	Compendial Reference(s)	Release Acceptance Criteria	Stability Acceptance Criteria
Appearance by Visual Observation	(b) (4)	Color: Colorless (b) (4) Clarity: Clear (b) (4) Practically free from visible particles	Color: Colorless (b) (4) Clarity: Clear (b) (4) ; Practically free from visible particles
pH by (b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)
Extractable Volume	(b) (4)	**For multidose container**: The volume should be such that each syringe delivers not less than stated doses.	NA
Identity by (b) (4) [1]	NA	Identity Confirmed	Identity Confirmed
Identity by (b) (4) [1]	NA	Identity Confirmed	Identity Confirmed
Relative Potency by (b) (4)	NA	(b) (4) Potency Relative to Reference Standard	(b) (4) Potency Relative to Reference Standard
Total Protein Content by (b) (4)	NA	(b) (4)	(b) (4)
Matrix-A Content by (b) (4)	NA	(b) (4)	(b) (4)
Matrix-C Content by (b) (4)	NA	(b) (4)	(b) (4)

Test Method	Compendial Reference(s)	Release Acceptance Criteria	Stability Acceptance Criteria
Sterility by (b) (4)	(b) (4)	No growth	No growth
Endotoxin by (b) (4)	(b) (4)	(b) (4)	NA
Container Closure Integrity Test (CCIT)	(b) (4)	NA	(b) (4) should be observed/no leak detected
Particle Size by (b) (4)	N/A	(b) (4)	(b) (4)

[1] Perform only one method for identity per batch.

[2] Release testing for Relative Potency is performed (b) (4) days from date of manufacture.

3.3.3.2 ANALYTICAL PROCEDURES

Test for appearance, pH, (b) (4), sterility, Endotoxin, expelled volume, Container Closure System, and (b) (4) assays and are performed following (b) (4) guidance. The relevant sections of (b) (4). And of (b) (4) are included in the table describing (b) (4) analytical methods for DP (Table 1 in section 3.2.P.5.2 in the submission).

(b) (4) methods include Identity by (b) (4), Identity by (b) (4), Relative Potency by (b) (4) (all reviewed by (b) (6)). Matrix-A content and Matrix-C content by (b) (4), and particle size by (b) (4) Below I provide review of the (b) (4) method (b) (4) for Matrix-A and Matrix-C content analysis and for the (b) (4) method (b) (4) for particle size determination in SARS-CoV-2 rS DP and method validation.

(b) (4)

8 pages determined to be not releasable: (b)(4)

(b) (6)

(b) (4)

3.3.3.5 BATCH ANALYSIS, IMPURITIES, (b) (4)

BATCH ANALYSIS Batch analysis data is shown for the three XBB1.5 PPQ DP batches filled in 5DV, batch (b) (4) (all at (b) (4) scale) and for the (b) (4) XBB1.5 DP clinical batches filled in (b) (4) (see section 3.3.1.2.2 "Manufacturing Process Development for XBB.1.5 DP" of the review memo). All analytical attributes including Matrix-A and Matrix-C content analysis by (b) (4) method met acceptance criteria. Particle size data is not shown in CoAs (see section 3.3.2.3 of the review memo about the release testing for PPQ batches).

IMPURITIES No new impurities / degradation products are formed during the drug product manufacturing process. All potential impurities originate from rS DS (reviewed by (b) (6)) and Matrix-M1 adjuvant (see information about impurities in Matrix adjuvant in section 3.1.5 of this review memo).

(b) (4)

Reviewer: I reviewed report QAG_07612 and I concur with the Firm's conclusion as no meaningful exposure to (b) (4) agents and negligible risk from (b) (4) impurities was identified during risk assessment. The confirmatory testing under EMA Article 5(3) was not deemed necessary.

3.3.4 STABILITY OF DP

Shelf-life of XBB.1.5 DP in 5DV: Based on statistical analysis, the established Wuhan stability data, comparability and supporting development data, **a shelf life of 9 months** at 5 ± 3°C is proposed.

The PPQ batches formulated at (b) (4) commercial scale and filled in 5DV, batch (b) (4) and the (b) (4) commercial batches formulated at (b) (4) scale and filled in 5DV, batch (b) (4) are monitored for stability at long-term 5 ± 3°C (b) (4) and at accelerated (b) (4) (Inverted) conditions for (b) (4) months and 6 months, respectively.

The stability protocol for long term conditions includes analytical testing for appearance, pH, (b) (4), Identity by (b) (4), protein concentration by (b) (4), Relative Potency by (b) (4), sterility by (b) (4), CCIT, Matrix-A and Matrix-C content and characterization tests for cholesterol, saponin integrity, phosphatidylcholine, particle size by (b) (4). The acceptance criteria for cholesterol, Phosphatidylcholine, and Saponin integrity is set as "report Result" and for particle size/ (b) (4) is set as (b) (4).

The stability protocol for accelerated conditions includes the same analytical tests and characterization tests except that acceptance criteria are set as "Report results".

The submission provides the stability data for the (b) (4) PPQ batches filled in 5DV (b) (4) for 9 and 6 months at long-term and accelerated conditions, respectively, and for 3 months and 1 month at long-term and accelerated conditions, for batches (b) (4). All attributes met acceptance criteria. The study is on-going.

Reviewer's note: The protocol for stability studies for the (b) (4) XBB.1.5 DP PPQ batches and for the (b) (4) additional commercial batches listed above (all formulated at (b) (4) scale and filled in 5DV) includes characterization tests (cholesterol content, phosphatidyl content, and Saponin Integrity test. The available stability data for these batches provides the results of characterization tests. However, section on Control of Drug Product that includes release testing and stability testing, includes particle size by (b) (4) but does not include cholesterol/phosphatidyl content or Saponin Integrity Index. The cholesterol/phosphatidyl content are tested in Matrix-A and Matrix-C DS at release and during stability by validated (b) (4) method. The Saponin Integrity Index (SII) measures a degree of saponin degradation by a non-validated (b) (4) method (b) (4) on (b) (4) Fraction-A and Fraction-C used as reference standards for Matrix-A, Matrix-C and SARS-CoV-2 DP. The description of (b) (4) method for lipids **in DP** and of (b) (4) in method for Saponin Integrity Index **in DP** is not included in the list of analytical procedures for XBB1.5 or JN.1 DP. There is no mention of characterization testing performed on DP at all anywhere in the dossier for XBB1.5 DP and JN.1 DP or the original Wuhan DP. It is my understanding that characterization tests do not need to be validated. But the information on method process including System Suitability and method quantitation should be included.

> **Reviewer: IR sent to the company on December 23, 2024, to provide a detailed information on the characterization methods (including controls and system suitability criteria) used for cholesterol, phosphatidyl content analysis, and Saponin Integrity Index for XBB1.5, JN.1 and Wuhan DP PPQ lots during stability studies (Amendment 4 and Amendment 42) and to indicate the validation status of these assays. The company response was received on January 3, 2025 (see section 2.0 Information Requests, IR2).**

Reviewer's note for stability of XBB1.5 DP formulated at (b) (4) scale and filled in single dose Pre-Filled Syringe (PFS), batches (b) (4), see section 3.4.5 of this memo.

POST APPROVAL STABILITY AND STABILITY COMMITMENT

Novavax expressed a commitment to continue the ongoing stability studies for the variant SARS-CoV-2 rS vaccine DP commercial batches per the protocols presented in Section 3.2.P.8.1 in the submission for the DP. In addition, (b) (4) of variant SARS-CoV-2 rS vaccine DP manufactured at each manufacturing

site will be placed on stability each year and will be tested using Analytical Procedures as described for DP in the dossier and tested per the Specifications. The stability studies will be conducted at 2 – 8°C (long term conditions) for up to (b) (4) months and under accelerated conditions at (b) (4) for up to 6 months.

Reviewer's note: Post approval monitoring of DP will include Matrix-A and Matrix-C content and particle size assessment. Other characterization tests (cholesterol and PC content and Saponin Integrity Index are not included in the protocol. Post approval stability protocol is acceptable.

3.4 SARS-COV-2 rS JN.1 DP

3.4.1 DESCRIPTION AND COMPOSITION OF JN.1 DRUG PRODUCT

Novavax COVID-19 Vaccine, Adjuvanted (2024 – 2025 Formula) contains 5 µg of SARS-CoV-2 recombinant (r) spike protein (Omicron JN.1 strain). Similar to XBB1.5 DP, the JN.1 DP is formulated with 50 µg of Matrix-M adjuvant per dose. Other ingredients that are included in the composition and their concentration are the same as included in the SARS-CoV-2 rS XBB.1.5 DP: cholesterol, phosphatidylcholine (including α-Tocopherol), potassium dihydrogen phosphate, potassium chloride, disodium hydrogen phosphate dihydrate, disodium hydrogen phosphate heptahydrate, sodium dihydrogen phosphate monohydrate, sodium chloride, polysorbate 80, sodium hydroxide (for adjustment of pH), hydrochloric acid (for adjustment of pH) and water for injection**.**

The finished product is presented as a dispersion for injection in a pre-filled syringe (PFS), containing a single dose of 0.5 mL. The storage conditions of DP in PFS is 2 to 8°C and the intended route of administration is intramuscular injection.

The SARS-CoV-2 rS Vaccine is manufactured with an (b) (4). The DP manufacturing sites use a range of (b) (4) µg/mL to (b) (4) µg/mL in the formulation process to achieve the nominal protein concentration of (b) (4) µg/mL at product release and throughout the full shelf life. An (b) (4) is applied to the Matrix-C target within the DP formulation process. The concentration target of (b) (4) µg/mL in the formulation is targeted to achieve the nominal Matrix-C concentration of (b) (4) µg/mL at product release and throughout the full shelf life. The (b) (4) does not impact the release specification.

The PFSs are the primary container closures and consist of 1 mL Standard, Round Flange, Siliconized Type I borosilicate glass syringe barrels with Luer lock and Plastic Rigid Tip Cap with (b) (4) Elastomer, sterile, ready to use (b) (4) and (b) (4) The suppliers of these primary packaging materials are qualified by SII Quality Assurance and are released at SII based on the supplier CoAs for filling and stoppering operations.

3.4.2 PHARMACEUTICAL DEVELOPMENT

The review covers the following sections:
3.4.2.1 Development of DP PFS presentation
3.4.2.2 Comparability studies for DP PFS presentation and comparability protocol
3.4.2.3 Analytical bridging between SIIPL JN.1 DP PFS and XBB.1.5 5DV
3.4.2.4 (b) (4) in phosphatidylcholine in Par DP clinical batches (b) (4)

1 page determined to be not releasable: (b)(4)

3.4.2.2 COMPARABILITY STUDIES FOR JN.1. DP PFS AND COMPARABILITY PROTOCOL

- Analytical comparability for Wuhan DP batches manufactured at EBSI and Par and at Par vs DP manufactured at SIIPL was established and reported in QAG_04829, QAG_07396 and QAG_20109. Following DS strain change to XBB.1.5, a comparability study was performed between XBB.1.5 DP in 5DV and Wuhan DP in 5DV, both manufactured at (b) (4) scale at SIIPL, reported in QAG_25113 (see section 3.2.1.2.2 in the review memo for XBB1.5 DP for adjuvant-related summary of these comparability reports).

- To support change from 5DV presentation to PFS, a comparability study was performed between XBB.1.5 DP in PFS (b) (4) scale) and XBB.1.5 DP in 5DV (b) (4) scale) per protocol QAG_25056 (see below review of the comparability protocol). The 5DV and PFS were manufactured at SIIPL and filled in (b) (4) , report QAG_27585. Data is provided for the (b) (4) SIIPL XBB.1.5 PPQ 5DV DP lots (b) (4)) and SIIPL XBB.1.5 **PPQ** PFS DP lots (b) (4) . In the comparability protocol QAG_25056, acceptance criteria were added for attributes related to adjuvant that were <u>not used in previous comparability protocols</u>: Saponin degradation, phosphatidylcholine (PC) content, cholesterol content, and (b) (4). The comparability study of SIIPL 5DV and PFS showed that all lots met the release specifications, and no significant differences were noted in the QA between the SIIPL XBB.1.5 5DV and PFS lots.

  Conclusion from the study: The manufacturing processes between SIIPL DP lots are considered equivalent. Both presentations utilize the same protein and Matrix formulation targets, protein concentration target of (b) (4) µg/mL and Matrix-M1 concentration target of (b) (4) µg/mL and use a Matrix-C formulation target of (b) (4) µg/mL. Both DP presentations use containers constructed from Type (b) (4) siliconized glass. Other than differences in scale and container closure system, there are no other operational differences between processes used to manufacture XBB.1.5 DP at SIIPL.

Comparability protocol QAG_25065

To accommodate the multiple manufacturing sites that manufacture DP and for transition from pandemic to endemic spread of SARS-CoV-2 virus, the number of doses in each container or/and change in container closure might be needed. The generic protocol was developed to assure developmental continuity of variant SARS-CoV-2 DP manufacturing by comparing analytical data of pre-change and post-change DP. Pre-change Wuhan DP lots serve as the reference lots used for comparability. This protocol compares release and characterization data for (b) (4) pre-change DP lots to at least (b) (4) post-change DP lots.

All lots must meet the current lot release specifications. Results from characterization assays need to be within the acceptance criteria that were set based on data from specific DP lots including (b) (4) Par DP lots (b) (4)) and a total of (b) (4) SIIPL DP lots (Table 25). Analytical comparability will also include generation of accelerated stability data to compare degradation pathways and rates of degradation between pre-change DP and post-change DP.

Table 25 Analytical Characterization Tests and Acceptance Criteria for Comparability Assessment of DP.

Test Method	Quality Attribute	Acceptance Criteria
Cholesterol Content	Excipient Quantity (µg/mL)	(b) (4)

Test Method	Quality Attribute	Acceptance Criteria
Phosphatidylcholine Content	Excipient Quantity (µg/mL)	(b) (4)
Saponin Degradation Index	Excipient Purity	Report Results
Particle Size (b) (4)	(b) (4)	Report Results [1] Report Results [1]
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)

[1] Initially the acceptance criteria for (b) (4) testing of DP will be "Report Results". Once sufficient data have been collected with the validated (b) (4) method, upper and lower limits for (b) (4) will be added to the DP specifications and used to evaluate analytical comparability.

If comparability is not established by the battery of tests included in this protocol, a mouse immunogenicity study may be performed according to ICH Q5E.

3.4.2.3 ANALYTICAL BRIDGING BETWEEN SIIPL JN.1 DP PFS AND SIIPL XBB.1.5 5DV

Analytical bridging between JN.1 PFS Variant DP and SIIPL XBB1.5 5DV and Wuhan 10DV was performed per comparability protocol QAG_25065 and the results are described in QAG 28508 (Amendment 42). Total protein concentration was consistent between the JN.1 PFS DP PPQ batches (b) (4) µg/mL), XBB.1.5 5DV DP batches (b) (4) µg/mL) and overlapped with Wuhan 10DV DP batches (b) (4) µg/mL) which included the Phase 3 clinical DP lots manufactured at Par. RP (measured using homologous reference standard) was consistent between the JN.1 PFS DP PPQ batches (b) (4) XBB.1.5 5DV DP batches (b) (4) and Wuhan 10DV DP batches (b) (4) This demonstrates that strain change did not impact the quality and integrity of the rS protein delivered in the vaccine dose.

All other DP attributes were consistent between XBB.1.5, JN.1 and Wuhan DP batches, including particle size (b) (4) As expected, Matrix-specific attributes (Matrix-A content, Matrix-C content, cholesterol content, PC content, saponin degradation index) were consistent between XBB.1.5, JN.1, and Wuhan DP batches and were not impacted by the change to XBB.1.5 and JN.1 Variant or change in container closure. The DP lots were added to accelerated stability and only T=0 release and characterization data is included in the bridging report QAG 28508.

COMPATIBILITY PFS

- Compatibility studied were performed for DP filled in syringes. Initial short-term stability studies (3 months) with Wuhan DP were performed at Novavax, where stability of DP was evaluated in (b) (4) types of syringes that included testing for phosphatidylcholine concentration, cholesterol concentration, Matrix-A and Matrix-C content, saponin integrity index, and particle size (b) (4) After three months, (b) (4) syringes were terminated based on vendor decision. No apparent differences

were noted for stability of DP between (b) (4) syringes that remain on the study for a total of (b) (4) months (b) (4) syringes of each).

- The follow-up compatibility study was performed at SIIPL using 1 mL Standard Barrel w/Luer Lock & PRTC (Plastic Rigid Tip Cap) syringes. Stability of Wuhan DP in PFS was monitored at 5 ± 3°C and at (b) (4) and (b) (4) months, respectively. Appearance, pH, (b) (4), protein concentration, relative potency, matrix content, sterility, and CCIT were evaluated. There was no noticeable impact on the stability and activity of the DP held in syringes at 2 – 8°C and (b) (4) (QAG_20596 and QAG_20597, effective October 2020).

Reviewer's note: compatibility study demonstrated that all QA of DP including attributes related to Matrix adjuvant remain stable in PFS for up to (b) (4) months confirming that selected container closure system will not negatively affect quality of DP.

Separate compatibility studies were not performed for variant DP as the PFS are constructed from the same material as was used for primary compatibility study with Wuhan DP and the DP is equivalent in pH, (b) (4), and overall product composition.

(b) (4)



3.4.3 MANUFACTURE AND PROCESS VALIDATION

3.4.3.1 MANUFACTURING PROCESS AND BATCH FORMULA

The JN.1 DP filled in PFS is manufactured at the same facility at SIIPL and using the same manufacturing process as manufacturing process used for XBB1.5 DP. The difference is in the scale, (b) (4) for JN.1 vs (b) (4) for XBB1.5 DP, and in the filling process. The batch formula was adjusted accordingly to the (b) (4) scale of the production, Table 26.

Table 26 SARS-CoV-2 rS protein Nanoparticle vaccine, batch formula JN.1 DP (b) (4) syringes

Name of Ingredient	Quantity Per Dose (0.5 mL)	Theoretical Quantity Per Batch Size
SARS-CoV-2 rS Protein Drug Substance	5 µg	(b) (4)
Matrix-M1	50 µg	
Disodium Hydrogen Phosphate Heptahydrate	2.465 mg	
Sodium Dihydrogen Phosphate Monohydrate	0.445 mg	
Sodium Chloride	8.766 mg	
Polysorbate 80	0.050 mg	
Hydrochloric Acid (pH adjustment)	Quantity Sufficient (q.s)	q.s
Sodium Hydroxide (pH adjustment)	Quantity Sufficient (q.s)	q.s
Water for Injections	Quantity Sufficient (q.s)	q.s

- (b) (4)

3.4.3.2 PROCESS VALIDATION JN.1 DP

(b) (4)

(b) (6)

(b) (4)

3.4.4 CONTROL OF DRUG PRODUCT (JN.1 DP PFS)

3.4.4.1 SPECIFICATIONS AND ANALYTICAL METHODS

Table 27 JN.1 DP PFS release and stability specifications

Test Method	Compendial Reference(s)	Release Acceptance Criteria	Stability Acceptance Criteria
Appearance by Visual Observation	(b) (4)	Color: Colorless (b) (4) Clarity: Clear (b) (4) Practically free from visible particles	Color: Colorless (b) (4) Clarity: Clear (b) (4) Practically free from visible particle
pH by (b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)
Particle Size	(b) (4)	(b) (4)	(b) (4)
Expelled Volume	(b) (4)	The volume measured for each container is not less than the nominal volume.	NA
Identity by (b) (4)	NA	Identity Confirmed	Identity Confirmed
Identity by (b) (4)	NA	Identity Confirmed	Identity Confirmed
Relative Potency by (b) (4)	NA	(b) (4) Potency Relative to RS	(b) (4) Potency Relative to RS
Total Protein Content by (b) (4)	NA	(b) (4)	(b) (4)
Matrix-A Content by (b) (4)	NA	(b) (4)	(b) (4)
Matrix-C Content by (b) (4)	NA	(b) (4)	(b) (4)
Sterility by (b) (4)	(b) (4)	No growth	No growth
Endotoxin by (b) (4)	(b) (4)	(b) (4)	NA
Container Closure Integrity Test (CCIT)	(b) (4)	NA	No failures allowed
(b) (4)	(b) (4)	NA	(b) (4)

RS, Reference Standard

ANALYTICAL METHODS RELATED TO MATRIX-M ADJUVANT IN JN.1 DP PFS

2 pages determined to be not releasable: (b)(4)

- (b) (4)

.

3.4.5 STABILITY JN.1 DP

Proposed shelf-life An initial shelf life of 3M at 5 ± 3°C is proposed. Stability will continue to be monitored per the protocol described within and additional data will be provided as available to extend shelf-life. (b) (4)

DP batches used for stability study The XBB.1.5 DP (b) (4) scale) filled in PFS, batches (b) (4) and JN.1 DP filled in PFS, batches (b) (4) (both manufactured with rS protein at (b) (4) µg/mL) and JN.1 DP batches with rS protein at (b) (4) µg/mL, batch (b) (4), are monitored for long-term and accelerated stability at 5 ± 3°C and (b) (4), respectively. JN.1 (b) (4) µg/mL batches (b) (4) are monitored in a limited stability study for (b) (4) months at long term conditions for potency and total protein by (b) (4) only.

Additionally, a photostability study on DP in PFS syringes was performed and found that storage of the product at (b) (4) hours, exposure to visible light is acceptable for both the intermediate and marketed packaging. DP in the marketed packaging can be stored under ICH conditions; however, PFS stored in intermediate packaging must be protected from exposure to direct and indirect daylight (ICH Q1B exposure). It is recommended to protect naked syringes from both direct and indirect daylight when possible (photostability report QAG_28284). Syringes are stored horizontally.

Stability data The following stability data is available at a time of submission in BLA 125817-42 (10-1-2024):

- For XBB.1.5 DP PFS batches (b) (4) µg/ml) (b) (4), stability is shown for (b) (4) 3 months for long-term and accelerated conditions, respectively.
- For JN.1 DP PFS batches (b) (4) µg/ml) (b) (4), stability is shown for 3 months for long-term and accelerated conditions,
- For JN.1 DP PFS batches (b) (4) µg/ml) (b) (4), stability is shown for (b) (4) months for long-term conditions and for 2 months at accelerated conditions; for up to 3 months for long-term conditions for batch (b) (4) (3 months), (b) (4) (3 months), (b) (4) (3 months), (b) (4) (2 months), (b) (4) (1 month), and (b) (4); and potency only data for the following batches stored at long-term

conditions is shown for the following batches: (b) (4) (3 months) (b) (4) (1 month) (b) (4) (1 month), and (b) (4) (1 month).

- **Conclusion from stability study** Quality attributes of adjuvant component were monitored in stability study of DP in PFS including Matrix-A/-C content, PC content, cholesterol content, Saponin Integrity Index, particle size, and (b) (4). All these analytical attributes met acceptance criteria in all tested batches at long-term conditions and at accelerated conditions at all time points available at a time of submission. The shown data confirmed stability of Matrix-M adjuvant in DP filled in PFS. [Adjuvant stability is not included in stability study of batches (b) (4)

3.5 SARS-COV-2 WUHAN DP

The SARS-CoV-2 rS protein nanoparticle vaccine formulated with rS DS from Wuhan-Hu-1 (Wuhan) strain was the original DP manufactured by Novavax. The SARS-CoV-2 rS Wuhan DP was granted Emergency Use Authorization for individuals aged 18 years of age and older on July 13, 2022. The manufacturing process for Wuhan rS protein DS is described in the BLA 125817-0 (January 31, 2024).

3.5.1 COMPOSITION, MANUFACTURERS, BATCH FORMULA, MANUFACTURING PROCESS, PROCESS VALIDATION

COMPOSITION The composition of the Wuhan DP including Matrix-M content (Matrix-A and Matrix-C adjuvant components) is the same as in the XBB.1.5 DP and JN.1 DP. The Wuhan DP is presented in 5 dose (5DV) or 10 dose (10DV) vials (5 mL clear (b) (4) type (b) (4) glass siliconized vials supplied from (b) (4)) covered with 13 mm bromobutyl uncoated siliconized rubber stopper (b) (4)) and sealed with aluminum seal (b) (4)).

MANUFACTURERS The following facilities are involved in manufacture and release of SARS CoV2 rS Nanoparticle vaccine DP batches in the PFS presentation:

- Serum Institute of India Pvt. Ltd. (SIIPL) (b) (4)
- Novavax AB Kungsgatan 109 SE-753 18 Uppsala, Sweden
    - Quality control testing: **Batch Release** and **Stability Testing**: *Matrix-A Content and Matrix-C content by* (b) (4) , *Particle Size by* (b) (4) .
- (b) (4)
    - Quality Control Testing: **Batch Release** (Appearance, pH, (b) (4) Identity by (b) (4) Relative Potency by (b) (4)

(b) (4)

(b) (6)

(b) (4)

.

PROCESS VALIDATION/OR EVALUATION

(b) (4)

3.5.2 CONTROL OF DP, ANALYTICAL PROCEDURES, VALIDATION OF ANALYTICAL PROCEDURES, BATCH ANALYSIS, STABILITY

CONTROL OF DRUG PRODUCT

Table 28 Release and stability Specifications for Wuhan DP (10DV and 5DV presentation)

Test Method	Release Acceptance Criteria	Stability Acceptance Criteria
Appearance by Visual Observation	Color: Colorless (b) (4) Clarity: Clear (b) (4) Practically free from visible particles	Color: Colorless (b) (4) Clarity: Clear (b) (4) Practically free from visible particles
pH by (b) (4) (b) (4)	(b) (4) (b) (4)	(b) (4) (b) (4)
Particle Size by (b) (4)	(b) (4)	(b) (4)

Test Method	Release Acceptance Criteria	Stability Acceptance Criteria
Extractable Volume	**For multidose container: The volume should be such that each syringe delivers not less than stated doses**	NA
Identity by (b) (4)	Identity Confirmed	Identity Confirmed
Relative Potency by (b) (4)	(b) (4) Potency Relative to Reference Standard	(b) (4) Potency Relative to Reference Standard
Total Protein Content by (b) (4)	(b) (4)	(b) (4)
Matrix-A Content by (b) (4)	(b) (4)	(b) (4)
Matrix-C Content by (b) (4)	(b) (4)	(b) (4)
Sterility by (b) (4)	No growth	No growth
Endotoxin by (b) (4)	(b) (4)	NA
Container Closure Integrity Test (CCIT)	NA	(b) (4) should be observed/no leak detected

Reviewer's note: For References for compendial methods used at release and stability, please see Table 24 of the review memo. Two attributes in the release and stability specifications for Wuhan DP (shown in Table 28 in this memo) differ from release specifications of XBB1.5 DP and JN.1 DP (Table 24 and Table 27, respectively in this memo), Relative Potency (RP) by (b) (4) and particle size/(b) (4) . In Wuhan DP and in JN.1 DP, the RP acceptance criteria is set at (b) (4) for release and stability, respectively, and in XBB1.5 DP, RP acceptance criteria is set at (b) (4) for release and stability, respectively.

The particle size (b) (4) acceptance criteria are set as: (b) (4) for Wuhan DP (Table 28) and (b) (4) for XBB1.5 and JN.1 DP (same for release and stability, Table 24 and Table 27, respectively). Thus, the acceptance criterion for RP is the same for Wuhan DP and JN.1 DP but is different for XBB.1.1 DP and particle size acceptance criteria is the same for XBB1.5 DP and JN.1 DP but is different from Wuhan DP suggesting that RP most likely is not related to particles size or/(b) (4).

ANALYTICAL PROCEDURES AND VALIDATION OF ANALYTICAL PROCEDURES Two non-compendial analytical methods assess Matrix-M adjuvant in DP: (b) (4) method (b) (4) for Matrix-A and Matrix-C content analysis and (b) (4) method (b) (4) for particle size (b) (4) Both methods were described in relation to XBB.1.5 DP (section 3.3.3.2 in the review memo for XBB.1.5 DP).

- The (b) (4) method for Matrix-A and Matrix-C content analysis was validated at SIIPL (b) (4) . The validation report QAG_07911 was reviewed (section 3.3.3.3 of this review memo for XBB1.5 DP).
- (b) (4) method (b) (4) was validated and the validation report QAG_20130 was reviewed (section 3.3.3.4 of the review memo for XBB.1.5 DP).
- Both methods were validated and found suitable for the intended purpose.

BATCH ANALYSIS This section provides information on the batches of SARS-CoV-2 rS Wuhan DP in 10DV and in 5DV presentation.

10DV presentation

- The following (b) (4) batches were manufactured at (b) (4) scale at (b) (4) premises SIIPL): (b) (4) batches for PV campaign and primary stability: batch (b) (4)); clinical/commercial batches (b) (4) that were used to support stability.
- (b) (4) batches were manufactured at (b) (4) scale for PV at (b) (4) Premises SIIPL) and (b) (4) PV/primary stability batches were manufactured at (b) (4) scale at (b) (4) Premises SIIPL) in (b) (4) and in (b) (4) respectively.
- The section also includes a list of (b) (4) clinical DP batches manufactured at Par (b) (4) scale, (b) (4) at each scale) and at EBSI facilities (b) (4) batches at (b) (4) scale).
- Representative CoAs are provided in the submission for the 10DV DP batch (b) (4) (batches to support stability).

5DV presentation

- For 5DV presentation, information is provided for (b) (4) PV/primary stability/commercial batches manufactured at (b) (4) scale in (b) (4) premises (SIIPL) in (b) (4) : batches (b) (4) (about (b) (4) vials per batch). The CoAs for these batches are included in the submission. All attributes including Matrix-A and Matrix-C content met acceptance criteria.

STABILITY

Conclusion on shelf-life for DP 10DV presentation For 10DV presentation, the proposed shelf-life of (b) (4) months is based on the results from the (b) (4) commercially representative primary SIIPL PPQ lots.

Stability data The stability data is provided for the PPQ SARS-CoV-2 rS Wuhan DP batches manufactured at (b) (4) facilities at (b) (4) and (b) (4) scale and filled in 10DV, vials are stored (b) (4) . Data are available for the long-term (5 ± 3°C) and accelerated (b) (4) conditions for the following nine batches:

- (b) (4)

Clinical Batches (b) (4) manufactured at PAR meet all acceptance criteria through (b) (4) months at long-term storage conditions (2 – 8°C). After the (b) (4) months OOS (on relative potency), the clinical use of this material was halted. Therefore, a shelf-life for (b) (4) months was established for these clinical

batches. Reviewer's note, the stability data from batches manufactured at PAR is not included in the basis for proposed shelf life as Potency Assay for PAR manufactured DP used earlier version of potency assay (b) (4) that was replaced with a (b) (4) potency assay used at SIIPL.
Reviewer's note: The OOS for RP was also recorded for SIIPL PPQ batches (b) (4) at 12-month time point. However, the RP at (b) (4) months was within acceptance criteria for potency after it was revised (I defer to (b) (6) regarding changes in the acceptance criteria for potency assay). Importantly, the attributes related to Matrix-M adjuvant, Matrix-A and Matrix-C content, met acceptance criteria in all SIIPL PPQ batches at all time points. The cholesterol content, PC content, and Saponin Integrity Index (SII) attributes were tested in Wuhan DP PPQ batches using characterization assays. The particle size parameters were also tested as characterization tests (no acceptance criteria) or with acceptance criteria in batches formulated (b) (4), batch (b) (4). The results of characterization testing showed that all these attributes maintained the same values during stability monitoring with very little or no change compared with release testing. These data confirmed that the Matrix-M1 adjuvant component of Wuhan DP is stable during proposed shelf-life.
Conclusion on shelf-life for DP 5DV presentation: For 5DV, a shelf life of (b) (4) months at 2 – 8ºC is proposed. The (b) (4) months shelf life is based on the supporting data from the 10DV, (b) (4)-month data from the 5DV vials, and comparability data for the 5DV vial presentation.

Stability data The PPQ batches of 5-dose vial batches manufactured at (b) (4) scale (batches (b) (4), manufactured in (b) (4) facility are monitored for long-term and accelerated stability for (b) (4) months and for 6 months, respectively. Stability study is completed. All analytical attributes met acceptance criteria. All attributes related to Matrix-M1 adjuvant met acceptance criteria with very little differences observed during stability monitoring suggesting that Matrix-M1 adjuvant is stable for (b) (4) months at recommended conditions for DP filled in 5DV and 10DV (2 – 8ºC).

3.6 NONCLINICAL STUDIES OF MATRIX-M ADJUVANT

The BLA 125817 includes a comprehensive package of nonclinical studies of SARS-CoV-2 rS adjuvanted vaccine. These studies include nonclinical pharmacology and nonclinical toxicology studies. The review of toxicology studies including toxicology studies of Matrix-M adjuvant alone are provided by (b) (6) (OVRR (b) (6). An extensive pharmacology program has been performed by Novavax and includes multiple immunogenicity studies evaluating both humoral and cell-mediated immune response in rodents and non-human primates (NHP), reviewed by (b) (6). Studies of Matrix-M adjuvant Mode of Action in rodents (including a biodistribution study) and in human monocytes in vitro are reviewed below.
Reports for four studies on the mode of action (MOA) of Matrix-M adjuvant are included in BLA125817. These reports that were previously included in the package for EUA request, were reviewed by adjuvant reviewer, and the review is available in CBER connect. High-level summary of these four studies and a short description of each study is included in 3.6.1 and 3.6.2, respectively. In addition, Novavax performed a biodistribution study of radiolabeled Matrix-M1 in mice, 22#330 (C1080121), that was not included in the EUA request. This study is reviewed in 3.6.3.

3.6.1 SUMMARY OF PHARMACOKINETICS STUDIES OF MATRIX-M ADJUVANT

Matrix-M adjuvant induces a rapid local transient activation of innate immune cells including upregulation of cytokine and chemokine levels (study STF 19#299A). The adjuvant effects are limited in both time and site of the vaccine injection: for an adjuvant effect to occur, Matrix-M adjuvant and the antigen need to be co-delivered at the same site, and likely targeting the same draining lymph node within a relatively short window (48 hours before or 24 after antigen injection). Contralateral injections of antigen and adjuvant do not result in any adjuvant effect on the antigen-specific immune response (STF 702-037). Cytokine and chemokine generation at the site of injection peaks starting at 6 hours and up to 48 hours after injection with a subsequent rapid decrease by 72 hours and return to background levels by 168 hours, illustrating the transient nature of the response.

An increase in key chemokines known to recruit monocytes/macrophages, dendritic cells, T cells, and neutrophils, such as MCP-1, IP-10, and KC/GRO, is detected between 6 and 48 hours after injection of Matrix-M adjuvant alone or with protein antigen at the injection site and draining lymph node, respectively. This results in an influx of various key immune cells to the injection site (neutrophils, monocytes, and CD11b+ dendritic cells), and to the draining lymph node (neutrophils, monocytes, dendritic cells, CD4+ T cells, CD8+ T cells, B cells, natural killer cells, and macrophages) at 48 hours after injection (STF 20#315A). In vitro studies showed that Matrix-M adjuvant triggers an active intracellular process dependent upon lysosomal acidification, indicating that that downstream lysosomal processing is required for Matrix-M to be able to exert its adjuvant effect (STF 80.08.0686). No specific Pattern Recognition Receptor (PRR) that is involved in Matrix-M-induced cell activation was identified.

3.6.2 DESCRIPTION OF PHARMACOLOGY STUDIES

STF 19-299A Cytokine and chemokine profile at the injection site draining Lymph Node and in plasma after Matrix-M adjuvant administration: (b) (4) mice were administered with Matrix-M adjuvant alone at 5 µg or with Matrix-M adjuvant with 6 µg of quadrivalent Nanoparticle Influenza Vaccine (NIV) (HA A/Michigan, A/Singapore, B/Phuket, B/Iowa), or with NIV alone (6 µg) IM in the hind leg. The cytokine and chemokine concentrations in the plasma and injection site muscle and in the draining lymph node (dLN) samples were measured.

STF 20#315A Cell recruitment and activation in draining lymph node and muscle upon intramuscular injection of Matrix-M: Female (b) (4) mice were immunized intramuscularly with 5 µg Matrix-M, Matrix-A or Matrix-C with 3 µg bivalent Nanoparticle Influenza Vaccine (NIV) (1.5 µg hemagglutinin (HA) from each A/Brisbane and B/Maryland strains) or with PBS. Injection site muscle and dLN were collected 48 hours post immunization (pi). The number of innate cells and of B and T cells were assessed in both tissues. Subcapsular sinus (SCS) and medullary sinus (MS) macrophages were quantified in dLN.

STF 702-037 Temporal and spatial effects of Matrix-M adjuvant administration on adaptive immune responses: (b) (4) mice were injected with 5 µg Matrix-M adjuvant IM prior or after injecting 5 µg of recombinant Ebola virus glycoprotein (EBOV GP) or co-administered at the same time at the same quadriceps muscle site. Additionally, mice were injected with Matrix-M adjuvant at the same site or in the opposite quadriceps muscle as EBOV GP. EBOV GP-specific IgG responses were evaluated at Day 14 and Day 21.

STF 80.08.0687 In vitro investigation into the mechanisms of action of Matrix-M: Cell viability, role of lysosomes, and hemolytic activity of Matrix-M or Matrix-A and Matrix-C were studied in U937 cells (human promonocytic cell line), in RAW 264.7 cells (mouse macrophage like cell line), and in primary

human monocytes treated with Matrix-M with doses from 0.27 µg/ml to 7.41 µg/mL. In all tested cells, the decreased viability following treatment with Matrix-M was completely blocked by pretreatment with inhibitors of lysosome acidification bafilomycin A1 and chloroquine. Fraction-A and Fraction-C induced a rapid reduction in cell viability in RAW 264.7 cells at 4°C or at 37°C due to the lytic nature of unformulated saponins. In contrast, Matrix-A and Matrix-C decreased cell viability at 37°C in a time-dependent manner and to a lesser degree compared with the unformulated fractions.

3.6.3 22#330 (C1080121) BIODISTRIBUTION STUDY OF MATRIX-M1 ADJUVANT FOLLOWING INTRAMUSCULAR INJECTION IN MICE (FINAL REPORT).

Also reported in Cecilia Carnrot et al., "Biodistribution of the saponin-based adjuvant Matrix-M following intramuscular injection in mice" Frontiers in Drug Delivery 2023 https://doi.org/10.3389/fddev.2023.1279710. Radiolabeled saponins or cholesterol (^{3}H) were incorporated into Matrix-M1 particles. Labeled Matrix-M1 adjuvant (10 µg) was given to CD-1 female and male mice by intramuscular injection with or without SARS-CoV-2 Spike protein (1 µg). Radioactivity of the adjuvant components was quantified in local and systemic tissues at seven timepoints over a period of 1 to 168 h (Figure 2, plasma, urine, bone marrow, brain, heart and injection site; Figure 3, axillary lymph nodes, iliac lymph nodes, inguinal lymph nodes, mandibular lymph nodes, popliteal lymph nodes, and mesenteric lymph nodes; Figure 4 intestine, kidney, liver, lungs, spleen, and testis; Figure 5, ovaries and uterus; Matrix-M1 alone, light blue circles; Matrix-M1 with SARS-CoV-2 rS protein, dark blue circles; cholesterol, green circles; data is shown as % of injected radioactivity/ml for plasma and urine or gram of tissue, normalized %ID/g).

Figure 2

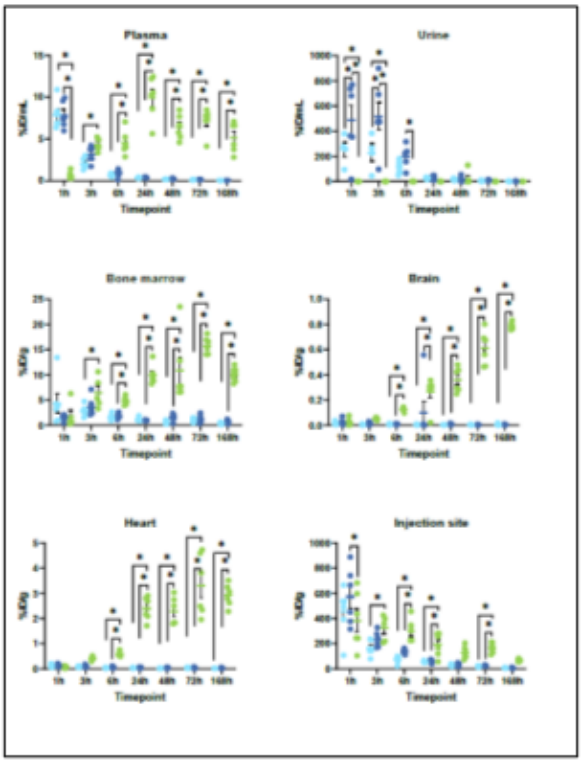


Figure 3

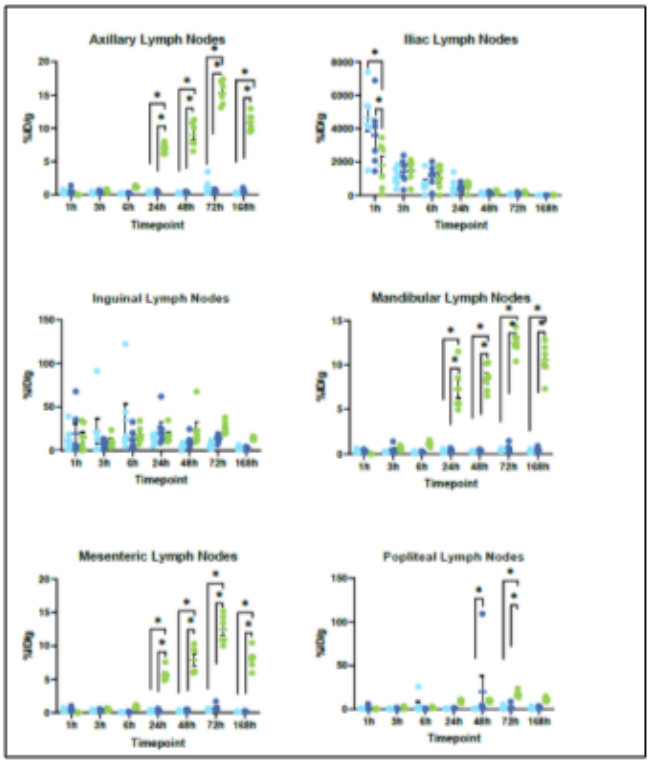


Figure 4

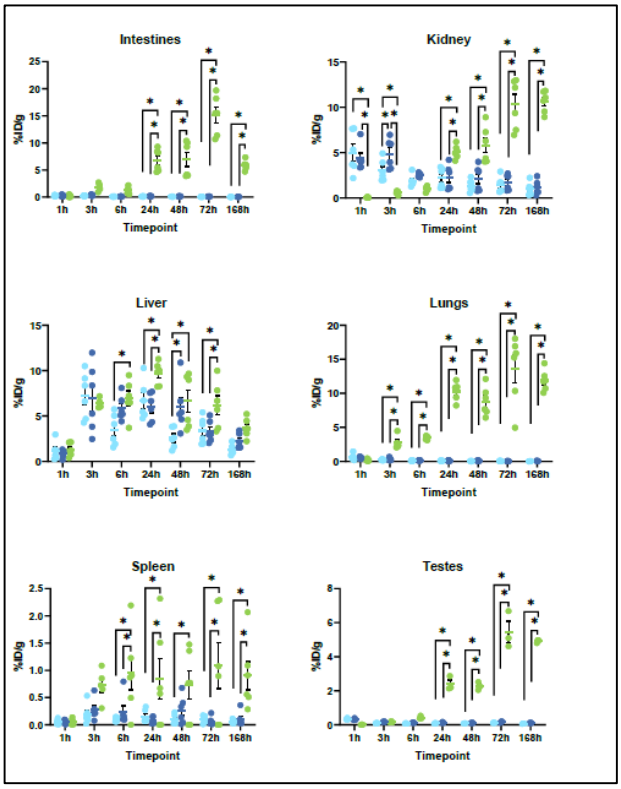
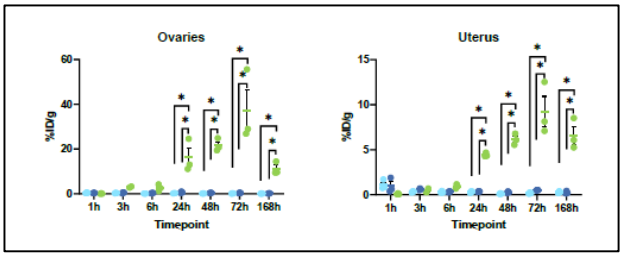


Figure 5



The highest saponin levels were found at the 1-h timepoint at the injection site, in the draining (iliac) lymph nodes, and in urine. Saponins were rapidly cleared from these tissues, reaching very low levels by 48–72 h. Systemically, saponins were found at low levels in the plasma, kidneys, liver, and bone marrow, and were barely detectable in other investigated tissues. No consistent statistically significant differences between mice that received Matrix-M1 adjuvant alone or in combination with rS protein was observed. Cholesterol (light green circles) was also found at high levels at the injection site and in the draining lymph nodes. These levels declined rapidly at first, then plateaued at 24–48 h. Radiolabeled cholesterol was found at low levels in other tissues at the earliest timepoints, until increasing and stabilizing after the 24-h timepoint, indicating entry into the endogenous cholesterol recycling pool**.**

Reviewer's note This study demonstrates a rapid distribution of Matrix-M1 adjuvant from the injection site to the draining lymph nodes, thus excluding a depot effect as central to the mechanism of action for this adjuvant. The difference in clearance patterns for saponins and cholesterol are suggestive of at least partial disassembly of the Matrix-particles.

Reviewer's note: the presence of cholesterol in most of the tissues at 7 days after administration may be consistent with the normal presence of cholesterol and its metabolites in all mammalian tissues.

Reviewer's note: for biodistribution study in mice, the sponsor used 10 µg dose of Matrix-M adjuvant (alone or with rS protein), which is 5 times lower than 50 µg of adjuvant used in a single dose of vaccine. Considering that the average weight ratio of human to mice (70 kg vs 20 g, respectively) is 3500 to 1.

Reviewer's assessment: In total, the biodistribution studies demonstrated the rapid local dissemination of Matrix M from the site of injection to draining lymph nodes, plasma urine and spleen, followed by

clearance of the saponin components after 24 hours. At that stage, the cholesterol component is released from cells and enters the endogenous cholesterol recycling pool.

APPENDIX 1 ABBREVIATIONS AND THE LIST OF REVIEWED REPORTS

ABBREVIATIONS

AcOH, Acetic acid
AGC-CPH, AGC Biologics A/S (CMO for manufacturing of Matrix-A/C in Copenhagen, Denmark)
Ardena-S, Ardena, Södertälje, Sweden
(b) (4)

BP, British Pharmacopoeia
BPR, Batch Production Records
CFU, colony forming units
CMO, Contract Manufacturing Organization
CPP, Critical Process Parameter:
CQA, Critical Quality Attribute
(b) (4)

IP, Indian Pharmacopoeia
IPC, In-Process Control
(b) (4)
IU, International unit of endotoxin
KPP, Key Process Parameter
(b) (4)

NOR, Normal Operating Range
NVX-AB, Novavax AB in Uppsala, Sweden
NVX-CoV2373 Vaccine antigen in Novavax vaccine against COVID-19 infection
PAR, Proven Acceptable Range
PBS, Phosphate Buffered Saline
PC, Phosphatidylcholine
(b) (4)
Permitted Daily exposure (PDE)
PE, Polyethylene
PETG, Polyethylene terephthalate glycol
PFS, Pre-Filled Syringe
Ph. Eur., European Pharmacopeia
(b) (4)

PPQ, Process Performance Qualification

STN 125817 Nuvaxovid
CMC: Matrix-M Adjuvant
(b) (6)

PQ, Performance Qualification
PV, Process Validation
(b) (4)

USP, United States Pharmacopeia
(b) (4)

REVIEWED REPORTS DS (72)

QAG_01802	QAG_04958	QAG_05787	QAG_05809	QAG_07973	QAG_09434
QAG_02093	QAG_05134	QAG_05788	QAG_05810	QAG_08193	QAG_09521
QAG_02220	QAG_05573	QAG_05789	QAG_06005	QAG_08194	QAG_09522
QAG_02474	QAG_05575	QAG_05790	QAG_06006	QAG_08195	QAG_09851
QAG_02475	QAG_05623	QAG_05791	QAG_06300	QAG_08196	QAG_09852
QAG_03328	QAG_05631	QAG_05792	QAG_07483	QAG_08197	QAG_10220
QAG_04083	QAG_05632	QAG_05793	QAG_07484	QAG_08521	QAG_19910
QAG_04085	QAG_05636	QAG_05801	QAG_07593	QAG_08522	QAG_20331
QAG_04278	QAG_07576	QAG_05803	QAG_07666	QAG_08530	QAG_20329
QAG_04279	QAG_05641	QAG_05804	QAG_07675	QAG_09198	QAG_21042
QAG_04926	QAG_05642	QAG_05807	QAG_07676	QAG_09292	QAG_26430
QAG_04957	QAG_05786	QAG_05808	QAG_07677	QAG_09294	D_OP_01230

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RDG_000216	QAG_07969	QAG_24081	QAG_25181	M_BC_TM_00663
QAG_04829	QAG_08393	QAG_24121	QAG_26531	RDG_000216
QAG_04964	QAG_10442	QAG_24186	QAG_27192	
QAG_07396	QAG_20109	QAG_24231	QAG 27585	
QAG_07612	QAG_20130	QAG_24815	QAG_27773	
QAG_07791	QAG_20312	QAG_25052	QAG_27777	
QAG_07911	QAG_20702	QAG_25062	QAG_28284	
QAG_07920	QAG_20596	QAG_25063	QAG_28508	
QAG_07967	QAG_20597	QAG_25064	QAG_28686	
QAG_07968	QAG_23637	QAG_25133	QAG_29209	