U.S. FOOD & DRUG ADMINISTRATION

DATE: March 23, 2025

TO: Biologics License Application File 125817/0

FROM: (b) (6)

THROUGH: (b) (6)
OVRR/CBER

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/OVRR/CBER

SPONSOR: Novavax, Inc.

STN: CMC Review/BLA 125817/0

PRODUCT: COVID-19 Vaccine, Adjuvanted [Nuvaxovid; SARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine (SARS-CoV-2 rS) with Matrix-M™ Adjuvant]

PROPOSED INDICATION: Active Immunization for the Prevention of COVID-19 Caused by SARS-CoV-2

ACTION DUE DATE: April 1, 2025

1. BLA STN: 125817/0

2. Applicant Name and License Number: Novavax, Inc., License #: 2349

3. Product Name/Product Type
Product name: COVID-19 Vaccine, Adjuvanted
Proprietary name: Nuvaxovid
Product Type: Recombinant spike (S) protein (SARS-CoV-2 rS) vaccine with Matrix-M adjuvant.

4. General Description of the Final Product
The COVID-19 Vaccine, Adjuvanted (Nuvaxovid) contains a recombinant full-length SARS-CoV-2 spike glycoprotein (rS) that is stabilized in its prefusion conformation, as the active ingredient. The Original monovalent (Wuhan strain; NVX-CoV2373) contains the rS protein of the prototype (Wuhan) SARS-CoV-2 and was authorized for emergency use on July 13, 2022. With the emergence of SARS-CoV-2 variants, the applicant developed vaccines in which the rS antigen of the prototype SARS-CoV-2 was substituted with the rS of a predominantly circulating variant recommended by expert advisory committees. Thus, vaccines containing the rS of various SARS-CoV-2 strains, including Omicron BA.1, Omicron BA.2, Omicron BA.5, Omicron XBB.1.5, and Omicron JN.1 were developed at various times. Like the Original monovalent (Wuhan), Omicron XBB.1.5 variant (2023-2024 Formula; NVX-CoV2601) and the Omicron JN.1 variant (2024-2025 Formula; NVX-CoV2705) were authorized October 3, 2023, and August 30, 2024, respectively, for emergency use under EUA 28237.
The SARS-CoV-2 rS antigen DS is manufactured as a recombinant glycoprotein in *Sf*9 insect cells using a baculovirus (BACV) vector platform. The drug product (DP) is a co-formulation of rS antigen with Matrix-M adjuvant, with Matrix-M as an enhancer of the immune response induced by rS. Following expression and purification, rS DS is stored at (b) (4)
Matrix-M is an adjuvant derived from saponin fractions (Fraction-A and Fraction-C) extracted from the bark of the soapbark tree, *Quillaja saponaria* Molina, phosphatidylcholine, and cholesterol. The two saponin fractions are formulated into approximately (b) (4) cage-like structures, Matrix-A and Matrix-C particles respectively, in a buffer containing potassium dihydrogen phosphate, potassium chloride, disodium hydrogen phosphate dihydrate, and sodium chloride. The final DP is a colorless to slightly yellow, clear to mildly opalescent solution, practically free from visible particles. COVID-19 Vaccine, Adjuvanted is a sterile, preservative-free, ready-to-use aqueous-buffered suspension. The Original monovalent (Wuhan) vaccine was filled as 10-dose (10DV) and 5-dose (5DV) multi-dose vials (MDV) presentations with (b) (4) , respectively. Both MDV presentations are filled into 5-mL clear (b) (4) type (b) (4) siliconized glass vials (with an average (b) (4) of (b) (4)) with 13-mm uncoated, siliconized bromobutyl rubber stoppers and 13-mm flip-off aluminum seal, and blue-colored plastic caps.

Following the emergence of SARS-CoV-2 variants and the observed reduction in clinical efficacy of Wuhan COVID-19 vaccines against emerging variants, particularly the Omicron variants, vaccine formulations containing variant-specific spike proteins were developed. Thus, the applicant initially developed and produced different clinical-trial materials (CTMs) of COVID-19 Vaccine, Adjuvanted of Omicron BA.1 and Omicron BA.5, in which the active ingredient was the rS protein from SARS-CoV-2 Omicron BA.1 and Omicron BA.5, respectively. The Omicron BA.1 and Omicron BA.5 CTMs were manufactured from the same Sf9 cells/BACV platform and with a similar manufacturing process as the Original monovalent,

including the production of the final DP container as a co-formulation of rS (BA.1 rS or BA.5 rS) with Matrix-M adjuvant. As the Omicron XBB.1.5 strain became the predominant circulating SARS-CoV-2 variant in the United States (U.S.) during the spring of 2023, COVID-19 vaccine development was re-directed to the XBB.1.5 vaccine resulting in the COVID-19 Vaccine, Adjuvanted (2023-2024 Formula; NVX-CoV2601). The 2023-2024 Formula, which contains the rS of Omicron XBB.1.5 SARS-CoV-2 as the active ingredient, was authorized as a 5-dose vial presentation for emergency vaccine supply in the U.S. on October 3, 2023.

With the continued evolution of new Omicron-lineage SARS-CoV-2 variants, the Vaccines and Related Biological Products Advisory Committee (VRBPAC) recommended the use of the Omicron JN.1 spike protein in formulating COVID-19 vaccines for the 2024-2025 season, leading to the development of the COVID-19 Vaccine, Adjuvanted (2024-2025 Formula). The 2024-2025 Formula contains rs pf the Omicron JN.1 SARS-CoV-2 as the active ingredient. The production of a single-dose pre-filled syringe (PFS) presentation of COVID-19 Vaccine, Adjuvanted was originally executed with the manufacture of PFS process-validation lots of the 2023-2024 Formula (Omicron XBB.1.5 vaccine). Subsequently, the 2024-2025 Formula was manufactured in the PFS presentation. The PFS presentation is filled at (b) (4) mL (per syringe) into 1-mL glass-barrel syringes with Luer-lock and plastic rigid tip caps. The syringe is stoppered with a plunger stopper which has a plunger rod made of polystyrene.

A single dose of the Novavax COVID-19 Vaccine, Adjuvanted (Nuvaxovid) is 0.5 mL and contains 5 µg of rS antigen and 50 µg of Matrix-M adjuvant. The COVID-19 Vaccine, Adjuvanted is indicated for active immunization in individuals 12 years of age and older for the prevention of COVID-19 caused by SARS-CoV-2. The vaccine is administered via the intramuscular route. The final DP is stores at 2° to 8°C, protected from light.

5. Major Milestones

This BLA was initially submitted in three rolls, with the third (final) roll submitted on April 1, 2024. A filing meeting was held on May 7, 2024, and an action date of April 1, 2025, was assigned for a standard review. FDA authorized the COVID-19 Vaccine, Adjuvanted (2024-2025 Formula) (JN.1 vaccine) on August 30, 2024, for vaccine supply for emergency use. At the mid-cycle review meeting with the applicant, September 30, 2024, FDA requested the applicant to submit Chemistry, Manufacturing, and Controls (CMC) information on the 2024-2025 Formula to the BLA. On October 31, 2024, the applicant submitted CMC information and nonclinical study data for the 2024-2025 Formula in amendment 42 to the BLA (BLA 125817/0.42; Serial # 0044).

6. CMC/Quality Review Team

The CMC quality review team comprises of the following reviewers:
(b) (6) [3.2.S Drug Substance Matrix-A and Matrix-C – NVX-AB (Sections 3.2.S.1 through 3.2.S.7); 3.2.S Drug Substance Matrix-A and Matrix-C – AGC-CPH (Sections 3.2.S.1 through 3.2.S.7)].

(b) (6) Control of Materials (b) (4) systems (b) (4), Characterization, and Testing], and (b) (4) (Section 3.2.S.2.3). Adventitious Agents safety evaluation and validation of viral clearance (Section 3.2.A.2).

(b) (6) (5.3.1 Reports of Biopharmaceutical Studies; and immunogenicity assays).

(b) (6) (rS Drug Substance (Sections 3.2.S.1 through 3.2.S.7); Drug Product, SARS-CoV-2 rS Vaccine, Adjuvanted (Section 3.2.P), and Module 4 (Non-clinical pharmacology); other CMC-related sections of the eCTD (Patent and Exclusivity [Section 1.3.5]; and Environmental Analysis [Section 1.12.14]).

7. Inter-Center Consults Requested

Not Applicable.

8. Submissions Reviewed

Date Received	Submission	Comments/ Status
January 31, 2024	Original submission, Roll 1 (STN 125817/0.0)	Reviewed by (b) (6)
February 27, 2024	Response to IR #2 of February 20, 2024 (STN 125817/0.1)	Reviewed by (b) (6)
February 29, 2024	Original submission, Roll 2 (STN 125817/0.2)	Reviewed by (b) (6)
April 1, 2024	Original submission, Roll 3 (STN 125817/0.4)	Reviewed by (b) (6)
April 18, 2024	Response to IR #8 of April 15, 2024 (STN 125817/0.10)	Reviewed by (b) (6)
May 9, 2024	Response to IR #12 of April 29, 2024, and FDA advice of May 3, 7, 8, 2025 (STN 125817/0.18)	Reviewed by (b) (6)
May 20, 2024	Response to IR #12 of April 29, 2024, and FDA advice of May 3, 7, 8, 2025 (STN 125817/0.22)	Reviewed by (b) (6)
May 15, 2024	Response to IR #14 of May 10, 2024 (STN 125817/0.21)	Reviewed by (b) (6)
December 20 2024	Response to IR #40 of December 16, 2024 (STN 125817/0.55)	Reviewed by (b) (6)
February 25, 2025	Response to FDA advice of February 23, 2025 (STN 125817/0.76)	Reviewed by (b) (6)
February 25, 2025	Response to IR #53 of February 24, 2025 (STN 125817/0.75)	Reviewed by (b) (6)
February 25, 2025	Response to IR #53a of February 25, 2025 (STN 125817/0.75)	Reviewed by (b) (6)
February 27, 2025	Response to IR #55 of February 26, 2025 (STN 125817/0.78)	Reviewed by (b) (6)
February 27, 2025	Response to FDA advice of February 26, 2025 (STN 125817/0.76)	Reviewed by (b) (6)
March 17, 2025	Response to IR #67 of March 14, 2025 (STN 125817/0.92)	Reviewed by (b) (6)
March 17, 2025	Response to IR #69 of March 17, 2025 (STN 125817/0.94)	Reviewed by (b) (6)

9. Referenced Regulatory Submissions

Master File # (b) (4) – Adventitious agents testing, (b) (4)
Master File # (b) (4) – SARS-CoV-2 Neutralizing Antibody Assays, (b) (4)

Drug Master File # (b) (4) – Phosphatidylcholine, (b) (4)

10. Reviewer Summary and Recommendation

A. Executive Summary

The COVID-19 Vaccine, Adjuvanted (Nuvaxovid) (Wuhan strain; Original monovalent) was authorized under an Emergency Use Authorization (EUA), July 13, 2022, for active immunization for the prevention of COVID-19 in individuals 12 years of age and older. As new SARS-CoV-2 variants emerged with divergent mutations in the receptor-binding domain of the spike protein, the likelihood of a reduction in the clinical efficacy of the Original monovalent against emerging variants became apparent. Thus, new vaccine formulations containing variant-specific spike proteins were developed. An updated vaccine was formulated with purified rS of the Omicron XBB.1.5 variant for the 2023/2024 vaccination campaign and was authorized October 3, 2023. As the JN.1 lineage of Omicron variants became dominant during 2024, the COVID-19 Vaccine, Adjuvanted was updated with the rS of the Omicron JN.1 subvariant as the active ingredient for the 2024-2025 Formula. Thus, the Original monovalent (Wuhan) vaccine, was developed as the first COVID-19 Vaccine, Adjuvanted, with the 2023-2024 Formula (XBB.1.5) and the 2024-2025 Formula (JN.1) introduced in 2023/2024 and 2024/2025, respectively, to implement a strain change.

The manufacturing process for Nuvaxovid is similar among different variant vaccines. However, while the authorized DP final container was in a multidose vial presentation (5- and 10-dose vials [5DV, 10DV] for the Original monovalent and 5DV for the 2023-2024 Formula), the JN.1 vaccine is in a PFS presentation.

The active ingredient in Nuvaxovid is the full-length SARS-CoV-2 spike protein produced as a baculovirus recombinant protein (rS) in Sf9 insect cells. The rS protein is extracted from Sf9 insect cells infected with recombinant baculovirus vector expressing rS and purified from the (b) (4) in a series of purification steps, including (b) (4) chromatography and (b) (4) chromatography. (b) (4) formulated into the rS (b) (4) in a formulation buffer composed of (b) (4) sodium phosphate (b) (4) sodium chloride, and (b) (4) (w/v) polysorbate-80.
The Nuvaxovid DP is a co-formulation of rS protein DS with Matrix-M adjuvant. The adjuvant is manufactured by mixing Matrix-A and Matrix-C, which are produced from purified Fraction-A and Fraction-C components, respectively, of saponin extracts of the soapbark tree, *Quillaja saponaria* Molina. Thus, the same vaccine technology platform is used in the production of Nuvaxovid, irrespective of the constituent rS.

The original Wuhan vaccine (BV2373) was extensively tested for immunogenicity and protective efficacy in animal models, including mice, hamsters, and non-human primates, as well as in Phases 1/2 and 3 clinical studies. A pivotal Phase 3 clinical trial conducted in the United States (U.S.) and Mexico provided the clinical efficacy data that supported the authorization of the Original monovalent. Following the emergence of the Omicron SARS-CoV-2 variants, a construct (BV2515) containing rS of Omicron BA.1 variant and BV2540, a construct containing rS of Omicron BA.5 variant were generated and tested for immunogenicity in nonclinical studies. Clinical trial materials of these constructs, NVX-CoV2515 and NVX-CoV2540, were also evaluated in clinical studies, including a Phase 3 study in which NVX-CoV2515 and NVX-CoV2540 were administered in individuals previously vaccinated with mRNA COVID-19 vaccines. Prior to the completion of the clinical development of the BA.5

vaccine, the SARS-CoV-2 Omicron XBB.1.5 variant superseded Omicron BA.5 as the dominant variant associated with SARS-CoV-2 infections. Thus, for the vaccine strain recommendation for the 2023-2024 Formula, the XBB.1.5 rS construct (BV2601) was evaluated for immunogenicity in nonclinical studies and under clinical study protocols.

Production of the rS DS and the Nuvaxovid drug product for commercial vaccine supply to the U.S. is performed at the Serum Institute of India Private Limited (SIIPL). The adjuvant component (Matrix-A and Matrix-C) drug substances are manufactured at Novavax AB, Uppsala Sweden (NVX-AB) and AGC Biologics, Copenhagen, Denmark (AGC-CPH).

This review memo covers manufacturing information for the rS DS (Wuhan rS, XBB.1.5 rS, and JN.1 rS) and the Nuvaxovid DP final container, the range of bioanalytical assays used in the characterization and quality control testing for the release and stability monitoring of the rS DS, and the Nuvaxovid final DP as well as the nonclinical pharmacological evaluation of immunogenicity of the rS DS (formulated with Matrix-M) in animals. This memo also includes the clinical immunogenicity and diagnostics assays that were used to support product development and reviewed by (b) (6)

CMC information for rS DS and DP manufacture, including quality control analytical testing, was originally submitted under Investigational New Drug (IND) application 22430/0 and in subsequent amendments to the IND. Manufacturing information was also provided under EUA 28237 to support the emergency use authorization requests for the Original monovalent (EUA 28237/0), 2023-2024 Formula (EUA 28237, Amendment 130), and 2024-2025 Formula (EUA 28237, Amendment 246). The marketing authorization request for Nuvaxovid under Biologics License Application (BLA) # 125817 was submitted under a rolling submission plan agreed between the applicant and FDA. CMC information was originally submitted in Roll 1 (January 31, 2024) and Roll 3 (April 1, 2024). Manufacturing information for the JN.1 rS DS and the JN.1 vaccine DP (2024-2025 Formula) was submitted in BLA 125817/0.42, October 31, 2024. Additional CMC, clinical assays, nonclinical pharmacology, or bioanalytical assay information provided in response to FDA information requests were submitted in amendments 1, 10, 18, 21, 22, 40, 53, 55, 59, 60, 65, 70, 71, 73, 75, 76, 77, 78, 92 and 94 to the BLA.
Issues relating to analytical assays were identified during product development and were resolved. During Phase 3 clinical development, the (b) (4) assay was used for measuring the total protein concentration of the Original monovalent (Wuhan) vaccine, as well as for the release of the Wuhan vaccine under the EUA. However, due to assay variability and shortage of the (b) (4) assay (b) (4), the assay could not be used in measuring the total protein concentration of Omicron vaccine drug products (BA.5, XBB.1.5). An (b) (4)) was developed and validated for the release and stability monitoring of the 2023-2024 Formula (XBB.1.5 vaccine DP), with a specification range of (b) (4) . Finally, a (b) (4) assay was developed and validated to replace the (b) (4) for total protein concentration for the release and stability monitoring of all COVID-19 Vaccine, Adjuvanted drug products. The release and stability specification for both (b) (4) are the same (i.e., (b) (4)). In bridging studies comparing the (b) (4) , and (b) (4) assays using XBB.1.5 DP lots, the results from the three assays were determined to be statistically equivalent.

Following the emergence of SARS-CoV-2 Omicron variants, the development of variant vaccines is constrained by the unavailability of variant-specific reference standards. Thus, for

the 2023-2024 Formula (XBB.1.5 vaccine), the Wuhan reference standard was used as the reference standard for relative potency testing of XBB.1.5 vaccine lots, resulting in potency values that were higher than the upper limit for the release of the Wuhan vaccine. After a homologous XBB.1.5 reference standard was developed and qualified, the release and stability specifications for the XBB.1.5 DP were revised. In addition, the lower release limit for relative potency for variant DP release must be re-evaluated as real-time stability data become available. Similarly, identity testing by (b) (4) is usually not possible in the early stage of development of variant vaccines. Thus, a (b) (4) method is used for identity testing until a qualified variant-specific antibody becomes available.

Both the Original monovalent (Wuhan) and the 2023-2024 Formula (XBB.1.5 vaccine) were manufactured at a target total protein concentration of (b) (4) µg/mL and authorized with a 9-month shelf life, supported with stability data. Using the same formulation strategy, the 2024-2025 Formula (JN.1 vaccine) was originally formulated at (b) (4) µg/mL. However, during an inspection of the SIIPL manufacturing facilities in June/July of 2024, FDA inspectors were informed that the release results for relative potency for (b) (4) process-performance qualification (PPQ) lots of the JN.1 vaccine formulated at (b) (4) µg/mL would not support a 9-month shelf life. Consequently, a new PPQ was initiated with a protein formulation target of (b) (4) µg/mL. The (b) (4) µg/mL formulation was authorized for vaccine supply for emergency use. The JN.1 vaccine DP under long-term storage, including the (b) (4) µg/mL formulation, rapidly loses potency in the (b) (4) month of manufacture (an average of (b) (4) potency loss for the (b) (4) µg/mL formulation). Based on the limited stability data available, a 3-month shelf life was authorized for the JN.1 vaccine and a 3-month shelf life is proposed in the licensure application submitted in BLA 125817/0. For the emergency supply of the JN.1 vaccine under the EUA, FDA required the applicant to provide real-time stability data for JN.1 DP lots on a monthly basis, and every commercial DP lot must be placed under stability monitoring. In addition, a statistically derived action limit of no less than (b) (4) at (b) (4) post-manufacture was implemented to assure vaccine potency at the proposed end of shelf life. JN.1 DP lots at the alert limit of (b) (4) are monitored in the stability program, but any JN.1 DP lot with a relative potency below (b) (4) at the (b) (4) stability timepoint is placed on hold and quarantined for an investigation. Real-time stability data for (b) (4) lots (b) (4) process-validation lots and (b) (4) commercial lots) of the JN.1 DP (b) (4) µg/mL formulation) through the (b) (4) month timepoint show an average relative potency of (b) (4), with (b) (4) of the (b) (4) lots having a relative potency of (b) (4) at (b) (4) months (i.e., below the (b) (4) lower limit of stability specification). Thus, the available real-time stability data support the 3-month shelf-life request for the JN.1 vaccine (2024-2025 Formula).

A rapid loss of potency occurs in the (b) (4) month of manufacture of the JN.1 vaccine. Therefore, FDA required the applicant to place all commercial lots of the 2024-2025 Formula (JN.1 vaccine; (b) (4) µg/mL formulation) on stability evaluation. A total of (b) (4) lots (comprised of (b) (4) PPQ lots and (b) (4) commercial lots) have been placed under long-term stability monitoring. In EUA 28237/0.348, the applicant requested to cease placing additional lots of the 2024-2025 Formula under long-term stability evaluation, but with the (b) (4) relative potency action limit at the (b) (4) timepoint remaining in place for newly manufactured DP lots. The average relative potency of the (b) (4) lots at release was (b) (4) Standard Deviation (lower limit of specification for DP release = (b) (4). In addition, real-time stability results for (b) (4) of the (b) (4) lots were provided through the (b) (4) month timepoint. (b) (4) of the (b) (4) lots had relative potency values in the range of (b) (4) at (b) (4) months (i.e., above the lower limit of stability specification of (b) (4)). Further, real-time stability data for al (b) (4) PPQ lots at the (b) (4) month timepoint show that (b) (4) lots had relative potency (b) (4) Based on these data, FDA concurred with the applicant that newly manufactured lots of the JN.1 vaccine need not be placed on long-term stability but

recommended continuous stability monitoring of lots with a relative potency of (b) (4) at the (b) (4) timepoint.

Regarding shipping of the COVID-19 Vaccine, Adjuvanted (2024-2025 Formula) in prefilled syringe presentation, FDA considers the shipping validation study data submitted in the original BLA to be inadequate and recommended a repeat of the study with a minimum of (b) (4) lots of recently manufactured 2024-2025 Formula (JN.1 vaccine). The study report will be submitted as a Post-marketing Commitment at the end of July 2025.

Information on nonclinical pharmacology studies of rS in animal species was submitted in Roll 2 (BLA 125817/0.2, February 29, 2024), and in BLA 125817/0.42. The review of information relating to Matrix-M adjuvant, its components, manufacture, and quality control testing is performed by (b) (6) the review of the adventitious agents' testing program for the rS DS, including Sf9 insect (b) (4) testing, (b) (4) testing is performed by (b) (6) and the review of diagnostic and immunogenicity assays supporting clinical evaluation of the COVID-19 Vaccine, Adjuvanted is performed by (b) (6)

B. Recommendation: The CMC information on the rS DS and DP processes for the manufacture of Nuvaxovid shows that they are adequately controlled to assure the purity and quality of the product, and consistency of manufacture. In analytical comparability studies, the variant rS drug substances (XBB.1.5 rS and JN.1 rS) are comparable in purity and quality with the Wuhan rS DS. Similarly, the variant vaccine drug products manufactured to date are comparable to the Wuhan vaccine DP, but the JN.1 DP (2024-2025 Formula) tends to undergo a rapid potency loss during the (b) (4) month of manufacture. The available long-term stability data for the Original monovalent and the 2023-2024 Formula presented in MDVs support a 9-month shelf-life but not for the 2024-2025 Formula presented in PFS. However, the available real-time stability data for the 2024-2025 Formula support a 3-month shelf-life as requested by the applicant in the BLA. Therefore, approval of Nuvaxovid as a vaccine for immunization for protection against COVID-19 is recommended.
The batch-analysis data and lot release results for DP lots intended for commercial distribution in the U. S. must be submitted for FDA review and approval prior to their distribution.

Manufacturing facilities
Serum Institute of India Pvt. Ltd., (b) (4), INDIA (b) (4)

Serum Institute of India Pvt. Ltd., (b) (4), INDIA (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Novavax – (b) (4)

Novavax AB, (b) (4) Uppsala, SWEDEN (b) (4)

(b) (4)

(b) (4)

(b) (4)

AGC Biologics A/S, (b) (4) , DENMARK (b) (4)

(b) (4)

(b) (4)

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3.2.S DRUG SUBSTANCE

3.2.S.1.1 – 1.3 Nomenclature, Structure and General Properties

The nomenclature for the three variants of the Novavax COVID-19 Vaccine drug substance (DS) included in this original BLA is as follows:

(b) (4)

(b) (4)

3.2.S.2 Manufacture

3.2.S.2.1 Manufacturers

The facilities involved in the manufacture of rS DS, the role of each facility and their registration information are provided in Table 1 (Adapted from Table 1, Section 3.2.S.2.1, BLA 125817/0.0, /0.4, and /0.42).

(b) (4)

(b) (4)

3.2.S.2.2 Description of Manufacturing Process

(b) (4)

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

(b) (4)



3.2.P DRUG PRODUCT

3.2.P.1 Description and Composition of Drug Product

Nuvaxovid (COVID-19 Vaccine, Adjuvanted) is a sterile, preservative-free suspension-for-injection containing recombinant spike (rS) protein of SARS-CoV-2 as the active ingredient. The Original monovalent (Wuhan strain) vaccine contains rS of the prototype Wuhan strain of SARS-CoV-2, the 2023-2024 Formula contains rS of the Omicron XBB.1.5 variant, and the 2024-2025 Formula contains rS of the Omicron JN.1 variant. Nuvaxovid is an aqueous-buffered suspension of rS DS co-formulated with Matrix-M adjuvant in a formulation buffer containing disodium hydrogen phosphate heptahydrate, sodium dihydrogen phosphate monohydrate, sodium chloride, polysorbate 80 (PS-80) and water-for-injection. The Matrix-M adjuvant contains Fraction-A and Fraction-C of saponin extracts derived from the soapbark tree, *Quillaja saponaria* Molina. The two fractions are separately formulated with cholesterol and phosphatidylcholine into Matrix complexes (Matrix-A and Matrix-C).

The Original monovalent (Wuhan vaccine) drug product (DP) was filled in multidose vial presentations of 10-dose vial (10DV) and 5-dose vial (5DV); the 2023-2024 Formula (XBB.1.5 vaccine) was filled in a 5DV presentation; and the 2024-2025 Formula (JN.1 vaccine) is filled as a single-dose pre-filled syringe (PFS) presentation.

Both the 10DV and 5DV presentations were filled in 5-mL (b) (4) type (b) (4) siliconized glass vials at a target of (b) (4) mL per vial and (b) (4) mL, respectively. The vial has an average (b) (4) of (b) (4). Vials are stoppered with uncoated, siliconized 13-mm bromobutyl rubber stoppers and crimped with 13-mm flip-off aluminum seal with blue plastic flip-off caps. For the PFS presentation, each syringe is filled at a target of (b) (4) mL in sterile, ready-to-fill 1-mL, round flange, siliconized Type (b) (4) borosilicate glass syringe barrel with Luer-lock and plastic rigid tip cap with (b) (4) elastomer. The syringe plunger stopper is a (b) (4) and the plunger rod is made of polystyrene.

The composition of Nuvaxovid, including excipients, is the same, irrespective of the vaccine strain/variant and presentation, except for the change in rS type. A dose of the vaccine contains 5 µg of rS antigen and 50 µg of Matrix-M (also known as Matrix-M1) and formulation excipients. The vaccine DP is stored at 2° to 8°C and administered by intramuscular injection in a volume of 0.5 mL. A list of the components of Nuvaxovid, including the active ingredient, adjuvant, and their quantities (per dose) and the function of each excipient is presented in Table 19 (Adapted from Table 1, Section 3.2.P.1, BLA 125817/0.4 and BLA 125817/0.42).

Table 19. Composition of Nuvaxovid drug product

Ingredient	Function	Quantity per 0.5-mL dose	Reference to Quality Standard
SARS-CoV-2 Omicron JN.1 rS protein[1]	Immunogen/active ingredient	5 µg[2]	In-house
Matrix-M Adjuvant (total saponin)[3] Also containing:	Adjuvant	50 µg	In-house
Cholesterol[4]	Formulation agent	30.5 µg	(b) (4)
PCL[4]	Formulation agent	23 µg	
Potassium dihydrogen phosphate[4]	Buffer	3.85 µg	
Potassium chloride[4]	Tonicity agent	2.25 µg	
Disodium hydrogen phosphate dihydrate[4]	Formulation buffer agent	14.7 µg	
Sodium Chloride[4]	Formulation buffer agent	(b) (4)	
Disodium hydrogen phosphate heptahydrate[5]	Formulation buffer agent	2.465 mg[6]	
Sodium dihydrogen phosphate monohydrate[5]	Formulation buffer agent	0.445 mg[6]	
Sodium Chloride[5]	Formulation Buffer agent –isotonicity adjuster	8.766 mg[6]	
Polysorbate 80[5]	Formulation Buffer agent – stabilizer	0.050 mg[6]	
Sodium hydroxide	pH adjustment	q.s.	
Hydrochloric acid	pH adjustment	q.s.	
Water for Injection	Vehicle	q.s.	

[1]SARS-CoV-2 rS of prototype Wuhan strain in the Original monovalent, Omicron XBB.1.5 variant rS in the 2023-2024 Formula, and Omicron JN.1 variant rS in the 2024-2025 Formula
[2]Nominal concentration.
[3]Matrix-M (Matrix-M1) = Matrix-A and Matrix-C components, in (b) (4) (by weight) proportion.
[4]Excipients used for Matrix; PCL is of (b) (4) origin and 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 ng of α-Tocopherol (DL-α-tocopherol).
[5]Excipients used for the DP formulation buffer. There are no (b) (4) for disodium hydrogen phosphate heptahydrate. Disodium hydrogen phosphate heptahydrate is referred to as Sodium phosphate dibasic heptahydrate on supplier's CoA. Sodium dihydrogen phosphate monohydrate is referred to as sodium phosphate monohydrate monobasic on supplier's CoA.
[6]Concentration of 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 (b) (4).
PCL = Phosphatidylcholine; q.s. = quantity sufficient; (b) (4)

3.2.P.2 Pharmaceutical Development

3.2.P.2.1 Components of the Drug Product

3.2.P.2.1.1 Drug Substance

The active ingredient in Nuvaxovid is a recombinant SARS-CoV-2 spike (rS) protein expressed in insect cells from a baculovirus vector, purified, and formulated in a formulation buffer (b) (4) sodium phosphate [pH (b) (4) sodium chloride; and PS-80 (b) (4) The DP is formulated at a nominal concentration of (b) (4) µg/mL rS. The rS DS is co-formulated with (b) (4)

µg/mL Matrix-M which serves as an adjuvant. Matrix-M is a saponin-derived adjuvant composed of two fractions (Fraction A and Fraction C), each of which is formed into nanoparticles (Matrix-A and Matrix-C, respectively) in phosphate-buffered saline (pH (b) (4), with cholesterol and phosphatidylcholine as excipients. In DP production, Matrix-A and Matrix-C are added at a ratio of (b) (4) in the DP formulation buffer and mixed to produce the (b) (4) DP.

3.2.P.2.1.2 Excipients

The DP is formulated in the same formulation buffer in which the rS DS is suspended. Thus, the formulation buffer (pH (b) (4)) contains disodium hydrogen phosphate heptahydrate (b) (4) mg/mL), sodium dihydrogen phosphate monohydrate (b) (4) mg/mL), sodium chloride (b) (4) mg/mL), and PS-80 (b) (4) mg/mL). The monobasic/dibasic sodium phosphate components maintain the pH around the target of (b) (4) at which the DP is stable, and PS-80, a non-ionic surfactant and emulsifier, is added as a stabilizing agent. The sodium chloride component provides stability and modifies tonicity.

3.2.P.2.2 Drug Product

3.2.P.2.2.1 Formulation Development

In Part 1 of Phase 1/2 clinical trial during early clinical development, the final DP was formulated at bedside by mixing rS DS with Matrix-M adjuvant at the point of vaccination. Starting from Part 2 of Phase 1/2 clinical trial till now, the DP clinical and commercial lots are a co-formulation of rS DS with the Matrix-M adjuvant. Co-formulation is performed during DP manufacture prior to filling of the final container. Thus, the co-formulated DP was used in the pivotal Phase 3 clinical study, as well as for vaccine supply under Emergency Use Authorization (EUA) and remains the form in licensure application. The DP formulation buffer has not changed since early product development except for the adjustment of the relative proportions of (b) (4) used to attain a final concentration of (b) (4) in the formulation buffer, to allow for minimal adjustment of pH to attain the target of (b) (4) During early clinical development, the (b) (4) PS-80 (b) (4) was used in the preparation of DP formulation buffer but was subsequently changed to the use of the higher quality (b) (4) PS-80 from the same vendor and PS-80 from (b) (4). Regarding container closure system for the DP, the original Phase 1/2 clinical-trial material (CTM) was filled at (b) (4)/vial into clear, sterile 3-mL single-dose borosilicate Type (b) (4) glass vials ((b) (4) stoppered with 13-mm (b) (4) coating stoppers, (b) (4) and crimped with yellow, flip-off crimp caps, (b) (4). In the latter part of Phase 1/2, the 3-mL single-dose borosilicate Type (b) (4) glass vials were sourced from a different vendor (b) (4). With the transition from single-dose vials in Phase 1/2 to a 10-dose multidose vial (10DV) presentation of the DP in Phase 3, the (b) (4) vials were used at PAR Sterile Products Rochester, Michigan (PAR) to produce the Phase 3 CTM. Following the transfer of manufacturing to the SIIPL, the DP was filled in 5-mL clear, (b) (4) type (b) (4) siliconized glass vials with an average (b) (4) of (b) (4), which was used for the 10DV presentation and the 5DV presentation (for both Wuhan vaccine [Original monovalent] and Omicron XBB.1.5 vaccine [2023-2024 Formula]).

A single-dose pre-filled syringe (PFS) presentation of the DP was originally evaluated with the XBB.1.5 vaccine. In stability evaluation of the PFS presentation of the XBB.1.5 vaccine (manufactured on a small scale) under long-term storage at 2° to 8°C, there were no significant changes in total protein concentration, pH, particle size, and (b) (4). However, there was a reduction in relative potency during the (b) (4) month post-manufacture. Analytical comparability

studies of the PFS and the 5DV presentations indicate that the DP filled in both container closure systems are comparable. The PFS container closure system is used for the JN.1 vaccine (2024-2025 Formula).

During Phase 1/2 and Phase 3 clinical development, production of the rS DS and DP CTMs was contracted to Contract Development and Manufacturing Organizations (CDMOs) located in the U.S., while adjuvant components (Matrix-A and Matrix-C) were manufactured by Novavax AB, (Uppsala, Sweden). In early Phase 1/2 clinical studies, the DP was used at formulations containing up to 50 µg/mL rS DS (i.e., a dose of 25 µg rS/50 µg Matrix-M1) and 70 µg/mL (i.e., 35 µg rS/50 µg Matrix-M1). However, for the latter part of Phase 1/2 through Phase 3 to authorization of the DP for emergency use, formulations targeting 10 µg/mL rS DS and 100 µg/mL Matrix-M1 have been used.
The rS DS and DP used in Phase 1/2 were manufactured at EBSI. For the pivotal Phase 3 clinical trial, production of the rS antigen DS was performed at FDBU (Research Triangle Park, North Carolina), while the DP was manufactured at PAR. In amendment 195 to IND 22430, the applicant informed FDA that production of both rS DS and the final DP container for emergency vaccine supply under the EUA will be performed at SIIPL, which will serve as the global supply node for the Novavax COVID-19 Vaccine, Adjuvanted. Manufacturing information for both rS DS and the final DP at the SIIPL site was submitted in the IND and CTMs manufactured at SIIPL were used in a Phase 2/3 clinical study in India.
Comparability evaluation of the rS DS CTM manufactured at FDBU and SIIPL was based on comparisons of analytical data for process-performance qualification (PPQ) lots manufactured at both sites, including in-process test results, product characterization and release test results. The data showed that rS DS from the FDBU and SIIPL sites are comparable. A similar comparability study of final DP lots manufactured at SIIPL and the DP lots manufactured at PAR for the Phase 3 CTM also showed that the DP lots were comparable.

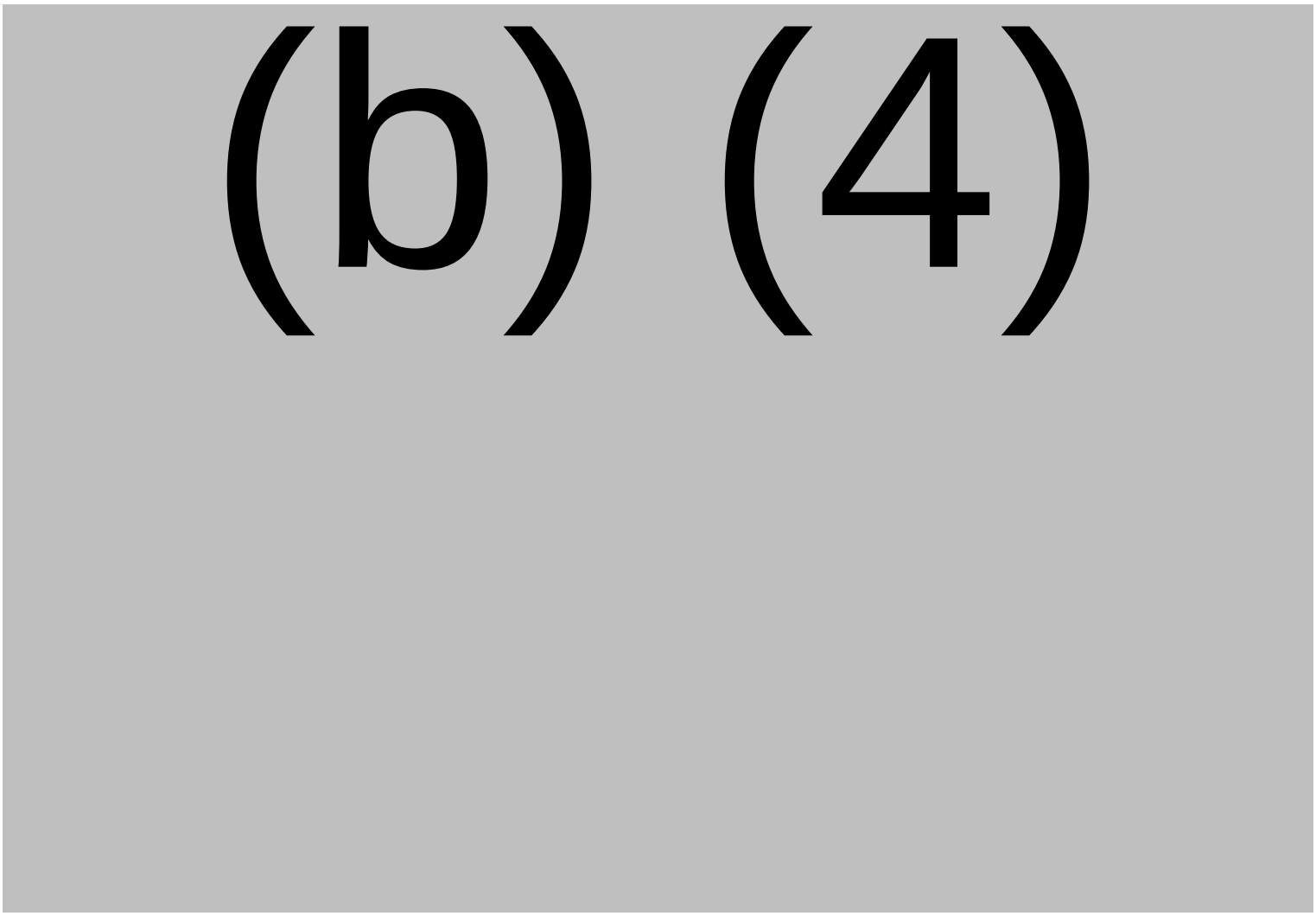
3.2.P.2.2.2 (b) (4)
The COVID-19 Vaccine, Adjuvanted is manufactured to achieve a label claim of (b) (4) µg/mL total protein concentration. To facilitate this, the DP is formulated over a range of (b) (4) µg/mL protein concentration (b) (4) µg/mL was used for the Wuhan and XBB.1.5 vaccines). Similarly, to achieve the target of (b) (4) µg/mL of the Matrix-C component of the adjuvant in the final DP, it is added to the DP (b) (4) during formulation at a target of (b) (4) µg/mL. These (b) (4) in the formulation process are within the acceptance ranges for protein concentration and Matrix-C, respectively, and do not affect their respective specifications for DP release.

3.2.P.2.2.3 Physicochemical and Biological Properties
The DP is formulated in the same formulation buffer in which rS DS is suspended and at the physiological pH of (b) (4). The formulation buffer contains (b) (4) sodium chloride to enhance stability of the rS antigen, with an (b) (4) of about (b) (4) . The rS antigen is the active ingredient in the DP and induces immune responses against SARS-CoV-2. The DP is adjuvanted with Matrix-M which is composed of Matrix-A and Matrix-C. Matrix-A and Matrix-C are nanoparticles of saponin fractions, Fraction-A and Fraction-C, respectively. Matrix nanoparticles are formed by complexing the saponin fractions with phosphatidylcholine and cholesterol in phosphate-buffered saline. Biologically, the saponin components produce the adjuvant effect to enhance the immune response induced by rS, with the nanoparticle form preventing the hemolytic potentials of saponin fractions and providing stability.

3.2.P.2.3 Manufacturing Process Development

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

(b) (4)



3.2.P.2.4 Container-Closure System

The Wuhan DP and the XBB.1.5 DP were filled in 5-mL clear, (b) (4) type (b) (4) siliconized glass vials with an average (b) (4) of (b) (4), stoppered (per (b) (4)) with 13-mm uncoated, siliconized, ready-for-sterilization rubber bromobutyl stoppers with 13-mm flip-off aluminum seal and blue-colored plastic caps. Container-closure integrity test (CCIT) is performed in the stability monitoring program to ensure that extraneous materials cannot breach the final container to contaminate the DP through its shelf life. To protect the DP from exposure to light, filled vials are packed in an outer carton. The glass tubing (b) (4) used in making the DP vials is compliant with requirements of the (b) (4) and (b) (4) of the (b) (4) . The manufacturer's CoA of the glass vials show that potential heavy metals ((b) (4) present in the (b) (4) glass tubing and packaging material are significantly below acceptable limits specified by (b) (4) regulations. Similarly, manufacturers' CoAs for rubber stoppers and aluminum seals indicate

that they meet specifications. Suitability of the container-closure system for the DP is further verified by in-house testing at SIIPL.

Regarding extractable/leachables, information on the glass vial DP container-closure system is derived primarily from the CoA provided by the vial manufacturer. In (b) (4) studies (per (b) (4) , the concentration of (b) (4) in the (b) (4) are compliant with (b) (4) , respectively. (b) (4) was detected by (b) (4) and reported as (b) (4) The levels of potentially leachable impurities in glass vials are below quantitation limits.
Potential leachables in the rubber stopper were evaluated by testing for extractables (b) (4) in (b) (4) different (b) (4) . Most of the categories of compounds detected in the analyses were at low levels in the (b) (4)

The PFS container-closure system comprises of a glass syringe barrel, a syringe plunger stopper, and a plunger rod. The syringe barrel is a(b) (4) Sterile, clean and ready-to-fill 1-mL, round flange, made of siliconized Type(b) (4) borosilicate glass, with Luer-lock and plastic rigid tip cap with (b) (4) elastomer. The plunger stopper is a (b) (4) , with the plunger rod made of polystyrene. No semi-volatile organic compounds, volatile organic compounds, non-volatile organic compounds, or potentially toxic elements were detected in leachable studies of the DP after 3 months storage at 2° to 8°C and at (b) (4) , indicating that the PFS container closure system complies with acceptable quality standards. In a shipping validation report for the PFS presentation (submitted in BLA 125817/0.51), packaging components including the outer carton and blister packs were not breached during shipment simulation, and quality attributes (pH, (b) (4) , and Matrix-A) met their respective acceptance criteria; the test syringes also passed (b) (4) evaluation. However, (b) (4) test samples of XBB.1.5 PFS lot (b) (4) used in the study were out-of-specification for total protein concentration and Matrix-C content. A repeat of the study with JN.1 DP lot (b) (4) did not provide clear results as the study was performed (b) (4) days after the DP lot was manufactured. FDA advised the applicant to repeat the shipping validation study.

Overall reviewer's assessment of Section 3.2.P.2.4

The two container-closure systems (5DV and PFS) for the Nuvaxovid DP are suitable for storage of the final DP at 2° to 8° C. Evaluation of both the 5DV and PFS container-closure systems for extractables/leachables indicate that none or negligible amounts of potential leachables were detected in the studies. The use of the 5DV and PFS container-closure systems for the DP does not have any apparent potentially negative effect on the quality and safety of the DP.

3.2.P.2.5 Microbiological Attributes

The DP is a sterile liquid formulation requiring no reconstitution and contains no preservative. However, the DP is filled in glass vials for the 10DV and 5DV multi-dose presentations, and in glass syringes for the PFS presentation. Thus, the final DP containers are evaluated for

microbiological attributes by the assessment of container-closure integrity (CCI) using a validated method, the CCIT.

For the vialed DP, CCI is assessed by the (b) (4) test, with (b) (4) *should be observed*" as the acceptance criterion. All (b) (4) early PPQ lots from the (b) (4) DP process at SIIPL met acceptance criterion.
During DP manufacture, the formulation buffer and rS DS are (b) (4)-sterilized (b) (4) prior to their addition to the (b) (4), the co-formulated DP (b) (4) is tested for bioburden and (b) (4) online during the filling of the DP final container.
Like the vialed DP, the PFS presentation is also tested for CCI by the (b) (4) method. All (b) (4) PPQ lots (b) (4) of XBB.1.5 PFS presentation met acceptance criterion for CCI.
In addition, the assessment of sterility by the (b) (4) method (per (b) (4) and (b) (4) is one of the release tests used for DP release. No microbial growth has been reported in any DP lot.

3.2.P.2.6 Compatibility

(b) (4)



(b) (4)

Overall Reviewer's Assessment of Section 3.2.P.2:

The development of the COVID-19 Vaccine, Adjuvanted DP has evolved through changes in manufacturing facility, scale of manufacture, final container closure system, modifications to some analytical assays used for product release and stability monitoring, and changes in acceptance criteria for some assays, as well as assay replacement (b) (4) and rS quantitation(b) (4) replaced with (b) (4) assay for protein concentration). Nonetheless, analytical comparisons among batches of the DP manufactured and tested at different times showed that the final DPs are similar in physicochemical properties (appearance, pH, (b) (4) and biological properties (protein concentration, Matrix-A and Matrix-C contents), as well as microbiological quality.
The process for DP manufacture at PAR for Phase 3 clinical development and the commercial process at SIIPL are comparable, demonstrating manufacturing consistency. The container-closure systems (both vials and PFS) are made of glass and bromobutyl rubber stoppers. Potential leachables were not detected in the DP stored at 2 to 8°C or (b) (4) through the 3-month shelf life of the DP in PFS presentation. Overall, the manufacturing process is well controlled to assure the quality and safety of the DP.

3.2.P.3 Manufacture

3.2.P.3.1 Manufacturers

Two presentations of Nuvaxovid DP are manufactured: a multidose vial presentation (10DV or 5DV) and a single-dose PFS presentation. The Wuhan vaccine, which was originally produced in a 10DV presentation was transitioned into a 5DV presentation. With the initiation of the SARS-CoV-2 variant-vaccine program, the XBB.1.5 vaccine was manufactured in the 5DV presentation. The 5DV presentation was transitioned to the single-dose PFS presentation for the JN.1 vaccine. All presentations of Nuvaxovid are primarily manufactured at SIIPL, with other supporting facilities providing QC testing services. The facilities involved in the manufacture of Nuvaxovid in 5DV and PFS presentations and their responsibilities are listed in Table 29 (Adapted from Table 1, Section 3.2.P.3.1, BLA 125817/0.4 and /0.42).

Table 29. Facilities for the manufacture of Nuvaxovid DP

Facility	DP Process/activity
SIIPL (b) (4)	DP Manufacture (Formulation, sterilization by (b) (4), aseptic filling, primary packaging) Secondary packaging (labelling & packaging). In-process QC testing (pH, (b) (4) bioburden)

	Batch release QC testing (Appearance; pH; (b) (4) Identity, by (b) (4) ; Protein concentration by (b) (4); Relative Potency, by (b) (4); Matrix-A content, by (b) (4) ; Matrix-C content, by (b) (4) ; Sterility; Endotoxin and [2]Extractable/Expelled volume). Stability testing (Appearance; pH; (b) (4) Identity, by (b) (4) ; Protein concentration by (b) (4); Relative Potency, by (b) (4); Matrix-A content, by (b) (4) ; Matrix-C content, by (b) (4) ; Sterility; and CCIT).
Novavax AB (b) (4) Uppsala, Sweden (b) (4)	Batch release and stability QC testing (Matrix-A content, by (b) (4) ; Matrix-C content, by (b) (4) ; and Particle size by (b) (4)
(b) (4)	Batch release and stability QC testing (Identity test, by (b) (4)).
(b) (4)	Batch release QC testing (Expelled volume) Stability QC testing (CCIT and (b) (4)

[1]Performs QC testing applicable to PFS presentation only.
[2]Verification of the volume of vaccine in the container closure is termed Extractable volume (5DV) or Expelled volume (PFS).
SIIPL = Serum Institute of India Pvt Ltd; FEI = FDA Establishment Identifier; DUNS = Data Universal Numbering System; DS = Drug Substance; QC = Quality Control; (b) (4) Assay; CCIT = Container Closure Integrity Test; (b) (4)

3.2.P.3.2 Batch Formula

The ingredients in Nuvaxovid and the quantity of each ingredient per dose of the vaccine are the same irrespective of the final container closure system (i.e., multidose vial or PFS). However, the 10DV (Wuhan vaccine) and the 5DV (Wuhan and XBB.1.5 vaccine) were formulated at a batch size of (b) (4) respectively, and the PFS (JN.1 vaccine) is formulated at a batch size of (b) (4). The batch formula for the multidose vials and PFS presentations is shown in Table 30 (Adapted from Table 1, Section 3.2.P.3.2, BLA 125817/0.4 and BLA 125817/0.42; see Section 3.2.P.1).

Table 30. Batch Formula of the 10DV, 5DV and PFS presentations of Nuvaxovid

Ingredient	Quantity per 0.5-mL dose	Theoretical quantity per Batch size			Reference to Quality Standards
		10DV	5DV	PFS	
SARS-CoV-2 rS protein	5 µg[1]	(b) (4)			In-house
Matrix-M Adjuvant	50 µg				In-house

Disodium hydrogen phosphate heptahydrate[2]	2.465 mg	(b) (4)
Sodium dihydrogen phosphate monohydrate[3]	0.445 mg	
Sodium Chloride[4]	8.766 mg	
Polysorbate 80[4]	0.050 mg	
Sodium hydroxide (for pH adjustment)	q.s.	
Hydrochloric acid (for pH adjustment)	q.s.	
Water for Injection	q.s.	

[1]Protein concentration (b) (4) for PFS) during DP manufacture.
[2]Sodium phosphate dibasic heptahydrate on supplier's Certificate of Analysis
[3]Sodium phosphate monohydrate monobasic on supplier's Certificate of Analysis
10DV = 10-Dose Vial; 5DV = 5-Dose Vial; PFS = Pre-Filled Syringe; V = Volume (batch size); q.s. = quantity sufficient; USP(b) (4)

Overall Reviewer's Assessment of Sections 3.2.P.3.1 and 3.2.P.3.2:

The quantity of each component of the DP is the same at formulation, with the (b) (4) formulations being scaled down versions of the Wuhan DP produced at a batch size of(b) (4) for emergency vaccine supply. The indicated quantity of each component for the different batch sizes is acceptable. All formulations are manufactured in the same facility, and each of the facilities involved, including facilities performing quality-control testing, have an FDA Establishment Identifier number. Overall, the information provided in Sections 3.2.P.3.1 and 3.2.P.3.2 is acceptable.

3.2.P.3.3 Description of Manufacturing Process

The process steps for the manufacture of Nuvaxovid are similar, irrespective of the vaccine strain, protein formulation target, or batch size. There are (b) (4) process steps in DP production. Except for differences in the batch size for commercial manufacture, Steps (b) (4) are the same for multidose vial and PFS presentations. Due to the difference in container closure systems (i.e., vials versus PFS), the filling process (Step (b) (4) is different for multidose vial and PFS presentations. Steps (b) (4) are the same for multidose and PFS presentations. Flow diagrams of the manufacturing processes for the multidose vials and PFS presentations are shown in Figure 2 (Adapted from Figure 1, Section 3.2.P.3.3, BLA 125817/0.4 and /0.42).

The process steps in the manufacture of the Nuvaxovid DP are as follows:

(b) (4)

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

(b) (4)

Overall Reviewer's Assessment of Section 3.2.P.3.3:

The DP manufacturing information provided is consistent with the information submitted the IND application and the EUA request. Formulation of the (b) (4) is similar for multidose vials and PFS, except for differences in the (b) (4) Manufacturing operations are similar for the MDVs and PFS except for the divergence at the DP filling step where different equipment and fill operations are used because of the differences in container-closure systems. The post-fill/finish steps (Steps (b) (4) are similar, and in both MDV and PFS operations, no material re-processing takes place. Operating parameters at each manufacturing process step are well defined and in-process testing during manufacturing assures product quality and safety. Thus, the information provided on the DP manufacturing process is acceptable.

3.2.P.3.4 Controls of Critical Steps and Intermediates

The formulation of the (b) (4) DP follows the same process steps, irrespective of the manufacturing batch-size. Therefore, the critical-process parameters (CPPs) for DP manufacture and their respective acceptable ranges for the DP process are similar, beginning with preparation of the DP formulation buffer through the preparation of the co-formulated (b) (4) DP. The operating parameters and their criticality for the DP process from the preparation of formulation buffer through the preparation of the formulated (b) (4) DP are summarized in Table 31 (Adapted from Table 1, Section 3.2.P.3.4, BLA 125817/0.4, and Table 1, Section 3.2.P.3.4, 125817/0.42).

(b) (4)

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

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Overall Reviewer's Assessment of Section 3.2.P.3.4:

Critical-process parameters in the manufacture of Nuvaxovid, from the preparation of the formulation buffer through the filling of the DP final container, have been established and their respective operational ranges or set points defined. The suitability of the defined CPPs for DP manufacture was evaluated with the testing of critical quality attributes of the final formulated DP (b) (4); all batches of formulated DP (b) (4) tested met acceptance criteria. In addition, in-process controls were established. Together, the CPPs and IPCs are adequate to ensure quality of the DP and are acceptable.

3.2.P.3.5 Process Validation and/or Evaluation

The validation of the DP manufacturing process was performed with the manufacture of (b) (4) process-performance qualification (PPQ) lots of each batch size (b) (4) for the 10DV, (b) (4) for the 5DV, (b) (4) for the PFS) at commercial scale. The (b) (4) scales were originally validated with the Wuhan vaccine. For the XBB.1.5 vaccine, which was

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

(b) (4)

(b) (4)



3.2.P.4 Control of Excipients

The excipients used in DP manufacture are disodium hydrogen phosphate heptahydrate, sodium dihydrogen phosphate monohydrate, sodium chloride, and PS-80. Water for injection is added *quantum satis* to attain the desired volume of buffer and sodium hydroxide and hydrochloric acid are used for pH adjustment as needed. These excipients are tested by the relevant (b) (4) methods.

Phosphatidylcholine, a component of the Matrix-M adjuvant is manufactured from (b) (4) The review of the adjuvant component is covered in (b) (6) memo.

No excipient of human origin is used in DP manufacture; no novel excipient is used in DP manufacture.

Overall Reviewer's Assessment of Section 3.2.P.4:

The chemical composition of the DP formulation buffer is well defined and are tested using the relevant (b) (4) methods. There is no novel excipient or excipient of human origin used in DP manufacture. Phosphatidylcholine, a component of Matrix-M adjuvant is covered in (b) (6) memo.

3.2.P.5 Control of Drug Product

3.2.P.5.1 and 3.2.P.5.6 Specifications and Justification of Specifications

The excipients listed in Table 39 (Adapted from Table 1, Section 3.2.P.4, BLA 125817/0.4 /0.42) are used in the (b) (4) DP manufacture. The CoA of each excipient from their manufacturers was provided, along with analytical reports on additional in-house testing performed on each excipient at SIIPL. The excipients in Table 39 comply with acceptance criteria for the various analytical tests, and there are no novel excipients used in the DP (b) (4).

Table 39. Excipients used in Nuvaxovid DP manufacture.

Ingredient	Function	Reference to Quality Standard
Disodium hydrogen phosphate heptahydrate[4]	(b) (4)	(b) (4)
Sodium dihydrogen phosphate monohydrate[4]	(b) (4)	
Sodium Chloride[4]	(b) (4) –isotonicity adjuster	
Polysorbate 80[4]	(b) (4) – stabilizer	
Sodium hydroxide	pH adjustment	
Hydrochloric acid	pH adjustment	
Water for Injection	Vehicle	

(b) (4).

The analytical test methods used for the release and stability evaluation of the Nuvaxovid DP are summarized in Table 40 (Adapted from Table 1, Section 3.2.P.5.1, BLA 125817/0.0, /0.4, and /0.42).

Table 40. Specifications for the release and stability evaluation of Nuvaxovid DP

Quality Attribute	Test Method	Final Acceptance Criteria for DP Release	Final Acceptance Criteria for DP Stability
Appearance	Visual observation (b) (4)	Color: Colorless (b) (4) Clarity: Clear (b) (4) Free from visible particles	Color: Colorless (b) (4) Clarity: Clear (b) (4) Free from visible particles
pH	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)
Particle size[1, 1a]	(b) (4)	(b) (4)	(b) (4)
Expelled volume[2]	(b) (4)	The volume measured for each container is not less than the nominal volume.	N.A.
Identity[3]	(b) (4)	Identity confirmed	Identity confirmed
Identity[3]	(b) (4)	Identity confirmed	Identity confirmed
Total Protein[4] Concentration	(b) (4)	(b) (4)	(b) (4)
Relative Potency[5]	(b) (4)	(b) (4) potency, relative to reference standard[2]	(b) (4) potency, relative to reference standard
Matrix-A Content	(b) (4)	(b) (4)	(b) (4)
Matrix-C Content	(b) (4)	(b) (4)	(b) (4)

Endotoxin	(b) (4)	(b) (4)	N.A.
Sterility	(b) (4)	No growth	No growth
Container-Closure Integrity	Container Closure Integrity Test (CCIT) (b) (4)	N.A.	No failure allowed
(b) (4)	(b) (4)	N.A.	(b) (4)

[1]Applicable to the release of the PFS presentation.
[1a]Particle size testing was used for the release of the XBB.1.5 DP and it is used in stability evaluation of Wuhan and variant DPs.
[2]The multidose 10DV and 5DV presentations are tested for "*Extractable Volume*" under the same (b) (4) reference, with the acceptance criterion as "*The volume should be such that each syringe delivers not less than stated doses*".
[3]Only one identity testing method is used at a time. (b) (4) is used during early product development. (b) (4) is used when specific antibody reagents become available.
[4]For emergency vaccine supply under the EUA, the Original monovalent (Wuhan) DP was tested for release by (b) (4) assay. Due to assay variability and shortage of the (b) (4) assay reagent, an (b) (4) was used for the release of the 2023-2024 Formula (XBB.1.5 vaccine DP). Subsequently, the (b) (4) assay was developed. In bridging studies performed using XBB.1.5 DP lots, the 3 assays (b) (4) were demonstrated to be statistically equivalent. The release and stability specification for both (b) (4) are the same. A specification range of (b) (4) was used for the release and stability evaluation of the XBB.1.5 vaccine by the (b) (4) method.
[5]For variant (XBB.1.5 and JN.1) DP release, relative potency testing is performed (b) (4) -manufacture to allow for (b) (4) . A specification of (b) (4) was originally requested for the JN.1 vaccine but was further revised to (b) (4) based on statistical analysis, with the lower release specification allowing for the JN.1 DP to be at or above the lower limit of (b) (4) relative potency in the stability program. The Original monovalent was released with a specification of (b) (4) for the EUA. The specification was revised to (b) (4) based on statistical analysis as more stability data became available. A specification of (b) (4) % was used for the release of the XBB.1.5 under the EUA when the XBB.1.5 vaccine was tested for relative potency against Wuhan interim reference standard (lot (b) (4) . The release specification was revised to (b) (4) after a qualified XBB.1.5 reference standard became available.
(b) (4)
; N.A. = Not Applicable, CCIT = Container Closure Integrity Test; (b) (4)

Justification of Specifications

Appearance:
Appearance is assessed by the evaluation of the DP for three quality attributes (color, clarity, and absence of visible particles). The acceptance criteria for appearance are "Colorless (b) (4) (color); "Clear (b) (4) (clarity); and "Practically free from visible particles" (visible particles). The evaluation of appearance (color, clarity, and visible particles) is performed by visual inspection. With relatively limited experience with the COVID-19 Vaccine, Adjuvanted DP, the acceptance criteria for appearance were initially based on experience with the applicant's quadrivalent influenza nanoparticle vaccine (Quad-NIV). Like the COVID-19 vaccine, the Quad-NIV is co-formulated with (b) (4) Matrix-M1 adjuvant.

pH:
The specification for pH was originally set at (b) (4) (i.e., pH target for DP formulation buffer (b) (4) With additional data availability for the Wuhan DP, all available data points for the Wuhan DP (n = (b) (4) were analyzed using (b) (4) which revealed an approximate (b) (4) , resulting in the revision of the acceptance range for pH to (b) (4)

(b) (4)

(b) (4)

Total protein concentration:
The current acceptance criterion of (b) (4) µg/mL (for both DP release and stability monitoring) was set with the lower limit similar to the rS content of the Phase 3 CTM (Wuhan strain) and accommodating assay variability, and the upper limit accommodating process variability.

Extractable volume/Expelled volume:
The specification for Extractable volume (for multidose vials) and Expelled volume (for PFS) was set to ensure the delivery of the intended dose of the vaccine.

Relative potency:
The specification for relative potency has evolved with product development and has been adjusted for each vaccine strain, including multiple adjustments for the Wuhan vaccine. The relative potency assay requires the use of a (b) (4) as the reference standard. The current specification ranges for the release and stability evaluation of the COVID-19 Vaccine, Adjuvanted (2024-2025 Formula) are (b) (4), respectively, with the upper limit aligned with that of the Wuhan vaccine. A (b) (4) is used in determining the relative potency for both (b) (4) DP. The lower stability specification of (b) (4) aligns with the potency of the Phase 3 DP lot (b) (4). In setting these specification ranges, relative potency results for XBB.1.5 vaccine lots (n = (b) (4)) were statistically analyzed using Log Normal distribution (with Goodness of Fit Test p-value of (b) (4)) and tolerance intervals criteria of (b) (4). Values of (b) (4) were obtained as the lower limit and upper limit, respectively. Thus, in BLA 125817/0.0, the applicant proposed relative potency specification ranges of (b) (4) (for release) and (b) (4) (for stability evaluation) of the XBB.1.5 vaccine DP. In IR #8 to the applicant, FDA recommended that the lower limit of the stability specification be aligned with the (b) (4) lower limit for the Original (Wuhan) monovalent. In their response submitted in BLA 125817/0.10, the applicant justified the proposed (b) (4) lower limit for the XBB.1.5 vaccine to the assignment of a nominal (b) (4) relative potency to the XBB.1.5 reference standard without purity adjustment. Without purity adjustment, the relative potency of the Wuhan IRS lot (b) (4) is (b) (4). The applicant further calculated (by simple mathematical equation) that if the relative potency value of (b) (4) was equated to a nominal (b) (4) relative potency, the lower limit of (b) (4) will be equivalent to a nominal (b) (4) (rounded to (b) (4) Since the monthly rate of potency loss of the XBB.1.5 DP had not been established due to limited real-time stability data at the time, FDA recommended that the lower specification limit be aligned with the clinically relevant (b) (4) potency of the Phase 3 DP. In BLA 125817/0.18, the applicant agreed to set the lower limit for the XBB.1.5 vaccine stability monitoring to (b) (4), along with assigning a nominal relative potency of (b) (4) to the XBB.1.5 reference standard. This strategy is also applicable to future variant vaccines, and the release and stability specifications will be based on available data from stability monitoring with homologous reference standards. In 125817/0.22, the applicant updated relevant Sections of BLA 125817/0 with the information.
For the release of JN.1 vaccine DP in PFS presentation, a common-slope statistical model was used to calculate a monthly potency loss of (b) (4) for variant rS DP from real-time stability data through the 3-month timepoint for the PFS presentation of the XBB.1.5 vaccine and the

JN.1 vaccine (both formulated at (b) (4) µg/mL). This rate of potency loss, along with the expected (b) (4) potency at the end of shelf-life was used to calculate a lower specification limit of (b) (4) (per (b) (4)) for the release of the JN.1 vaccine formulated at (b) (4) µg/mL total protein concentration. Thus, the relative potency specification for the release of the JN.1 DP formulated at a target of (b) (4) µg/mL is (b) (4)

Matrix-A and Matrix-C Contents:
The specification ranges of (b) (4) µg/mL – (b) (4) µg/mL (Matrix-A) and (b) (4) µg/mL (Matrix-C) were set based on preclinical and clinical experience with the use of Matrix-M adjuvant. With the target concentration of Matrix-M in the DP being (b) (4) µg/mL, at an (b) (4) ratio of Matrix-A to Matrix-C, the specification limits were based on a (b) (4) of the target. Thus, for Matrix-A, (b) (4) µg/mL (b) (4) or (b) (4) µg/mL. The specification range for Matrix-A was derived as (b) (4) µg/mL (b) (4) µg/mL, lower limit) and (b) (4) µg/mL (b) (4) µg/mL, upper limit). Similarly, for Matrix-C, (b) (4) of (b) (4) µg/mL is (b) (4) µg/mL. The specification range for Matrix-C was derived as (b) (4) µg/mL (b) (4) µg/mL, lower limit) and (b) (4) µg/mL (b) (4) µg/mL, upper limit).

Identity ((b) (4) *)*:
Product identity confirmation is required per (b) (4) (b) (4) (identity confirmed).

Sterility:
Confirmation of product sterility is required per (b) (4) , with the acceptance criterion of “No growth” indicating the absence of microbial contamination.

Endotoxin:
The specification of (b) (4) was set based on the applicant’s experience using the Sf9/BACV manufacturing platform. In addition, the specification is below the limit of (b) (4) body weight prescribed in (b) (4)

Container-Closure Integrity Test:
The specification of (b) (4) is meant to assure that the DP container closure remains intact and no infiltration of potential contaminants through the shelf-life of the DP.

(b) (4)

Particle Size:
The specification of a (b) (4) of (b) (4) and a (b) (4) of (b) (4) was based on statistical analysis of data for (b) (4) lots of the XBB.1.5 vaccine. In the analysis, based on the lowest (b) (4) value, the (b) (4) data and the (b) (4) data for the (b) (4) DP lots were fitted by normal distribution and long-normal distribution, respectively. By using the goodness-of-fit test and diagnostic plots, the suitability of the distributions was verified. Since the number of samples (n = (b) (4)) was (b) (4), the (b) (4) and

(b) (4) limits were calculated by tolerance interval (T.I.), with a T.I. criterion of (b) (4) confidence, and (b) (4) (for (b) (4)) and (b) (4) (for (b) (4)).

Reviewer's Assessment of Sections 3.2.P.5.1 and 3.2.P.5.6:

Analytical test methods for the testing and release and stability monitoring of the final DP of Nuvaxovid were established. The acceptance criteria for some of the test methods were originally leveraged from the applicant's experience with the manufacture of vaccines using the same manufacturing platform. The test methods and their acceptance criteria have also evolved with product development and specification ranges for some analytical tests (e.g., (b) (4) and pH) have been tightened as more data for Nuvaxovid became available and were analyzed. Like the test methods for the rS drug substance, test methods for the release and stability monitoring of Nuvaxovid have been established and used for DP testing across strains. However, the acceptance criteria for relative potency for the release of the DP, particularly the lower limit of release, may need to be revised for each new variant vaccine. This is necessary because of the need to develop and qualify a reference standard and determine the potency loss profile for each variant vaccine. Overall, the specifications provided for each release and/or stability test and the justification of each specification are acceptable.

3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures

Several analytical tests are used to measure a set of defined quality attributes for the release and stability evaluation of the COVID-19 Vaccine, Adjuvanted DP. Test methods for the adjuvant component of the DP are reviewed by (b) (6) Some analytical tests have been modified or replaced over the course of product development. The range of test methods used in product evaluation include (b) (4) methods, with some of the test methods being used in common for both rS DS and DP testing. The test methods used in common for rS DS and DP testing and their validation are described under Sections 3.2.S.4.2 and 3.2.S.4.3. The (b) (4) methods used for (b) (4) DP testing are visual inspection (for appearance), (b) (4) (for pH), endotoxin test, and sterility test. The (b) (4) tests used for (b) (4) DP testing are identity test, and relative potency assay.

Assays that are specifically used for DP testing (and their validation) are described below.

(b) (4) *analytical methods specific for DP testing*.

(b) (4)

Extractable Volume.
The number of doses that can be effectively obtained from the final DP container is evaluated according to (b) (4) for both multidose vial and PFS presentations.

With the change of container-closure system for the JN.1 DP final container from a multi-dose vial to a PFS, the (b) (4) test methods for the volume of the DP in the container-closure (b) (4)); and container closure integrity ((b) (4) remain unchanged but the acceptance criterion for the volume of the DP in the container (Extractable volume for multi-dose vials; Expelled volume for PFS) changed from **"**The volume should be such that each syringe delivers not less than stated doses" (Extractable volume) to "The volume measured for each container is not less than the nominal volume" (Expelled volume).

Container-Closure Integrity Test.
The container-closure integrity test (CCIT) is used in the stability program to evaluate the integrity of the container-closure system by determining that the container closure is leak-proof and remains uncompromised to preserve the sterility of the DP. Verification of the integrity of closure of the final DP container is performed as described in (b) (4) A validation of the (b) (4) method for the prefilled syringe presentation was performed at (b) (4) ., and the report was reviewed under IND 22430, Amendment 632. The study established a (b) (4)

(b) (4)

Sterility test
Sterility test is performed for DP release, as well as at specific timepoints in the DP stability program. Sterility test is performed by the (b) (4) according to (b) (4) .

Non-compendial analytical methods for the DP process

(b) (4)

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

(b) (4)



3.2.P.5.5 Characterization of Impurities

The impurity profile of Nuvaxovid is expected to be the same, irrespective of the rS variant (Wuhan, XBB.1.5 or JN.1) since the (b) (4) of all variants are manufactured with the same baculovirus/Sf9 platform (see Section 3.2.S.3.2 for the impurity profiles of (b) (4)). Process-related impurities are removed during (b) (4) purification of (b) (4), including (b) (4) chromatography and (b) (4) chromatography. The impurity profiles of Matrix-A and Matrix-C (b) (4) are described in (b) (6) review memo (see Section 3.2.S.3.2 for the impurity profiles of Matrix-A and Matrix-C). A risk assessment of the presence of (b) (4) in Matrix-A and Matrix-C did not identify any risk. Process-related

impurities from the (b) (4) Matrix-M may be present in trace amounts in the formulated (b) (4) DP. There are no new process-related impurities introduced during DP manufacture. A risk assessment of the presence of (b) (4) in Nuvaxovid was performed per guidance provided by the (b) (4) and the FDA; no potential source of (b) (4) was identified. (b) (4) are not used in the DP process. In addition, there are no new raw materials or reagents used in the DP process.

Overall Reviewer's Assessment of Sections 3.2.P.5.4 and 3.2.P.5.5:

The batch release analysis shows consistency in manufacture of the JN.1 DP. Except for relative potency, which tends to be strain-specific, all other quality attributes that are evaluated for DP release have been consistent and within their respective acceptance criteria, indicating manufacturing consistency. Therefore, the batch analysis data are acceptable.
Regarding the impurity profile of the DP, there are no new impurities introduced in the DP process. Trace amounts of impurities from the rS DS, Matrix-A and Matrix-C may be present in the DP.

3.2.P.6 Reference Standards

Information on the reference standards for the Wuhan, XBB.1.5, and JN.1 vaccine is provided in Section 3.2.S.5.

3.2.P.7 Container-Closure System

The Wuhan DP was originally filled as a 10DV presentation and transitioned into a 5DV presentation, which was also used for the XBB.1.5 DP. The JN.1 DP is manufactured as a single-dose presentation in a pre-filled syringe primary container.
Multidose 5DV/10DV:
The container closure system for the 5DV presentation comprises of a 5-mL clear, (b) (4) type (b) (4) siliconized glass vial (per (b) (4) with an average (b) (4) of (b) (4); 13-mm bromobutyl, uncoated, siliconized, ready-for-sterilization rubber stopper (per (b) (4) ; and a 13-mm flip-off aluminum seal with a blue-colored plastic cap (b) (4) . Container-closure integrity test (CCIT) is performed for the release of the final DP container and filled vials are packed in an outer carton to protect from light. The manufacturer's CoA of the glass vials indicate (b) (4) contents (b) (4) tubing and packaging (i.e., significantly below acceptable limits of (b) (4) regulations). Similarly, manufacturers' CoAs for the rubber stopper and the aluminum seal show that they meet specifications. Suitability of the container-closure system for the DP is further verified by in-house testing at SIIPL.

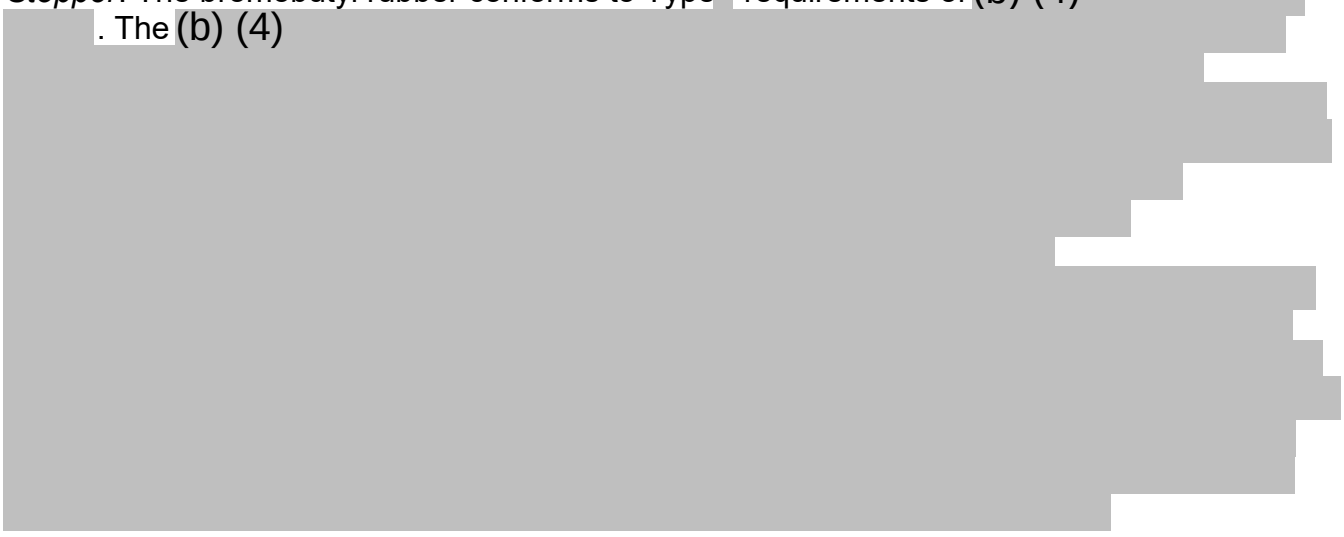
The extractable/leachable information on the DP container-closure system is based on information provided by the vial manufacturer. The glass tubing (b) (4) used in making the DP vials is compliant with (b) (4) requirements.

Glass vials: Vials were evaluated for extractables (by the manufacturer). For this purpose, representative vials were (b) (4)

(b) (4)



Stopper: The bromobutyl rubber conforms to Type (b) (4) requirements of (b) (4) . The (b) (4)



Single-dose PFS:
The PFS container closure system comprises of a syringe barrel, a syringe plunger stopper, and a plunger rod. All 3 components are manufactured by (b) (4) . A letter of authorization to cross-reference (b) (4) Drug Master File (b) (4) for the (b) (4) *Glass Pre-fillable Syringe (PFS)* was included in Section 3.2.P.7, BLA 125817/0.42.

The syringe barrel is a (b) (4) Sterile, clean and ready-to-fill 1-mL, round flange, made of siliconized Type (b) (4) borosilicate glass, with Luer-lock and plastic rigid tip cap with (b) (4) elastomer. The plunger stopper is a (b) (4) , with the plunger rod made of polystyrene. A (b) (4) Certificate of Conformance (document QAG_27852) and analytical test reports (document QAG_29821) on the PFS were included in Section 3.2.P.7, BLA 125817/0.42. These documents show that the PFS container closure system comply with acceptable quality standards.

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



Overall reviewer's assessment of Section 3.2.P.7
The two container-closure systems (5DV and PFS) for the Nuvaxovid DP are suitable for storage of the final DP at 2° to 8° C. Evaluation of both the 5DV and PFS container closure systems for extractables/leachable (E/L) indicate that none or negligible amounts of potential leachables were detected in the E/L studies. The use of the 5DV and PFS container closure systems for the DP do not have any apparent potentially negative effect on the quality and safety of the DP. Regarding shipping validation, FDA concluded that the shipping validation data submitted in the BLA for the PFS presentation were inadequate and recommended that the shipping validation study be repeated with a minimum of (b) (4) commercial lots of JN.1 PFS, as a Post-marketing Commitment. In BLA 125817/0.92 the applicant agreed to repeat the shipping validation study and provide the study report by July 31, 2025.

3.2.P.8 Stability

3.2.P.8.1 Stability Summary and Conclusion and 3.2.P.8.3 Stability Data

The Wuhan DP and the XBB.1.5 DP were authorized for a shelf life of 9 months, and the JN.1 vaccine was proposed for a shelf life of 3 months. In IR #69, FDA requested the applicant to define what is considered the "Date of Manufacture" for the purpose of determining an expiry date for the DP. In their response submitted in BLA 125817/0.94, March 17, 2025, the

applicant indicated that the date of initiation of filling of syringes is the date of manufacture. DP lots are placed on stability monitoring under long-term storage condition at 2° to 8°C, as well as under accelerated storage conditions of (b) (4) relative humidity (RH). The Phase 3 (Wuhan) clinical DP (lots (b) (4) , manufactured at PAR) were evaluated for stability under long-term and accelerated storage conditions in both (b) (4) and (b) (4) storage positions. The Wuhan DP and XBB.1.5 DP lots manufactured at SIIPL were evaluated for stability (both long-term and accelerated) in the (b) (4) storage position. In addition, the Phase 3 clinical lots (b) (4) were evaluated for stability under (b) (4) relative humidity) and the Wuhan, XBB.1.5, and JN.1 vaccines were evaluated for photostability by exposure of the (b) (4) . Based on the photostability study results, both vialed and PFS presentations of the DP, irrespective of the rS variant, should be protected from direct and indirect light.
The Wuhan DP lots in the stability program were 10DV PPQ lots (b) (4) , and (b) (4) (manufactured on a (b) (4) scale at SIIPL); 10DV PPQ lots (b) (4) (manufactured on a (b) (4) scale at SIIPL, using (b) (4) produced at (b) (4) scale); 10DV Clinical/commercial lots (b) (4) (manufactured on a (b) (4) scale at SIIPL, using (b) (4) produced at (b) (4) scale); and 10DV Phase 3 clinical lots (b) (4) (manufactured at (b) (4) scale, respectively, at PAR); as well as Wuhan vaccine 5DV PPQ lots (b) (4) (manufactured at (b) (4) scale at SIIPL). Stability studies with all Wuhan DP (10DV and 5DV), including long-term stability studies, have been completed.
There were (b) (4) XBB.1.5 vaccine lots in the stability program. These were 5DV commercial lots (b) (4) (released with Wuhan IRS lot (b) (4) (released with XBB.1.5 reference standard lot XBB230531). The long-term stability evaluation of lots (b) (4) was originally planned for (b) (4) months. However, because of the change in vaccine strain to the JN.1 vaccine for the 2024-2025 Formula, stability monitoring of the XBB.1.5 vaccine was terminated at the (b) (4) -month timepoint). Stability evaluation of lots (b) (4) was also terminated. The final stability data (both under long-term and accelerated) for lots (b) (4) were submitted in EUA 28237/282. All lots met stability acceptance criteria for all quality attributes (including relative potency) through the (b) (4) -month timepoint under long-term storage condition. Under accelerated conditions, relative potency decreased below the lower release acceptance criteria in (b) (4) 3 months.

The quality attributes evaluated in the stability program are appearance (color, clarity, presence of visible particles); pH; (b) (4) ; total protein concentration; relative potency; sterility; and CCIT. Except for CCIT, PAR DP batches (b) (4) were also evaluated for stability based on the assessment of the same set of quality attributes. In addition, the acceptance criteria for the assessment of pH (b) (4) total protein concentration (b) (4) µg/mL), and relative potency (b) (4) for batches (b) (4) , were based on the set of existing acceptance criteria (in parenthesis) at the time of their release.

Based on the stability data available, relative potency appears to be the major stability indicator for the COVID-19 Vaccine, Adjuvanted. Although the specifications (both for DP release and stability monitoring) have changes throughout the course of product development, the changes have no major impact on the assessment of DP stability.

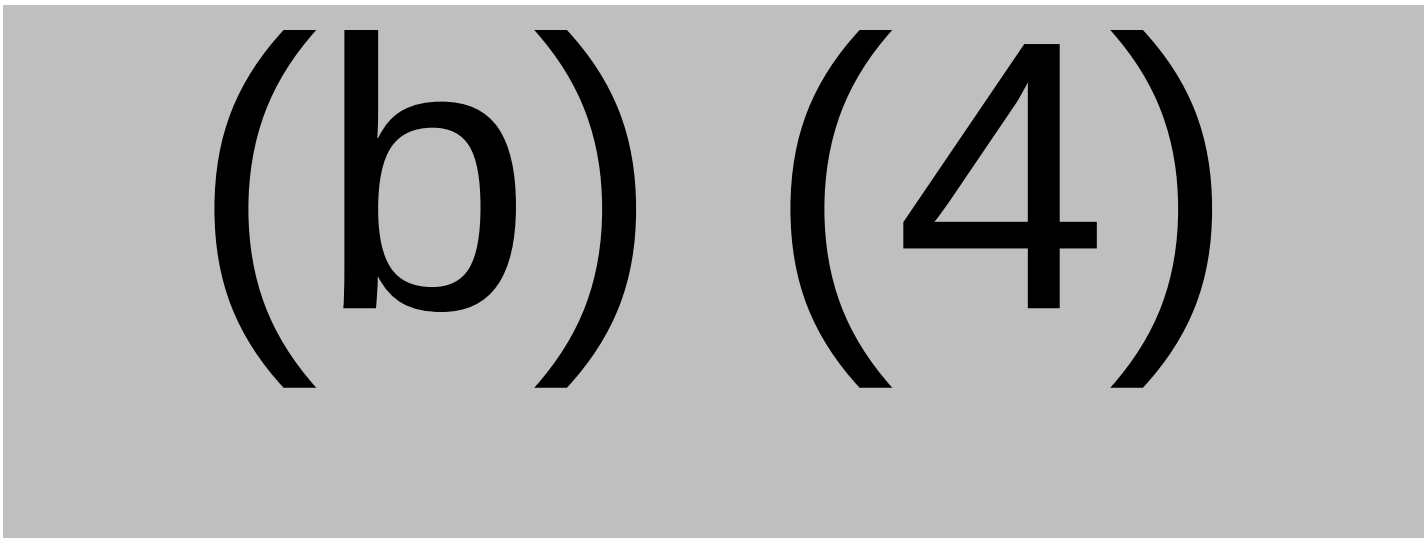
Under accelerated storage conditions, loss of potency below the current specification limit (b) (4) occurred from (b) (4)-month to (b) (4) month for the Wuhan 5DV, from (b) (4) month to (b) (4) months for the 10DV stability lots, from (b) (4) month to (b) (4) months for the XBB.1.5 vaccine and at the (b) (4) month timepoint for the JN.1 vaccine.

Although statistical analyses of stability data for the Wuhan and XBB.1.5 vaccine indicate that the loss of potency over the course of stability evaluation is significant, real-time stability data for the stability lots show that relative potency values are above the lower specification limit of (b) (4). Real-time stability data for all (b) (4) PPQ lots of the (b) (4) µg/mL formulation of the JN.1 vaccine are above the (b) (4) lower limit at the (b) (4) month timepoint. Thus, the shelf-life request of 3 months for the JN.1 vaccine is acceptable.

3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment

The applicant commits to continue stability evaluation of the vaccine DP according to the stability study protocol for the (b) (4) µg/mL formulation of the JN.1 vaccine (Table 41; Adapted from Table 4, Section 3.2.P.8.1, BLA 125817/0.4). The stability evaluation will continue under long-term storage condition (2° to 8°C) for (b) (4) months and under accelerated conditions (b) (4)

(b) (4)



Stability lots of the JN.1 vaccine (b) (4) µg/mL) under accelerated storage conditions (b) (4) , will be tested using the same set of analytical tests in Table 40 and test results will be reported as obtained (i.e., there are no specifications for test parameters). In the original post-approval stability commitment, the applicant had planned to perform stability monitoring under long-term storage condition and stability evaluation under accelerated storage condition will be performed only when a significant change is made to the manufacturing process. In BLA 125817/0.77, the applicant modified the condition for stability testing to indicate that annual stability batches will be placed on long-term stability and the decision to evaluate a batch under accelerated storage conditions in addition, due to a significant change in process, will be made on a case-by-case basis.

3.2.P.8.3 Stability Data

Under long-term storage, stability testing timepoints for SIIPL Wuhan lots (b) (4) were at 1-month, 3-, (b) (4) -months post-manufacture. Similarly, SIIPL Wuhan lots (b) (4) were tested at the same timepoints but terminated at the (b) (4) month timepoint. The Phase 3 DP lots were tested at 1-month, 2-, 3-, (b) (4) months post-manufacture. Under accelerated conditions, all stability lots were tested at 1-month, 2-, 3-, (b) (4) months timepoints. In addition, all SIIPL DP lots were tested at 0.5-month timepoint and the SIIPL DP lots produced (b) (4) were further tested on day-(b) (4) post-manufacture. DP appearance, pH, (b) (4) , total protein concentration and relative potency were tested at all time points for the SIIPL stability batches. The remaining tests were performed at release and at the end of stability monitoring. For the PAR stability lots, all stability tests were performed at all time points except for sterility, which was evaluated at release and at the (b) (4) -month timepoints.

The stability data for the Wuhan DP lots (b) (4) PAR and (b) (4) SIIPL lots) show statistically significant loss of potency for the SIIPL and PAR lots (except for SIIPL lot (b) (4)); the statistically calculated rate of degradation of the 5DV presentation of the Original monovalent during the first (b) (4) months post-manufacture is (b) (4) . However, the real-time stability data show that all (b) (4) Wuhan DP lots in the 10DV presentation and the (b) (4) Wuhan DP lots (b) (4) in the 5DV presentation had relative potency values above the

lower specification limit of (b) (4) at the (b) (4)-month timepoint, supporting a proposed shelf-life of (b) (4) months for the Wuhan DP stored at 2° to 8°C.

Like the Wuhan vaccine, the 5DV presentation of the XBB.1.5 vaccine (2023-2024 Formula) showed statistically significant degradation but real-time stability data show that it remains stable through the (b) (4)-month timepoint, including meeting relative potency specifications, under long-term storage condition. In the first 6 months post-manufacture, a degradation rate of -(b) (4) was calculated for the XBB.1.5 vaccine 5DV. The real-time stability data through the (b) (4) month timepoint support a proposed 9-months shelf-life for the XBB.1.5 vaccine (2023-2024 Formula) under long-term storage condition. Data from stability evaluation under accelerated conditions show that the relative potency of SIIPL DP batches begins to decrease from about (b) (4) months (b) (4) batches) and from about (b) (4) days (b) (4) batches (b) (4) , albeit still meeting acceptance criteria.

For the PFS presentation of COVID-19 Vaccine, Adjuvanted, the XBB.1.5 DP lots (b) (4) , JN.1 DP lots (b) (4) (all formulated at (b) (4) µg/mL protein concentration), as well as the JN.1 DP lots formulated at (b) (4) µg/mL (lots (b) (4) are evaluated in the stability program in a (b) (4) storage position at 2° to 8°C (long-term) and at (b) (4) (accelerated). In BLA 125817/0.45, the applicant provided real-time stability data through the 6-month timepoint for XBB.1.5 DP lots (b) (4) , (b) (4) , and relative potency and total protein concentration data for lots (b) (4) . Based on statistical calculations, the XBB.1.5 vaccine (2023-3034 Formula) in PFS presentation was estimated to have a monthly rate of potency loss of (b) (4). Stability data through the 3-month timepoint were provided for the for JN.1 DP lots (b) (4) . Under accelerated conditions, all PFS lots were below (b) (4) relative potency at the 1-month timepoint. Under long-term storage, XBB.1.5 DP lot (b) (4) had a relative potency of (b) (4) at the 6-month timepoint, lot (b) (4) was below the specification limit of (b) (4) at 6 months, and lot (b) (4) was (b) (4) at the 2-month timepoint. JN.1 DP lot (b) (4) were below specification at the 2- and 3- month timepoint, respectively, and lot (b) (4) was at (b) (4) at the 3-month timepoint. From the statistical analysis update submitted in EUA 28237/0.356, the PPQ lots of the JN.1 vaccine (2024-2025 Formula) in PFS presentation formulated at (b) (4) µg/mL and (b) (4) µg/mL have estimated monthly degradation rates of (b) (4) respectively, during the first (b) (4) months post-manufacture. Similarly, commercial lots of the 2024-2025 Formula (b) (4) µg/mL formulation) have an estimated degradation rate of (b) (4) in the first 6 months post-manufacture. The increase in formulation target to (b) (4) µg/mL formulation was intended to support a 3-month shelf-life request for the JN.1 vaccine. Due to the rapid loss of potency of the JN.1 vaccine (2024-2025 Formula) in the (b) (4) month of manufacture, FDA required the applicant to submit real-time stability data for JN.1 DP lots in the stability program monthly, and place all newly manufactured lots under stability monitoring. In addition, a statistically derived alert limit of (b) (4) was established and implemented to prevent the release of any JN.1 DP lot with a relative potency below (b) (4) post-manufacture. Thus, under the EUA, lots of the 2023-2024 Formula intended for distribution in the U.S. must have a relative potency of (b) (4) post-manufacture. The requirement to place all manufactured lots on stability ended, at the applicant's request and with FDA concurrence, after a total of (b) (4) lots of the (b) (4) µg/mL formulation of the 2024-2025 Formula had been placed under long-term stability monitoring and real-time stability data of (b) (4) months had been provided, with real-time stability data for (b) (4) lots being in the range of (b) (4) (average

= (b) (4)) at the (b) (4)-month timepoint. The (b) (4) lot (commercial lot (b) (4)) which had a relative potency of (b) (4) at release (prior to the implementation of the (b) (4) alert limit) had a relative potency of (b) (4) at the (b) (4) month timepoint.
The stability updates submitted in BLA 125817/0.45 show that all (b) (4) JN.1 DP lots formulated at (b) (4) µg/mL had relative potency values above the stability lower limit of (b) (4) at the (b) (4)-month timepoint. The real-time stability data for the (b) (4) µg/mL formulation of the JN.1 vaccine supports the requested shelf-life of 3 months.

Overall Reviewer's Assessment of Section 3.2.P.8:

Based on the real-time stability data through the (b) (4) month timepoint submitted in BLA 125817/0.45, all (b) (4) PPQ lots of the (b) (4) µg/mL formulation of the JN.1 vaccine have relative potency values above the lower limit of (b) (4). Therefore, the proposed shelf-life of 3 months for the COVID-19 Vaccine, Adjuvanted (2024-2025 Formula), is acceptable.

Other eCTD Modules
Module 1
A. Environmental Assessment or Claim of Categorical Exclusion
The COVID-19 Vaccine, Adjuvanted (Nuvaxovid) is a protein-based vaccine manufactured for immunization for protection against COVID-19 caused by SARS-CoV-2. In the marketing authorization request for Nuvaxovid submitted in BLA 125817/0, the applicant requested a Categorical Exclusion from the requirement of an Environmental Assessment for the product. Based on the manufacturing information submitted for the antigen (active ingredient; recombinant SARS-CoV-2 spike protein [rS]) and the adjuvant drug substances (saponin extracts from the soapbark tree), the amount of potential waste generated from the manufacturing process is negligible and should have no environmental impact. As a protein-based vaccine, Nuvaxovid has no potential to establish latency and/or reactivation, or recombination, and would degrade rapidly in the environment. Therefore, the request for Categorical Exclusion of Nuvaxovid from the requirement of an Environmental Assessment is acceptable and should be granted. The applicant commits to complying with the categorical exclusion criteria as detailed in 21 CFR 25.31.

B. Reference Product Designation Request
In Section 1.3.5.3 of the eCTD for BLA 125817/0, the applicant included a request for reference product designation for COVID-19 Vaccine, Adjuvanted DP. Based on the information provided, granting of a reference product exclusivity period for the DP is recommended.

C. Labelling Review

The COVID-19 Vaccine, Adjuvanted is a preservative-free, colorless to slightly yellow, clear to mildly opalescent suspension for intramuscular injection that is free from visible particles. A dose is 0.5 mL and contains 5 µg of rS protein of the SARS-CoV-2 and 50 mcg Matrix-M adjuvant prepared from saponin extracts derived from the soapbark tree, *Quillaja saponaria* Molina. The saponin extracts (Fraction-A and Fraction-C) are mixed with cholesterol and phosphatidylcholine to form Matrix particles. The Matrix particles are mixed and co-formulated with the rS drug substance. The rS protein is produced by recombinant DNA technology using a baculovirus expression system in the Sf9 insect cell line that is derived from the *Spodoptera frugiperda* species. The 2024-2025 Formula contains rS from the

Omicron JN.1 variant. The excipients in Nuvaxovid are components of the drug substance and drug product formulation buffers, and are potassium dihydrogen phosphate (3.85 mcg), potassium chloride (2.25 mcg), disodium hydrogen phosphate dihydrate (14.7 mcg), disodium hydrogen phosphate heptahydrate (2.465 mg), sodium dihydrogen phosphate monohydrate (0.445 mg), sodium chloride (8.766 mg in the DP formulation buffer and (b) (4) mcg in the Matrix-M adjuvant), polysorbate 80 (0.050 mg), and Water for Injection. The pH is adjusted with sodium hydroxide or hydrochloric acid. The DP may contain trace amounts of process-related impurities (b) (4)

The Nuvaxovid prefilled syringes are supplied at 10 per carton and stored in the refrigerator at 2° to 8°C, protected from light.

4 Nonclinical Study Reports
4.2.1 Pharmacology
4.2.1.1. Primary Pharmacodynamics

Since the beginning of the development of the COVID-19 vaccine, Adjuvanted, various constructs of the active ingredient (rS), including Wuhan rS, XBB.1.5 rS, and JN.1 rS, have been tested in several nonclinical studies to assess immunogenicity, and in some instances, protective efficacy in animal models. In all animal pharmacology studies, Discovery lots of rS DS manufactured on a small scale (b) (4) at Novavax were used. These small-scale lots were not clinical-grade but are produced using materials and manufacturing processes similar to the manufacture of clinical-trial materials. Most of the non-clinical studies were controlled with a placebo group; the placebo being the rS DS formulation buffer (also used as the diluent in preparing dosing regimens) or (b) (4). Matrix-M1 lot (b) (4) was used in most of the studies, with lot (b) (4) used in a couple of studies in NHPs.

The in-life phase of the nonclinical pharmacology studies was contracted to contract research organizations or research institutions and testing of immune responses was performed by the applicant. The assays for measuring antibody and cell-mediated immune responses in the nonclinical studies are not validated. Validation of the assays for nonclinical immunogenicity assessment in different animal species would be challenging in part due to the low volume of test samples (e.g. serum) obtainable from small animals. However, the assay procedures used in testing antibody responses in the nonclinical studies are similar to the procedures of the validated assays for measuring antibody responses in clinical studies.

In the assessment of protective efficacy in mice, hamsters, and non-human primates, vaccinated animals and controls were challenged with SARS-CoV-2. The evaluation of protective efficacy included the assessment of protection against weight loss, pathogenesis, assessment of viral replication in the upper and lower respiratory tracts, and assessment of lung histopathology for evidence of potential vaccine-enhanced disease. In all animal models, the immune response induced by adjuvanted BV2373 administered at sub-optimal to high doses of rS conferred partial to full protection against disease and pathology and no evidence of enhanced disease was observed. In all nonclinical pharmacology studies, animals were inoculated by the intramuscular route as proposed for the clinical administration of COVID-19 Vaccine, Adjuvanted in humans.

Nonclinical evaluation of BV2373 (Wuhan rS)

Originally, (b) (4) In preliminary immunogenicity studies in mice (*Mus musculus*), BV2373 induced the most robust immune responses, including antibodies that inhibit the binding of S to human ACE2 (ACE2) and the immune response was enhanced in the presence of Matrix-M1 adjuvant. The BV2373/Matrix-M adjuvant combination also induced a balanced Th1/Th2 response as determined by IgG1/IgG2a ratios. Thus, BV2373 was further evaluated in a series of immunogenicity and protective efficacy studies in different animal models (mice, hamsters, and non-human primates [NHPs]). In all studies, BV2373 was formulated with Matrix-M on the day of inoculation of animals. Nonclinical study reports were originally submitted to IND 22430.

A range of doses (0.01 µg to 10 µg) of the Wuhan rS vaccine BV2373, with or without 5 µg of Matrix-M was tested in (b) (4) mice. In hamsters (b) (4) , doses of 1 µg and 10 µg of rS with 15 µg Matrix-M were tested. In NHPs, (b) (4) baboons (b) (4) cynomolgus macaques (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*), BV2373 was tested at doses in the range of 1 µg to 25 µg, with or without 25 µg or 50 µg of Matrix-M. In most non-clinical studies, animals were inoculated on a prime/boost schedule at intervals of 14- or 21- days (mice), 14 days interval (hamsters), or 21 days interval (non-human primates). In some studies, a single dose of rS, with or without Matrix-M, was also used, and a couple of studies in NHPs (702-087 and 702-115) were long-term pharmacology studies in (b) (4) baboons and rhesus macaques, respectively. Immunogenicity assessment was based on the quantitation of antibody responses (binding antibodies by ELISA, ACE2-inhibiting antibodies by ELISA, neutralizing antibodies by inhibition of infectivity of wild-type SARS-CoV-2 isolate or psedoviruses expressing the S protein), as well as cell-mediated immune responses. The nonclinical studies conducted with the Wuhan rS and their outcomes are as follows:

Study 702-089
In this preliminary study, the immunogenicity of three constructs of recombinant Wuhan S protein was evaluated in mice to identify an appropriate candidate for development into a COVID-19 vaccine. (b) (4) . Female (b) (4) mice (n = 8 in 12 groups, and a placebo group of 4) were injected on days 0 and 14 with 1 or 10 µg of each construct, each dose administered with and without Matrix-M. Serum samples obtained on days 13, 21, and 28 were tested for rS-specific IgG and ACE2 binding inhibiting antibodies by ELISA. Mice in the placebo and RBD treatment groups did not induce an IgG or ACE2-binding inhibiting antibody response. BV2369 induced an IgG response but not ACE2-binding inhibiting response. BV2365 at both 1 and 10 µg induced both IgG and ACE2-binding inhibiting antibody responses that increased after the 2nd dose. The antibody responses were dose-dependent and enhanced by Matrix-M.

Study 702-090

Study 702-090 was another preliminary study similar in design to study 702-089 but with the (b) (4)-treatment cohort replaced with BV2373 (1 and 10 µg, with and without 5 µg Matrix-M). BV2373 (b) (4)), which carries (in addition to the(b) (4) substitutions) a (b) (4) substitutions in the (b) (4) for enhanced stability in the pre-fusion protein conformation.

Female (b) (4) mice (n = 8 in 12 groups, and a placebo group of 4) were injected on days 0 and 14 with 1 or 10 µg of each construct, each dose administered with and without Matrix-M. Serum samples obtained on days 13, 21, 28, and 42, were tested for rS-specific IgG and ACE2-binding inhibiting antibodies by ELISA, as well as neutralizing antibody (by testing capacity to block SARS-CoV-2 infectivity in (b) (4) Mice in the placebo treatment group did not induce any antibody responses. BV2369 induced an IgG response but not ACE2 inhibiting and neutralizing antibody responses. At both 1 and 10 µg dose levels, BV2365 and BV2373 induced measurable levels of IgG and antibodies inhibitory to SARS-CoV-2 spike protein binding to ACE2. Serum samples were pooled for the treatment groups tested for neutralizing antibodies (i.e., the Day-13 and Day-28 sera of the adjuvanted 10-µg dose of BV2365 and BV2373). The Day-13 neutralizing antibody titers were 320 and 1280 for the BV2365 and BV2373 pools, respectively, but both had the same titer of 10240 in the Day 28 serum pool. The antibody responses were increased after the 2nd dose and highest in the groups injected with adjuvanted 10 µg BV2373. The antibody responses were dose-dependent, highest in the Day 28 serum samples, and enhanced by Matrix-M. In summary, BV2373 induced the highest levels of antibody responses, the antibody response is higher with the prime/boost regime and enhanced by Matrix-M adjuvant.

Study 702-095

This study was designed to evaluate antibody and cell-mediated immune responses induced by Wuhan rS in mice after a prime and a boost at an interval of 21 days. A group of female (b) (4) mice (n = 6) was injected with 10 µg of BV2373. A second group (n = 6) was injected with 10 µg of BV2373 adjuvanted with of 5 µg Matrix-M. A third group (n = 3) was injected with placebo. Serum samples obtained a day before the priming injection and on study days 20 and 28 were tested for rS-specific IgG and ACE2 binding-inhibiting antibodies. Splenocytes were obtained from mice on Day 28 were tested for T-cell responses by IFN-γ enzyme-linked immunospot (ELISpot) assay. The results show that no rS-specific IgG antibodies were detected in the placebo treatment group. rS-specific IgG response was elicited in both groups inoculated with BV2373, with about 48-fold and 35-fold increase after the 2nd dose in the groups injected with and without Matrix-M, respectively. IgG levels were > 50-fold higher in the adjuvanted BV2373 group than the unadjuvanted group. A similar pattern was obtained for ACE2-binding inhibiting antibody response, with the adjuvanted treatment group having > 30-fold higher antibody level than the unadjuvanted BV2373 treatment group. In the assay for cellular immune response, no IFN-γ-secreting cells (immunospots) were detected per 10^6 splenocytes from the placebo group. The average number of IFN-γ-secreting splenocytes (310 per 10^6 splenocytes) in the adjuvanted treatment group was >19-fold higher than the number of positive cells (16) in the unadjuvanted group. By intracellular-cytokine staining, numbers of $CD4^+$ and $CD8^+$ T-cells expressing Th-1 cytokines were higher in the adjuvated BV2373 treatment group than the unadjuvanted group. In summary, this study demonstrated that BV2373 induces both human and cell-mediated immune responses in mice. The immune responses are enhanced by Matrix-M adjuvant.

Study 702-092

This was a dose-ranging study of BV2373. Female (b) (4) mice in groups of 10 were inoculated with a range of doses (0.01-, 0.1-, 1.0-, and 10- µg) of BV2373 in 2 sets. Each injectate was adjuvanted with 5 µg Matrix-M1. On study Day 14, the 1st set of mice received a booster dose in the same amount of BV2373/Matrix-M1 as the priming dose; the 2nd set did not receive boosters. A group inoculated with placebo and a group inoculated with 10 µg of unadjuvanted BV2373 were included as controls; both controls were inoculated on the prime and boost schedule. Serum samples obtained a day before the 1st dose and on study days 13, 21, and 28 were tested for S protein-specific binding IgG, ACE2-binding inhibiting antibodies, and neutralizing antibodies. On study Day 56, mice were challenged with 1.5 x 10^5 pfu/mouse of SAR-CoV-2 (2019 nCoV/USAWA1/2020) via the intranasal route, and necropsy and histopathology were performed on Day 60 or Day 63. The results show a dose response to BV2373, with mice injected with adjuvanted BV2373 ≥ 1 µg having the highest antibody responses. Similarly, there was a dose-dependent protection of mice after intranasal challenge with SARS-CoV-2. Lung histopathology at 4 and 7 days post-challenge did not show any evidence of vaccine-associated enhanced-respiratory disease in mice in the low (BV2373) dose-treatment groups.

Study 702-093

The immunogenicity and protective effect of BV2373 in (b) (4) hamsters was evaluated. Wild-type male and female (b) (4) hamsters were assigned to 4 treatment groups (n = 8 per group). Two groups were injected with 1 and 10 µg of BV2373 with 15 µg Matrix-M on Day 0 and Day 14. The 3rd group was injected with 10- µg of BV2373 with 15 µg Matrix-M only on Day 0. The 4th group was injected with placebo on Day 0 and Day 14. Serum samples were collected prior to Day 0, and on days 14, 21, and 29 and tested for IgG antibody by ELISA, ACE2 binding inhibiting antibodies by ELISA and neutralizing antibodies against SARS-CoV-2 (2019-nCoV/USA-WA1/2020) by inhibition of formation of cytopathic effect in (b) (4) . On Day 35, each hamster was challenged with 5.6 × 10^4 pfu of SARS-CoV-2 (2019-nCoV/USA-WA1/2020 (obtained from the CDC) and weighed daily over 2 weeks. Oral swabs were for the determination of the levels of SARS-CoV-2 total RNA (qRT-PCR) and sub-genomic RNA (sgRNA, by qRT-PCR) were collected 2, 4, 7, and 14- or 15-days post-challenge. At 4 days post challenge, 3 or 4 hamsters from each group was euthanized 4 days pot-challenge (the remaining hamsters were euthanized on 14- or 15-days post-challenge) for histopathology. The results show that antibody levels (total IgG, ACE2 binding-inhibiting, and neutralizing antibodies) were below limits of detection in the placebo group. BV2373 induced high IgG titers in all treatment groups, with the adjuvanted 10 µg prime/boost treatment group having the highest IgG-antibody titers. A similar trend was observed in the ACE2-binding inhibiting antibody response, with the prime/boost treatment groups having the highest Day-21 and Day-28 titers compared with the single-dose group, and the 10 µg prime/boost treatment group having the highest ACE2-binding inhibiting antibody titers. A reduction in the level of activity occurred in all treatment groups in the two days post-challenge, which increased and peaked 5 days post-challenge in the placebo. All hamsters in the BV2373 treatment groups returned to baseline activity levels by Day 3. High lung viral load occurred in all treatment groups 2 days post-challenge, but highest in the placebo group. The levels decreased by Day 4 post-challenge but remained highest in the placebo group and continued to decrease in all groups through days 14/15. In the Day-4 histopathology, the placebo group had pronounced alveolar edema, alveolar hyperplasia, alveolar mixed cell inflammation, mononuclear cell inflammation, and the presence of syncytial cells. Except for a hamster in the 10-µg single-dose BV2373 group with a histopathological score of 1, all other hamsters had no obvious histopathology. In

summary, BV2373 induced antibody responses, including neutralizing antibodies, and protected (b) (4) hamsters from disease and lung pathology caused by SARS-CoV-2, and no evidence of vaccine-enhanced disease was detected.

Study 702-094

The immunogenicity and protective effect of BV2373 in cynomolgus macaques (*Macaca fascicularis*) were evaluated. Male (9) and female (15) macaques were randomly assigned to 6 treatment groups (n = 4, with 1 or 2 male per treatment group). The 1st group was inoculated with placebo on days 0 and 21; the 2nd group was inoculated with 2.5 µg BV2373, with 25 µg Matrix-M on days 0 and 21; the 3rd group was inoculated with a single dose (Day 0) of 5 µg BV2373, with 25 µg Matrix-M; the 4th group was inoculated with 5 µg BV2373, with 50 µg Matrix-M on Day- 0 and 21 (clinical dose); the 5th group was inoculated with a single dose (Day 0) of 5 µg BV2373, with 50 µg Matrix-M; and the 6th group was inoculated with 25 µg BV2373, with 50 µg Matrix-M on Day- 0 and 21. Serum samples were collected on days 0, and 21 and tested for SARS-CoV-2 spike protein-specific IgG and ACE2 binding-inhibiting antibody by ELISA. Serum samples collected on Day 33 were tested for neutralizing antibody by (b) (4) and by the inhibition of infectivity (CPE) in (b) (4). Macaques were challenged with SARS-CoV-2 (2019-nCoV/USA-WA1/2020) on study Day 35, with the challenge dose of 1.1×10^4 pfu split between intranasal and intratracheal routes. Necropsy and histopathology were performed on Day 42, and bronchoalveolar lavage (BAL) fluid and nasal swabs were tested for viral RNA by reverse transcription quantitative polymerase chain reaction (RT-qPCR). The levels of sgRNA evaluated by RT-qPCR.

No rS-specific IgG antibodies were detected in the placebo serum samples. High titers of antibodies (IgG and ACE2-binding-inhibiting) were detected in the Day 21 serum samples from macaques in all BV2373-treatment groups, with comparable Day-21 antibody titers between the 5 µg BV2373/50 µg Matrix-M and the 25 µg BV2373/50 µg Matrix-M treatment groups. The level of antibodies induced by a dose of 2.5 µg BV2373 with 25 µg Matrix-M prime/boost were about 2-fold to 3-fold lower than the levels induced by 5 µg BV2373/50 µg Matrix-M prime/boost (clinical dose) treatment group in Day-21 and Day-33 serum samples, respectively. Neutralizing antibodies were detected in BV2373 groups but were significantly lower in the single-dose treatment groups (for example, a CPE_{100} neutralizing antibody titer of 19840 ± 7564 standard error of the mean [SEM] for the 5 µg BV2373/50 µg Matrix-M prime/boost group versus 1320 ± 548 SEM for the single-dose 5 µg BV2373/50 µg Matrix-M prime/boost group). The RNA copies/mL of BAL fluid from the placebo group was ≥ 576-fold higher than any of the BV2373 immunization groups on Day-2 post-challenge. Except for the group immunized with 2.5 µg BV2373/25 µg Matrix-M which had detectable sgRNA (43.5 copies/mL) at 2 days post-challenge, sgRNA was below detection in all BV2373 treatment groups. In the placebo group, 9131 sgRNA copies/mL of BAL were detected on Day 2. A similar pattern of significantly higher RNA levels in nasal swabs of macaques in the placebo treatment group compared with macaques in BV2373-treatment groups was obtained. In summary, adjuvanted BV2373 induced protective immune responses in cynomolgus macaques.

Study 702-099

The immunogenicity and protective effect of BV2373 in rhesus macaques (*Macaca mulatta*) was evaluated in Study 702-099. Macaques were assigned to 6 groups (n = 2 to 5 per group) in two treatment cohorts. The 3 groups in the 1st treatment cohort were inoculated on a prime/boost schedule on Day 0 and Day 21, with placebo, 5 µg BV2373/50 µg Matrix-M, and 25 µg BV2373/50 µg Matrix-M. Macaques in the 2nd treatment cohort were inoculated with a

single dose (Day 0) of placebo, 5 µg BV2373/50 µg Matrix-M, and 25 µg BV2373/50 µg Matrix-M. Serum samples were collected on days 0, 21, and 31/32 were tested for SARS-CoV-2 spike-specific IgG and ACE2-binding-inhibiting antibody by ELISA. Serum samples collected on Day 31/32 were also tested for neutralizing antibody by the inhibition of infectivity (CPE) in (b) (4) . BAL and nasal washes collected on days 31 or 32 were tested for spike-specific IgG and IgA. On Day 38, macaques were challenged with SARS-CoV-2 (SARS-CoV-2/human/USA/WA-CDC-02982586-001/2020) at a target of 1.05×10^6 pfu/animal split evenly between the intranasal and intratracheal routes. Antibodies were not detected in any Day-0 and placebo serum samples at any timepoint. High titers of IgG were detected in the Day-21 serum samples from all BV2373 treatment groups. While these titers had decreased in the single-dose treatment groups, increases of > 20- or > 35-fold in IgG titers was obtained for the BV2373 treatment group after the booster doses. Similarly, ACE2 binding-inhibiting antibodies increased by > 18- or > 29-fold in the prime/boost groups but decreased in the single-dose groups. Low levels of S-specific IgG and IgA were detected in BAL and nasal washes in all BV2373 treatment groups but not in placebo groups, with higher titers in the prime/boost groups. A mean neutralizing-antibody titer of 34 was detected in the placebo group but neutralizing antibodies were > 19- to >971-fold higher in BV2373 treatment groups, with the prime/boost groups having the highest antibody titers. Lung, nasal cavity, and trachea samples from necropsy were tested for viral load.

Viral RNA levels were between 17- to > 692-fold higher in placebo nasal washes than the BV2373 treatment groups on day-2 post-challenge. A similar pattern was evident for sgRNA. Although viral RNA and sgRNA levels decreased steadily through days 7/8 post-challenge, their levels in the placebo samples (e.g., a geometric mean of 88277 for sgRNA on days 7/8) were still >223-fold higher than the BV2373 treatment group with the highest sgRNA level (a geometric mean of 394.2 on days 7/8). A similar pattern was obtained for the BAL samples and nasopharyngeal swab samples, with the placebo treatment group having significantly higher viral RNA and sgRNA than BV2373 treatment groups. In summary, Matrix-M adjuvanted BV2373 induced antibody responses, including neutralizing antibodies, with comparable levels of antibody between macaques in the 5 µg and 25 µg BV2373 treatment groups. Rhesus macaques were protected from SARS-CoV-2 infection, with the prime/boost treatment group conferring a more robust protection than the single-dose treatment groups, and no evidence of vaccine-enhanced disease was seen.

Study 702-111.

The immunogenicity and protective effect of fractional doses of BV2373 was evaluated in rhesus macaques (*Macaca mulatta*). Macaques (males and females) were assigned to 8 treatment groups (n = 5 per group). The placebo group was inoculated with PBS on Day 0 and 21. Groups 2, 3, 4, 5, and 6 were inoculated with BV2373 at doses of 5, 1, 0.2, 0.04, and 0.008 µg, on Day- 0 and 21; all doses were adjuvanted with 50 µg Matrix-M. Group 7 was inoculated with 50 µg Matrix-M (without antigen) and group 8 was inoculated once (Day 0) with 5 µg BV2373 with 50 µg Matrix-M. For immunogenicity analyses, blood samples were collected on study days 0, 21, 35, 42, 44, 46, 48 and on the day of euthanasia. Peripheral blood mononuclear cells (PBMCs) were collected on Day 28 for cell-mediated immune-response evaluation. BAL fluid samples were collected on days 35, 44, 46, 48 and the day of euthanasia, nasal washes on day 35, nasopharyngeal swabs on days 42, 44, 46, 48 and the day of euthanasia, with necropsy from Day 49 to 52. IgG antibody was not detected in any Day 0 serum samples of any treatment group nor in the Day 21 and Day 35 serum samples from the placebo and Matrix-M-only treatment groups. All treatment groups inoculated with BV2373 had measurable IgG levels that increased from Day 21 to Day 35, including the dose of 0.008

μg (Day 21 Geometric Mean Titer [GMT] = 438, Day 35 GMT = 26405) except in the single-dose 5 μg BV2373/50 μg Matrix-M treatment group where IgG titer waned (Day 21 GMT = 9428, Day 35 GMT = 6203). The ACE2-binding inhibiting antibody levels followed a similar pattern. The Day 35 IgG and ACE2-binding inhibiting antibodies induced by the 5 and 1 μg BV2373 treatment groups cross-reacted with SARS-CoV-2 variant rS proteins (Alpha, Beta, Delta, Omicron BA.1, Omicron BA.2, and Omicron BA.5), with comparable or higher titers than the Day 21 antibody levels against the homologous Wuhan rS. Low levels of S-specific IgG and IgA were also detected in Day 35 BAL fluid and nasal wash samples for all BV2373 treatment groups but not in placebo or adjuvant-only groups. Neutralizing antibodies were detected in all BV2373 treatment group, including a GMT of 1305 in the 0.008 μg BV2373 treatment group. The single-dose group had the lowest GMT of 173. Th1 cytokine-producing cells were detected in day 28 splenocytes of all BV2373 groups except in the single-dose group which is at baseline level and comparable to the placebo group. Viral RNA and sgRNA in BAL and nasopharyngeal swabs correlated with the antibody responses, with the placebo and adjuvant-only groups having significantly higher RNA levels. In summary, suboptimal doses of BV2373 adjuvanted with Matrix-M induced protective immune responses in rhesus macaques.

Study 702-115

The long-term immunogenicity and protective efficacy of rS was evaluated in rhesus macaques. Two groups of macaques (n = 6 per group) were vaccinated as follows: Group 1 was vaccinated with 5 μg BV2373 adjuvanted with 50 μg Matrix-M on Day 0 and Day 28, followed by a boost with 5 μg of BV2443 (Gamma rS) on Day 245. Macaques in group 2 were split into 2 sub-groups of 3, the 1st sub-group was vaccinated with 5 μg BV2373 adjuvanted with 50 μg Matrix-M on Day 245 and Day 273, and the 2nd sub-group was vaccinated with 5 μg BV2443 adjuvanted with 50 μg Matrix-M on Day 245 and Day 273. Group 3 contained 9 macaques as placebo control. Pre- and post-vaccination blood samples were collected at multiple timepoints for the evaluation of immune responses. Serum samples were collected 7 days before vaccination and on days 14, 28, 32, 42, and 57 post-vaccination and tested for total IgG against S protein and RBD, and cell-mediated immune response was evaluated by ELISpot and intracellular cytokine staining. Macaques were challenged with SARS-CoV-2 on Day 365. On Day 462, macaques in group 1 were split into 2 sub-groups (n = 3 per sub-group), a sub-group, along with a macaque in group 3, were challenged with Wuhan strain (SARS-CoV-2 USAWA1/2020) and the 2nd sub-group was challenged with SARS-CoV-2 Gamma strain (hCoV-19/Japan/TY7-503/2021). All 6 macaques in group 2, and 2 macaques in group 3 were challenged with the Gamma strain. On Day 539, the remaining 3 macaques in group 3 were split into 2 sub-groups (n = 3 per subgroup); a sub-group was challenged with the Wuhan strain and the 2nd sub-group was challenged with the Gamma strain. Each macaque was challenged with 6 x 10^5 PFU of virus in 3 mL PBS via the intratracheal route and 2 x 10^5 PFU in 1 mL through the intranasal route. At several timepoints pre- and post-challenge, specimens (bone marrow, nasal swabs, and bronchoalveolar lavage) were collected.

Both anti-S and anti-RBD antibodies were detectable in vaccinated macaques at timepoints post-vaccination. In naïve animals, S-specific IgG was detected at high titers beginning from Day 7 post-challenge.

Study 702-087

In study 702-087, 4 groups of (b) (4) baboons (n = 2 or 3 per group) were vaccinated with BV2373 on Day 0 and Day 21. Group 1 was vaccinated with 25 μg of BV2373 without adjuvant; groups 2, 3, and 4 were vaccinated with 1, 5, and 25 μg of BV2373, respectively, all

adjuvanted with 50 µg Matrix-M. Pre-vaccination (Day 0) serum samples, as well as serum samples obtained on Days 21, 28, 35, 49, 120, 182, 245, and 303 post-vaccination were tested for S-specific IgG, ACE2-binding-inhibiting antibodies, and SARS-CoV-2-neutralizing antibodies. Peripheral blood mononuclear cells (PBMCs) obtained on Days 28, 49, 182, and 245 were tested for cell-mediated immune responses by ELISpot and intracellular cytokine staining. No spike-specific IgG was detected in pre-bleed serum samples. The mean IgG titer in the unadjuvanted BV2373 was 117 on Day 21, and no significant increase in titers after the booster dose, with Days 28, 35 and 49, IgG titers of 120, 125 and 110, respectively. In group 2, mean IgG titers of 1700, 33126, 64781 and 54141 were recorded on Days 21, 28, 35 and 49, respectively. The highest IgG titers were recorded in group 3, with mean titers of 29249, 237432, 215587 and 76076 on Days 21, 28, 35 and 49, respectively. IgG titers of 8404, 174436, 130630 and 35027 on Days 21, 28, 35 and 49, respectively, were obtained for baboons in group 4. ACE2 binding inhibiting antibodies were not detected at any timepoint in the unadjuvanted treatment group. ACE2 antibody was detected at low titers of 52 and 22 on Day 21 in groups 3 and 4, respectively. Antibodies were detected in group 2 on day 28 and peaked at a titer of 390 on Day 35. Peak ACE2 binding inhibiting antibody titers of 877 and 433 against Wuhan rS were recorded on Day 28 in both groups 3 and 4. Neutralizing antibodies (measured by inhibition of CPE) peaked on Day 35, with titers of 6400, 17920, and 17067 in groups 2, 3, and 4, respectively, which rapidly decreased to 330, 300, and 373, respectively by Day 120 and were at or close to baseline levels by Day 182. Neutralizing antibodies were not detected in Day 245 and Day 303 serum samples (tested by (b) (4) . Low numbers (range, 9 to 58 per 10^6 PBMCs) of IFN-γ producing cells were detected in Day 28 PBMCs, with the lowest number in the unadjuvanted group. A similar trend was obtained for the number of $CD4^+$ T-cells expressing Th1 cytokines (IFN-γ^+, IL-2^+, or TNF-α^+) singly, doubly, or triply, with group 3 having the highest numbers, as determined by intracellular cytokine staining. By day 182, the numbers of cytokine-producing cells had decreased substantially (e.g., 171 IFN-γ^+ producing $CD4^+$ cells/10^6 PBMCs on Day 49 to 35 cells on Day 182). Thus, BV2373 induces antibody and cell-mediated immune responses in baboons which is enhanced with Matrix-M adjuvant. Neutralizing antibody response peaked at 2 weeks after a booster dose with adjuvanted BV2373.

With the emergence of pre-Omicron SARS-CoV-2 variants of concern (VOCs), each macaque in this study was inoculated with 3 µg of BV2426 (rS of SARS-CoV-2 strain B.1.351; Beta strain) adjuvanted with 50 µg of Matrix-M1 on Day 318. A second booster of 3 µg BV2426/50 µg Matrix-M was administered to a subset of baboons (1 of 2 baboons in groups 1 to 3, and 2 of 3 baboons in group 4) on Day 339. Serum samples obtained on Day 325 (1 week after BV2426 boost), Day 339 (3 weeks after BV2426 boost), and Day 353 (5 weeks after BV2426 boost) were tested for S-specific IgG and ACE2-binding-inhibiting antibodies against the Wuhan SARS-CoV-2 (USA/WA-1) and Beta strain. Serum samples were also tested for the neutralization of SARS-CoV-2 USA/WA-1, Beta, and strain Alpha (B.1.1.17). PBMCs obtained on Days 325 and 353 were tested for cell-mediated immune responses by ELISpot and ICS assays. The results show significant increases in total IgG titer (> 186.9 folds) over pre-boost (Day 303) titers, in both Wuhan S and Beta S ELISAs, with a mean titer of 480,015 and 362,640, respectively, on Day 325. IgG titers were also higher than titers obtained at peak response on Day 35. On Day 339, mean IgG titers of 600,138 and 541,903 were obtained against Wuhan S and Beta S, respectively, and by Day 353 the mean IgG titers decreased to 514,423 and 454,649, respectively.

ACE2-inhibiting antibody level against Wuhan rS increased from a titer of 10 on Day 303 to 911.4 on Day 325 (i.e.,1 week after BV2426 boost) for serum samples obtained one week after the Day 318 with BV2426 (US-WA1 rS IC_{50} mean titers = 911.4 and Beta rS mean IC_{50} titer =

423.6), but with minimal increases in the subset of animals inoculated with a second booster of BV2426.

Neutralizing antibodies against the Wuhan, Beta, and Alpha strains were assessed by (b) (4)], with mean (b) (4) titers on Day 325 as 2,491; 7,517; and 3,879, respectively, representing > 31- to 94-folds increase above the Day 303 (baseline level) titers. Neutralizing antibody titers remained high through Day 353. Increase in neutralizing antibody titers in the subset of baboons inoculated with a second boost was modest.

A 10.6- to 29- fold increase in IFN-γ-producing cells was obtained on Day 325 when compared with pre-boost levels, with 145- and 275- IFN-$\gamma^+/10^6$ PBMCs re-stimulated with Wuhan rS and Beta rS, respectively. There was only a slight increase two weeks after the second boost. IL-4 producing $CD4^+$ cells also increased from baseline level on Day 245 by 45- to 51.5-fold but declined in all baboons. Overall, the ratio of IFN-γ to IL-4 producing cells was 1.4 and 2 on Day 325; 20.2 and 28.7 on Day 353 when PBMCs were restimulated with Wuhan rS and Beta rS, respectively, indicating a predominantly Th1-type cytokine response.

In (b) (4) assay, the average number of polyfunctional $CD4^+$ T cells producing single, double, and triple Th1 cytokines (IFN-γ^+, IL-2^+, or TNF-α^+) increased by approximately 11 to 43.3 folds (Wuhan rS) and 8.7 to 30.7 folds (Beta rS) on Day 325, compared to the Day 245 levels. There was a 4.6- to 7.5-fold increase in $CD4^+$ T cells expressing IL-5 and IL-13 (Th2 cytokines) upon re-stimulation with Wuhan rS, and their levels did not increase significantly after the 2nd boost of BV2426.

On Day 660, a booster dose of 5 µg BV2373 (Wuhan) rS adjuvanted with 50 µg Matrix-M was injected into 1 of 2 baboons in groups 1 to 3, and 1 of 3 baboons in group 4. The second sub-set of 1 of 2 baboons in groups 1 to 3, and 2 of 3 baboons in group 4 were injected with 5 µg BV2515 (Omicron BA.1 rS) adjuvanted with 50 µg Matrix-M. Serum samples collected on Days 667 and 681were tested for S-specific IgG against Wuhan-, Alpha-, Beta-, Delta-, Omicron BA.1-, Omicron BA.2-, and Omicron BA.5- rS. IgG titers were generally high across the board, irrespective of the antigen used in IgG ELISA. ACE2-binding-inhibiting antibody was level was higher for the 3 baboons from groups 2,3, & 4 boosted on Day 660 than the baboon boosted in group 1 but was generally detectable against Wuhan, Alpha, Beta, Delta, Omicron BA.1 and Omicron BA.5, with overlapping titers in all baboons. The ACE2 inhibiting antibody titers ranged from 129 to 846 in the baboon from group 1, and from 211 to 1353 in the baboon from groups 2,3, & 4.

Neutralizing antibody (by (b) (4)) in the Day 660 serum samples were at baseline level but increased substantially by Day 681 after the Day 660 boost with BV2373, with the highest titer recorded against Omicron BA.1 and the least titer against Delta. The Day 681 neutralizing antibody titer of the subset of baboons boosted with BV2515 increased from a baseline titer of 80 to a range of 1280 to 99840 in all baboons and was highest against Omicron BA.1. Overall, Study 207-087 demonstrated that the Wuhan rS induced both humoral and cell-mediated immune responses when injected Matrix-M adjuvant into baboons. The antibody response peaked between 1 to 2 weeks after the booster dose of the primary series. The antibody response waned substantially by Day 120, but there is a rapid recall of the antibody response with a booster dose of a homologous or heterologous rS > 9 months after the primary vaccination. The induced antibody response after a sequential boost with two doses of Beta rS (at an interval of 3 weeks) > 9 months (Day 318 and Day 339) after the primary series, as well as additional boosters of subsets with Wuhan rS or Omicron BA.1 rS > 21 months (Day 660) after the primary series cross-neutralized SARS-CoV-2 strains (Alpha, Beta, Delta) and Omicron BA.1.

Nonclinical evaluation of BV2601 (XBB.1.5 rS)
Following the emergence of the Omicron variants of SARS-CoV-2, the applicant began the development of a candidate Omicron BA.1 vaccine. As the SARS-CoV-2 Omicron BA.5 variant became predominant, vaccine development efforts were re-focused on a candidate BA.5 vaccine. In nonclinical study 702-171/172, the immunogenicity of candidate Omicron subvariant spike proteins (BA.1 rS, BA.12.1 rS, and BA.5 rS), along with the prototype Wuhan rS) was demonstrated in mice. However, before the development of the BA.5 vaccine was completed, the Omicron XBB.1.5 variant became the predominant SARS-CoV-2 variant causing infections and attention was re-directed to the development of an XBB.1.5 vaccine. The XBB.1.5 rS (BV2601) was evaluated in three studies in mice. In all studies in mice, animals were inoculated with 1 µg (0.1 µg rS was also evaluated in one study) of monovalent BV2601 formulated with 5 µg of Matrix-M adjuvant. Where a bivalent formulation was included, each mouse received 0.5 µg of the constituent rS adjuvanted with 5 µg of Matrix-M.

Study 702-191
This study was designed to generate nonclinical immunogenicity data to support the proposed strain change from the Original monovalent (Wuhan) vaccine to the COVID-19 Vaccine, Adjuvanted 2023-2024 Formula (XBB.1.5 vaccine) for the Fall 2023/2024 vaccination campaign. In the study, the immunogenicity of the monovalent XBB.1.5 rS (BV2601) as a booster dose was evaluated. Mice in groups of 10 were vaccinated prime/boost at an interval of 2 weeks, using 1 µg of adjuvanted BV2373 (Wuhan) rS (10 treatment groups). A second set of 10 treatment groups received a bivalent formulation (0.5 µg BV2373 rS + 0.5 µg BV2601 rS + 5 µg Matrix-M). On Study Day 47 (i.e., 33 days after the 2nd dose), monovalent formulations of BV2373 rS, BV2540 (BA.5) rS, XBB.1.5 (BV2601) rS, BQ.1.1 (BV2589) rS, CH.1.1 (BV2619) rS, or XBB.1.16 (BV2633) rS were administered as booster doses to a group of mice each previously vaccinated with a primary series of Wuhan rS or a bivalent (Wuhan rS + BA.5 rS) rS in the initial prime/boost series. Mice in the remaining treatment groups (4 groups each in the monovalent Wuhan prime/boost and bivalent (Wuhan rS + BA.5 rS) prime/boost groups) received a Day 47 booster of bivalent rS formulations (Wuhan rS + BA.5 rS, Wuhan rS + XBB.1.5 rS, Wuhan rS + BQ.1.1 rS, and Wuhan rS + XBB.1.16 rS). Serum samples were collected on Study days -1, 13, 21, and 61 for antibody assays. On Study Day 61, spleen cells were collected from 5 mice from each of the treatment groups boosted with a bivalent formulation of XBB.1.5 rS or XBB.1.16 rS for the evaluation of cell-mediated immune responses. Data from this study indicate that the inoculation of a booster dose of the monovalent XBB.1.5 rS > 30 days after a prime/boost with Wuhan rS or a bivalent Wuhan/BA.5 rS formulation induced robust IgG, ACE2-binding inhibiting and neutralizing antibody responses, and cell-mediated immune responses. Neutralizing antibody (50% pseudovirus neutralizing antibody [pVN_{50}]) GMTs were measured against lentivirus pseudotypes expressing the S protein of SARS-CoV-2 strains or variants. The neutralizing-antibody data are shown in Tables 42a & 42b (Extracted from Table 7, document TR # 2023-54-702-191, BLA 125817/0.2).

Table 42a. Day-21 GMT of pVN_{50} antibody against pseudotyped viruses

1° series (Day 0, 14)	**Day 21 pVN_{50} GMT Wuhan S pV [95% CI]**	**Day 21 pVN_{50} GMT BA.5 S pV [95% CI]**	**Day 21 pVN_{50} GMT BQ.1.1 S pV [95% CI]**	**Day 21 pVN_{50} GMT CH.1.1 S pV [95% CI]**	**Day 21 pVN_{50} GMT XBB.1.5 S pV [95% CI]**	**Day 21 pVN_{50} GMT XBB.1.16^2 S pV [95% CI]**	**Day 21 pVN_{50} GMT XBB.2.3^2 S pV [95% CI]**
Wuhan rS (n = 100)	9111 [7751, 10708]	74.4 [63.2, 87.6]	51.2 [49.7, 52.8]	< LOD	< LOD	< LOD	56.2 [53.7, 58.7]

Wuhan rS + BA.5 rS (n = 98)[1]	7043 [5794, 8562]	7457 [6202, 8966]	2870 [2445, 3368]	144.2 [116.4, 178.6]	134.3 [107.0, 168.5]	474.3 [362.0, 621.4]	1199 [968.9, 1483]

Table 42b. Day-61 GMT of pVN_{50} antibody against pseudotyped viruses (n = 89 to 100)

1° series (Day 0, 14)	Day 47 Boost	Day 61 pVN_{50} GMT Wuhan S pV	Day 61 pVN_{50} GMT BA.5 S pV	Day 61 pVN_{50} GMT BQ.1.1 S pV	Day 61 pVN_{50} GMT CH.1.1 S pV	Day 61 pVN_{50} GMT XBB.1.5 S pV	Day 61 pVN_{50} GMT XBB.1.16[2] S pV	Day 61 pVN_{50} GMT XBB.2.3[2] S pV
Wuhan rS	Wuhan rS	13912	373.2	99.4	61.7	53.2	55.8	94.9
Wuhan rS	XBB.1.5 rS	7943	220.6	76.8	89.0	135.0	177.7	215.5
Wuhan rS	XBB.1.16 rS	6570	357.9	136.5	143.0	178.5	210.8	297.1
Wuhan rS + BA.5 rS	Wuhan rS	19126	15061	4447	147.1	354.5	285.3	439.7
Wuhan rS + BA.5 rS	XBB.1.5 rS	4722	41286	31258	5865	5763	8838	4425
Wuhan rS + BA.5 rS	XBB.1.16 rS	3671	23520	14292	1157	6367	4778	2938
Wuhan rS	Wuhan rS + XBB.1.5 rS	12931	815.6	143.2	73.8	119.1	93.3	162.1
Wuhan rS	Wuhan rS + XBB.1.16 rS	16665	712.5	163.7	143.0	133.1	173.8	191.8
Wuhan rS + BA.5 rS	Wuhan rS + XBB.1.5 rS	21797	27362	28855	3419	3597	2862	4443
Wuhan rS + BA.5 rS	Wuhan rS + XBB.1.16 rS	7185	44284	25021	2813	2115	2626	4579

[1]Results for 2 mice were excluded from GMT computation due to low antibody response.
[2]Serum samples were tested individually or in pools.
pVN_{50} GMT = 50% Pseudovirus Neutralization Geometric Mean Titer; S PV = Spike Pseudotype virus.

Pools of the Day 21 serum samples from the Wuhan/BA.5 bivalent treatment group also neutralized pseudoviruses expressing the S proteins of Omicron EG.1.5.1, Omicron FL.1.5.1, Omicron HV.1 and Omicron HK.3, but not Omicron BA.2.86.

Following the Day 47 booster, neutralizing antibodies against the homologous rS administered on Day 47 increased to higher GMTs. For example, neutralizing GMTs for the group vaccinated with Wuhan rS in the primary series and boosted with Wuhan rS on Day 47, neutralizing antibody GMT against Wuhan S-pseudotyped virus increased from 9111 on Day 21 to 13912 on Day 61 but neutralizing antibody GMTs against heterologous BA.5 S pseudovirus was 373 and < 100 for other Omicron (BQ.1.1, CH.1.1, XBB.1.5, XBB.1.16, and

XBB.2.3) S-pseudotyped viruses. A similar pattern was evident among the groups primed with Wuhan rS in the primary series followed by booster doses of bivalent rS. For example, in the group that received a booster of bivalent Wuhan/XBB.1.5 rS after a Wuhan primary series, neutralizing antibody GMT against XBB.1.5 pseudovirus was 119 but 13736 against Wuhan pseudovirus. The treatment groups vaccinated with bivalent Wuhan/BA.5 in the primary series followed by boosters of monovalent rS or bivalent rS generated higher GMTs of more broadly neutralizing antibodies. For example, the group vaccinated with bivalent Wuhan/BA.5 rS in the primary series followed by a boost with XBB.1.5 rS on Day 47 had neutralizing antibody GMTs of > 4000 against pseudoviruses of Wuhan and all other Omicron variant pseudoviruses above except BA.2.86 where the neutralizing antibody GMT was 466 (LOD = 50).
In the evaluation of cell-mediated immune responses by intracellular cytokine staining, splenocytes (collected on Day 61) from mice inoculated with XBB.1.5 rS- or XBB.1.16 rS-containing booster on Day 47 were re-stimulated with various rS proteins (Wuhan, BA.5, BQ.1.1, XBB.1.5, or XBB.1.16) and tested for $CD4^+$ cells expressing Th-1 cytokines (IFNγ, IL-2 and TNF-α) and a Th-2 cytokine (IL-4). For the evaluation of $CD8^+$ response, splenocytes from the Day 47 XBB.1.5 rS- or XBB.1.16 rS-containing booster groups were re-stimulated with a peptide pool of Wuhan spike protein. The results show that $IFN\gamma^+$, $IL\text{-}2^+$ and $TNF\text{-}\alpha^+$ CD4 cells, as well as triple-staining ($IFN\gamma^+/IL\text{-}2^+/TNF\text{-}\alpha^+$) CD4 cells were detected at comparable numbers to $IL4^+$ $CD4^+$ cells, suggesting a balanced Th1/Th2 response. Similarly, across the groups boosted with XBB.1.5 rS- or XBB.1.16 rS-containing regimens on Day 47, the percentage of $CD8^+$ cells expressing IFNγ, IL-2 and TNF-α ranged from 3 – 10%, 1.5 – 6.5%, and 2.3 – 8.1%, respectively. Thus, XBB.1.5 rS induced both CD4- and CD8- T-cell responses when inoculated as a booster dose in mice.

Study 702-173
This study was originally initiated to evaluate the immunogenicity of the Omicron BA.5 variant rS in rhesus macaques (*Macaca mulatta*). Subsequently, new-variant rS vaccine constructs in the applicant's COVID-19 vaccine development program were incorporated into Study 702-173 for immunogenicity evaluation, including the XBB.1.5 rS vaccine. In the evaluation of the XBB.1.5 rS, rhesus macaques were inoculated with the human dose of 5 µg rS with 50 µg Matrix-M by prime/boost at an interval of 21 days. Macaques previously vaccinated with Wuhan rS, BA.5 rS, and a bivalent formulation (2.5 µg Wuhan rS + 2.5 µg BA.5 rS + 50 µg Matrix-M) received a booster dose with Omicron BQ1.1 rS or XBB.1.5 rS on Day 246 (i.e., about 8 months after the original primary series). Inoculation of a group of macaques with a primary series of monovalent XBB.1.5 rS was also initiated on Study Day 246, with the 2nd dose of adjuvanted XBB.1.5 rS administered on day 267. Serum samples obtained on Days 0, 21, 35, 210, and 260 were tested for antibody responses and PBMCs collected on Days 0, 35, 210, and 260 were tested for cell-mediated immune responses. IgG antibody responses against Wuhan, BA.1., BA.2, and BA.5 rS proteins were detected in all serum samples on Day 21, with significant increases in titers 2 weeks after the 2nd dose of the primary series, as well as high antibody responses against additional Omicron variant rS proteins (BA.4.6, BA.2.75, BQ.1.1, XBB, XBB.1.5, CH.1.1, XBB.1.16, and XBB.2.3). By Day 210, IgG titers had declined significantly across groups, but following a booster with Omicron BQ1.1 rS or XBB.1.5 rS Day 246, IgG antibody GMTs against Wuhan rS, and Omicron rS variants (BA.5, BQ.1.1, XBB.1.5, CH.1.1, XBB.1.16, and XBB.2.3) increased across the board by up to > 50-fold by Day 260. A similar trend was obtained for ACE2-binding inhibiting antibodies, with increases in GMTs on Day 260.
Neutralizing antibody titers in Day 21 serum samples were measured by the inhibition of live-virus infection of cells and formation of CPE. Serum samples from the bivalent rS

(Wuhan/BA.5 rS) treatment group had neutralizing antibody GMTs of 3265 and 13060 against live Wuhan (USA-WA1/2020) and Omicron BA.5, respectively. With the monovalent Wuhan rS and BA.5 rS, neutralizing antibody GMTs were strain biased. For example, macaques in the monovalent BA.5 rS group had a GMT of 41936 against the homologous SARS-CoV-2 Omicron BA.5 virus and a titer of 167.7 against the prototype USA-WA1/2020, and vice versa. The Day 35 serum samples were tested by pseudovirus neutralization assays. Serum samples against Wuhan rS neutralized the pseudovirus expressing the homologous Wuhan rS (GMT of 21280) but had low GMTs of < 100 against most Omicron variants (including XBB.1.5) except BA.2 (GMT of 370). Like the total IgG and ACE2-binding inhibiting antibodies, neutralizing antibody GMTs had waned across the board by Day 210 (the homologous Wuhan rS GMT of 757). Following the Day 247 booster with BQ.1.1 rS or XBB.1.5 rS neutralizing antibody titers increased, including neutralizing antibody GMTs of between 466 to 2529 against Omicron rS pseudoviruses in the group boosted with BQ.1.1 rS. Among macaques boosted with XBB.1.5, neutralizing antibody GMTs against other Omicron rS pseudoviruses were higher than those boosted with BQ.1.1 rS.

The T-cell response data show the induction of Th1 T-cell responses (IFNγ$^+$, IL-2$^+$ and TNF-α$^+$), with IL-2$^+$ and TNF-α$^+$ CD4 T-cell being predominant; induction of polyfunctional (IFNγ$^+$/IL-2$^+$/TNF-α$^+$) Th1 CD4 T-cells, irrespective of the rS antigen used in in vitro stimulation of PBMCs (including Wuhan rS), and comparable levels of Th2 (IL-5$^+$ and IL-13$^+$) cytokine-producing CD4 T cells.

Study 702-186
Naïve mice in groups of 10 were vaccinated prime/boost at an interval of 2 weeks. A set of 3 groups of mice received 0.1 µg of adjuvanted original rS, BA.2 rS, or XBB.1.5 rS, and a second set of 3 groups of mice received 1 µg of adjuvanted Original rS, BA.2 rS, or XBB.1.5 rS. Serum samples were collected on Study Day -1, 13, and 21 for antibody assays and spleen cells were collected on Study Day 21 for the evaluation of cell-mediated immune responses. The results show a dose-dependent antibody response, with the highest IgG titers induced against homologous rS.
IgG antibodies induced by XBB.1.5 rS had minimal cross-reactivity with Wuhan rS, and vice versa. The XBB.1.5 rS induced higher titers of cross-reacting antibodies against the rS proteins of Omicron BA.5, BN.1, BQ.1.1, XBB.1.16, and CH.1.1 than IgG induced by Wuhan rS or Omicron BA.2 rS. There was no cross-neutralization between Wuhan and XBB.1.5 antibodies, but XBB.1.5 induced modest levels of neutralizing antibodies against other Omicron variants (BA.2, BA.5, BQ.1.1, and CH.1.1), with the highest antibody titers against XBB.1.5 and XBB.1.16. The T-cell response shows a balanced Th1/Th2 CD4$^+$ T cell response, and the induction of polyfunctional effector T cells (IFN-γ$^+$/IL-2$^+$/ TNF-α$^+$) CD4 T cells.

Study 702-188
In this study, mice in groups of 20 were vaccinated prime/boost on Days 0 and 14, using 1 µg of adjuvanted Wuhan rS, BA.2 rS, BA.5 rS, XBB.1.5 rS and a bivalent formulation (0.5 µg Wuhan rS + 0.5 µg XBB.1.5 rS). Each dose was formulated with 5 µg Matrix-M. Pooled serum samples (from each treatment group), or 10 randomly selected serum samples collected on Study Days -3 (pre-vaccination), 13, and 21. The Day 21 serum samples were tested for antibody responses (total IgG, ACE2-binding inhibiting, and neutralizing). All treatment groups induced total IgG responses against Wuhan, and Omicron variants, with varying levels of IgG, but with antibody responses highest against homologous rS.

Similarly, ACE2 binding inhibiting antibodies were highest against homologous rS. For example, the ACE2 binding antibody titer against Wuhan rS in the group vaccinated with Wuhan rS was 2080, but at the LOD for most Omicron rS variants (BQ.1.1, CH.1.1, XBB.1.5, XBB.1.16, and XBB.2.3). The ACE2 binding inhibiting antibody induced by XBB.1.5 rS was highest against the homologous XBB.1.5 rS (titer = 634) and ranged from 143 (BA.2) to 730 (XBB.1.16) for other Omicron variants, but at LOD for Wuhan rS. The bivalent (Wuhan/XBB.1.5) vaccination group induced ACE2 binding inhibiting antibody against all rS variants tested (Wuhan, and Omicron BA.2, BA.5, BQ.1.1, CH.1.1, XBB.1.5, XBB.1.16, and XBB.2.3), with titers ranging from 166 (BQ.1.1) to 544 (XBB.1.16) for the Omicron variants, and the highest titer (1808) was against Wuhan rS.
Like the ACE2 binding inhibiting antibody response, the neutralizing antibody response, as determined by pseudovirus neutralization test, shows that the antibody response induced by XBB.1.5 did not neutralize Wuhan S-pseudotyped virus, and vice versa, it neutralized other Omicron variants at relatively lower titers (titer range, 85 to 572) against BA.2, BA.5, BQ.1.1, CH.1.1 but at higher titers against XBB-lineage Omicron variants. The neutralizing antibody titers against XBB.2.3, XBB.1.5, and XBB.1.16 were 2058, 4156, and 4554, respectively. Serum pool from the bivalent rS treatment group had neutralizing antibodies against all S pseudotypes, with titers ranging from 52 (CH.1.1) to 2748 (Wuhan).
In summary, data from Study 702-188 show that the XBB.1.5 rS induced antibody responses, including neutralizing antibodies, that neutralized pseudoviruses expressing the S protein of Omicron variants, with highest titers against XBB-lineage S proteins.

Nonclinical evaluation of BV2705 (JN.1 rS)
BV2705 was tested for immunogenicity in two nonclinical studies (Study 702-207 and Study 702-173).

Study 702-207
In Study 702-207, the immunogenicity of Omicron variant rS constructs, including BV2705 (JN.1 rS) was evaluated in female BALB/c mice. Mice were assigned to treatment groups (n = 10, 19 or 20/group) and vaccinated with various formulations of Omicron variant rS (1 µg) adjuvanted with 5 µg Matrix-M in a 2-dose primary series on study Days 0 and 14 (Table 43; Adapted from Table 1, document TR # 2023-56-702-207 v2.0, BLA 125817/0.42). On study Day 77, a booster dose of XBB.1.5 rS or JN.1 rS was administered to 4 of the treatment groups and on study Day 121, a booster dose of JN.1 rS or JN.1.11.1 rS was administered to 4 other treatment groups. Two treatment groups that received a primary vaccination with JN.1 rS or HV.1 rS did not receive additional booster doses.
Serum samples obtained from mice on study days 21, 77, and 90 were tested for S protein-specific IgG responses (50% Effective Concentration [EC_{50}]) against the prototype rS and Omicron JN.1-lineage rS proteins (XBB.1.5, HV.1, JN.1, JN.1.11.1) by ELISA. All treatment groups had high IgG GMTs against the S protein of Omicron variants (XBB.1.5, HV.1, JN.1, and JN.1.11.1) on Day 21, as well as against the prototype rS; antibody GMTs were highest against homologous antigens (Tables 44a & 44b; Adapted from Tables 3, 4, and 5, document TR # 2023-56-702-207 v2.0, BLA 125817/0.42). The IgG GMTs depreciated by 1 to 2-fold by Day 77. Following the Day-77 inoculation of XBB.1.5 rS or JN.1 rS boosters to groups 1, 2, 4, and 5, IgG GMTs appreciated across the board in the range of 4.3 to 18.6-fold. The Day-90

Table 43: Design of Study 702-207

Group (n)	rS type (1 µg) + 5 µg Matrix-M (Days 0 and 14)	rS type (1 µg) + 5 µg Matrix-M (Day 77)	Blood sampling timepoints (Day)[3]	Spleen collection; 6 mice/group (Day 90)
1 (20)	XBB.1.5 rS	XBB.1.5 rS	0, 13, 21, 77, 90	✓
2 (20)	XBB.1.5 rS	JN.1 rS	0, 13, 21, 77, 90	✓
3 (20)	XBB.1.5 rS	-	0, 13, 21, 77, 90[4]	-
4 (20)	[2]Wuhan rS + BA.5 rS + XBB.1.5 rS	XBB.1.5 rS	0, 13, 21, 77, 90	✓
5 (20)	[2] Wuhan rS + BA.5 rS + XBB.1.5 rS	JN.1 rS	0, 13, 21, 77, 90	✓
6a (10)	[2] Wuhan rS + BA.5 rS + XBB.1.5 rS	-	0, 13, 21, 77, 90[4]	-
6b (10)	[2] Wuhan rS + BA.5 rS + XBB.1.5 rS	-	0, 13, 21, 77, 90	-
7 (20)	XBB.1.5 rS	-	0, 13, 21, 77	-
8 (20)	JN.1 rS	-	0, 13, 21	-
9 (19)[1]	HV.1 rS	-	0, 13, 21	-

[1]A mouse died in quarantine prior to Study Day 0
[2]A dose of the trivalent formulation contained 1 µg each of Wuhan rS, BA.5 rS and XBB.1.5 rS, adjuvanted with 5 µg Matrix-M
[3]The antibody response data included in this report are through the Day 90 timepoint.
[4]Blood samples were obtained only from the 6 mice euthanized for the preparation of splenocytes.
n = Number of mice

serum samples from groups 1, 2, 4, and 5 (Table 43) were also tested for IgG GMTs against Omicron KP.2 rS. The GMTs of anti-KP.2 rS antigen in the 4 groups were relatively higher than any of the pre-boost GMTs measured on Day 77, with the JN.1 rS booster groups (groups 2 and 5) having 1.4 to 2.2-fold higher IgG GMTs against KP.2 than the groups treated with an XBB.1.5 rS boost (groups 1 and 4).

Table 44a. Day-21 IgG antibody response against JN.1 rS.

Group (n)	1° series (Day 0, 14)	Day 21 IgG GMT Wuhan rS antigen [95% CI]	Day 21 IgG GMT XBB.1.5 rS antigen [95% CI]	Day 21 IgG GMT JN.1 rS antigen [95% CI]	Day 21 IgG GMT JN.1.11.1 rS antigen [95% CI]	Day 21 IgG GMT XBB.1.5 rS antigen [95% CI]	Day 21 IgG GMT JN.1 rS antigen [95% CI]	Day 21 IgG GMT JN.1.11.1 rS antigen [95% CI]
1 (20)	XBB.1.5 rS	13605 [9382,19729]	90899 [71230,115999]	10722 [7178,16014]	9052 [6379,12846]	63272 [48332,82830]	9891 [7713,12684]	16080 [11822,21870]
2 (20)	XBB.1.5 rS	10393[2] [6478,47880]	59266 [47880,73358]	8738 [6323,12074]	27131 [5140,9894]	48907 [40678,58800]	10161 [7840,13168]	16956 [12717,22608]
4 (20)	Trivalent[1]	62077 [51663,74590]	58364 [47971,71008]	11834 [9804,14283]	9130 [6066,13742]	29259 [23698,36126]	8538 [6940,10505]	13764 [11038,17165]
5 (20)	Trivalent[1]	73337 [58482,91966]	65098 [50301,84249]	15546 [12494,19344]	16278 [12758,20770]	36508 [26789,49754]	10880 [8034,14733]	16425 [12107,22282]
8 (20)	JN.1 rS	3516 [2287,5406]	5273 [3871,7183]	114059 [86709,150035]	107641 [81173,142740]	N.A.	N.A.	N.A.
9 (19)	HV.1 rS	14432 [9633,21623]	65062 [49550,85429]	12026 [7625,18968]	12966 [8598,19553]	N.A.	N.A.	N.A.

Table 44b. Day-90 IgG antibody response against JN.1 rS.

Group (n)	1° series (Day 0, 14)	Day 77 Boost	Day 90 IgG GMT XBB.1.5 rS antigen [95% CI	Day 90 IgG GMT JN.1 rS antigen [95% CI]	Day 90 IgG GMT JN.1.11.1 rS antigen [95% CI]	Day 90 IgG GMT KP.2 rS antigen [95% CI]
1 (20)	XBB.1.5 rS	XBB.1.5 rS	272934 [223153,333821]	78549 [63307,97461]	112362 [90738,139140]	79654 [66103,95984]
2 (20)	XBB.1.5 rS	JN.1 rS	358284 [302938,423742]	159044 [132252,191264]	253548 [201029,319786]	176929 [148653,210583]
4 (20)	Trivalent[1]	XBB.1.5 rS	224487 [181185,278138]	74322 [59715,92502]	91710 [74961,112202]	65927 [53168,81749]
5 (20)	Trivalent[1]	JN.1 rS	237403 [188959,298265]	115382 [85411,155869]	165213 [126769,215317]	94017 [70527,125330]

[1]A dose of the trivalent formulation contained 1 µg each of prototype rS, BA.5 rS and XBB.1.5 rS, adjuvanted with 5 µg Matrix-M
[2]A serum sample was excluded due to depleted sample volume.
N.A. = Not Applicable, group was terminated after the primary series; GMT = Geometric Mean Titer; CI = Confidence Interval; 1° series = Primary series

Antibody responses induced by XBB.1.5 rS, HV.1 rS, or the trivalent formulation (Wuhan rS + BA.5 rS + XBB.1.5 rS) neutralized pseudoviruses expressing the respective homologous S protein but had no neutralizing antibodies (or had low titers near the LOD) against pseudoviruses expressing the S protein of JN.1-lineage Omicron variants (Tables 45a, 45b, & 45c; Adapted from Tables 6, 7, and 8, document TR # 2023-56-702-207 v2.0, BLA 125817/0.42).

Table 45a. Day-21 Neutralizing antibody response against JN.1 rS.

Group (n)	1° series (Day 0, 14)	Day 21 pVN_{50} GMT Wuhan S pV	Day 21 pVN_{50} GMT XBB.1.5 S pV [95% CI]	Day 21 pVN_{50} GMT HV.1 S pV [95% CI]	Day 21 pVN_{50} GMT JN.1 S pV [95% CI]	Day 21 pVN_{50} GMT JN.4 S pV [95% CI]	Day 21 pVN_{50} GMT JN.1.11.1 S pV [95% CI]	Day 21 pVN_{50} GMT JN.1.7 S pV [95% CI]	Day 21 pVN_{50} GMT JN.1.13.1 S pV [95% CI]
1 (20)	XBB.1.5 rS	50 [50, 50]	4921 [3492, 6934]	1218 [834.8, 1776]	50 [50, 50]	NT	57.4 [46.5, 70.9]	50.7 [49.3, 52.2]	NT
2 (20)	XBB.1.5 rS	50[2] [50, 50]	5680 [3929, 8211]	1109 [736.3, 1670]	61.4 [46.5, 81]	51.4[2] [48.3, 54.7]	58.3[3] [46.6, 72.9]	61.4[3] [45.6, 82.8]	59.3[2] [40.3, 87.3]
4 (20)	Trivalent[1]	NT	3442 [2285, 5184]	739.1 [455.1, 1200]	57.5 [43.3, 76.2]	NT	51.5 [48.5, 54.7]	52.4 [47.5, 58]	NT
5 (20)	Trivalent[1]	3709[2] [1502, 9158]	5556 [3501, 8815]	1887 [1142, 3120]	51.2 [48.7, 53.9]	50[2] [50, 50]	56.1 [49, 64.3]	53.4 [48.6, 58.6]	NT
8 (20)	JN.1 rS	50[2] [50, 50]	50.4 [49.6, 51.1]	56 [44.2, 70.9]	11215 [6640, 18944]	7185 [3352, 15404]	7650 [4491, 13029]	7001 [4852, 10103]	14583 [8870, 23976]
9 (19)	HV.1 rS	50[2] [50, 50]	2495 [1425, 4369]	3223 [1979, 5249]	77.1 [45.5, 130.5]	120.1[2] [42.9, 336.6]	104.5 [54, 202.4]	72.2 [45, 116]	67.4 [43.9, 103.3]

Table 45b. Day-77 Neutralizing antibody response against JN.1 rS.

Group (n)	1° series (Day 0, 14) series	Day-77 Boost	Day 77 pVN_{50} GMT XBB.1.5 S pV [95% CI]	Day 77 pVN_{50} GMT HV.1 S pV [95% CI]	Day 77 pVN_{50} GMT JN.1 S pV [95% CI]	Day 77 pVN_{50} GMT JN.4 S pV [95% CI]	Day 77 pVN_{50} GMT JN.1.11.1 S pV [95% CI]	Day 77 pVN_{50} GMT JN.1.7 S pV [95% CI]
1 (20)	XBB.1.5 rS	XBB.1.5 rS	3537 [2438, 5132]	1367 [886, 2109]	57.2 [49.7, 65.8]	NT	56.9 [48.2, 67.1]	54.5 [45.9, 64.7]
2 (20)	XBB.1.5 rS	JN.1 rS	4614 [3393, 6273]	2004 [1212, 3313]	59 [44.7, 77.9]	NT	58.9 [48.1, 72]	58.8 [47.9, 72.2]
4 (20)	Trivalent[1]	XBB.1.5 rS	3375 [2220, 5132]	1559 [980.8, 2479]	83.8 [54.5, 128.9]	66.6[2] [47.3, 93.6]	61 [46.8, 79.5]	68.1 [50.3, 92.1]
5 (20)	Trivalent[1]	JN.1 rS	3625 [1915, 6863]	2185 [1165, 4097]	74.8 [53.2, 105.1]	65.5[2] [39.8, 107.8]	91.2 [51.2, 162.4]	99.3 [62.9, 156.9]

Table 45c. Day-90 Neutralizing antibody response against JN.1 rS.

Group (n)	1° series (Day 0, 14) series	Day-77 Boost	Day 90 pVN_{50} GMT XBB.1.5 S pV [95% CI]	Day 90 pVN_{50} GMT HV.1 S pV [95% CI]	Day 90 pVN_{50} GMT JN.1 S pV [95% CI]	Day 90 pVN_{50} GMT JN.1.11.1 S pV [95% CI]	Day 90 pVN_{50} GMT JN.1.7 S pV [95% CI]	Day 90 pVN_{50} GMT JN.1.13.1 S pV [95% CI]
1 (20)	XBB.1.5 rS	XBB.1.5 rS	37885 [19709, 72824]	30178 [15330, 59407]	447.1 [199.6, 1001]	588.1 [251.7, 1374]	487.1 [119.6, 1189]	NT
2 (20)	XBB.1.5 rS	JN.1 rS	46668 [28558, 76262]	31899 [17535, 58028]	2839 [1323, 6090]	2204 [1044, 4653]	2118 [1050, 4272]	2131 [876.8, 5178]
4 (20)	Trivalent[1]	XBB.1.5 rS	41901 [27662, 63469]	18341 [11914, 28234]	153.6 [103.3, 228.5]	231.6 [128.6, 417.3)	221.8 [133.3, 369.0]	NT
5 (20)	Trivalent[1]	JN.1 rS	30035 [19731, 45720]	18341 [10758, 31268]	1219 [684.4, 2171]	1836 [923.6, 3650]	1434 [801.4, 2565]	NT

[1]A dose of the trivalent formulation contained 1 µg each of Wuhan rS, BA.5 rS and XBB.1.5 rS, adjuvanted with 5 µg Matrix-M
[2]GMT was calculated from the data for 10 serum samples.
[3]A serum sample was excluded due to depleted sample volume.
NT = Not Tested; GMT = Geometric Mean Titer; CI = Confidence Interval; 1° series = Primary series

Serum samples from this study were tested for the neutralization of pseudoviruses expressing the S protein of newly emerged JN.1-lineage variants, including KP.2 and KP.3. For this purpose, the Day-21 serum samples from groups 2 and 8 and the Day-90 serum samples from group 2 (Table 43) were tested for neutralizing antibodies against pseudoviruses expressing the S protein of Omicron variants JN.1.16 (only group 8 sera), KQ.1, KP.2, KP.1.1, KP.3, and LA.2. The results show that the Day-21 serum samples from naïve mice that were vaccinated with the primary series of JN.1 rS (group 8) neutralized all JN.1-lineage variants, including KP.2 and KP.3, to higher GMTs when compared to Day-21 serum samples obtained after a primary series of XBB.1.5 rS (group 2) (Table 46; Adapted from Tables 6, 7, and 8; document TR # 2023-56-702-207 v2.0, BLA 125817/0.42). Neutralizing antibody levels were at baseline on Day 77 but increased significantly by Day-90 following a booster dose of JN.1 rS on Day 77.

Table 46. Neutralizing antibody against JN.1-lineage variants.

Group (n)	Treatment	Day-21 pVN_{50} GMT JN.1.16 S pV [95% CI]	Day-21 pVN_{50} GMT KQ.1 S pV [95% CI]	Day-21 pVN_{50} GMT KP.2 S pV [95% CI]	Day-21 pVN_{50} GMT KP.1.1 S pV [95% CI]	Day-21 pVN_{50} GMT KP.3 S pV [95% CI]	Day-21 pVN_{50} GMT LA.2 S pV [95% CI]
2 (20)	XBB.1.5 rS (Day 0, 14)	NT	64.2[1] [42.6, 96.7]	70.9[1] [38.2, 131.4]	64.8[1] [42.2, 99.5]	60.0[2] [39.0, 92.1]	60.3[2] [40.4, 90.0]
8 (20)	JN.1 rS (Day 0, 14)	8139[1] [5192, 12761]	2868[1] [1829, 4497]	2707[1] [1501, 4881]	4248[1] [2211, 8164]	2028[2] [1140, 3605]	3229[2] [1841, 5666]

Group (n)	Treatment	Day-77 pVN_{50} GMT JN.1.16 S pV [95% CI]	Day-77 pVN_{50} GMT KQ.1 S pV [95% CI]	Day-77 pVN_{50} GMT KP.2 S pV [95% CI]	Day-77 pVN_{50} GMT KP.1.1 S pV [95% CI]	Day-77 pVN_{50} GMT KP.3 S pV [95% CI]	Day-77 pVN_{50} GMT LA.2 S pV [95% CI]
2 (20)	XBB.1.5 rS (Day 0, 14)	NT	62.0[3] [48.5, 79.2]	62.4[3] [49.6, 78.4]	70.3[3] [44.1, 112.1]	50[2] [50, 50]	50[2] [50, 50]

Group (n)	Treatment	Day-90 pVN_{50} GMT JN.1.16 S pV [95% CI]	Day-90 pVN_{50} GMT KQ.1 S pV [95% CI]	Day-90 pVN_{50} GMT KP.2 S pV [95% CI]	Day-90 pVN_{50} GMT KP.1.1 S pV [95% CI]	Day-90 pVN_{50} GMT KP.3 S pV [95% CI]	Day-90 pVN_{50} GMT LA.2 S pV [95% CI]
2 (20)	XBB.1.5 rS (Day 0, 14) JN.1 rS (Day 77)	927.7[3] [323.1, 2664]	1496[3] [613.8, 3648]	1713[3] [463.5, 6333]	1317[3] [440.7, 3939]	2357[2] [783.1, 7096]	2960[2] [782.6, 11195]

[1]Data are missing from 1 animal due to depleted serum volume.
[2]GMT was calculated from n = 8 animals.
[3]GMT was calculated from n = 10 animals.

Thus, the antibody response induced by JN.1 rS given as a 2-dose primary series in naïve mice or as a booster dose in mice previously vaccinated with the rS of earlier SARS-CoV-2 strains neutralized JN.1-lineage Omicron variants, including the KP.2 and KP.3 variants. Antibodies induced by XBB.1.5 rS did not neutralize pseudoviruses expressing JN.1-lineage spike proteins.

On Day 90, six mice from each of groups 1, 2, 4, and 5, were euthanized, splenocytes were isolated and re-stimulated with rS antigens for the evaluation of $CD4^+$ T-cell response (cytokine-secretion profile) by intracellular-cytokine staining. Consistent with results from earlier non-clinical studies, the $CD4^+$ T-cell response data suggest that re-stimulation of splenocytes with homologous or heterologous rS induced similar levels of Th1 cytokines, as well as similar levels of Th2 $CD4^+$ cells.

Th-1 $CD8^+$ T-cell responses were evaluated using splenocytes that were re-stimulated with peptide pools of the XBB.1.5 S protein. The results show high numbers of $CD8^+$ cells expressing Th1 type cytokines (IFN-γ^+, IL2$^+$, and TNF-α^+) (Table 47; Adapted from Table 16, document TR # 2023-56-702-207 v2.0, BLA 125817/0.42).

Table 47. Th-1 $CD8^+$ T-cell responses (Day 90)

Group (n)	1° series (Days 0, 14)	Booster dose	GMT of IFN-γ^+ cells/10^6 $CD8^+$ cells [95% CI]	GMT of IL-2^+ cells/10^6 $CD8^+$ cells [95% CI]	TNF-α^+ cells/10^6 $CD8^+$ cells [95% CI]
1 (6)	XBB.1.5 rS	XBB.1.5 rS	132490	99393	124542

			[85922, 204295]	[70868, 139399]	[82840, 187235]
2 (6)	XBB.1.5 rS	JN.1 rS	146973 [89897, 240284]	93409 [57491, 151767]	141881 [99190, 202946]
4 (6)	Trivalent[1]	XBB.1.5 rS	172600 [118589, 251209]	90993 [70733, 117056]	141881 [99190, 202946]
5 (6)	Trivalent[1]	JN.1 rS	199693 [172567, 231083]	110324 [92529, 131542]	165312 [140615, 194346]

Regarding Tfh and germinal-center B cells, the geometric mean percent (GM%) of Tfh cells detected were 0.37% and 0.27% for the XBB.1.5 rS primary series/XBB.1.5 rS boost and trivalent rS primary series/XBB.1.5 rS boost treatment groups, respectively. Similarly, the GM% of germinal center B cells in these treatment groups were 0.54% and 0.56%, respectively. In the groups boosted with JN.1 rS, the GM% of Tfh cells detected were 0.39% and 0.34% in the XBB.1.5 rS primary series/JN.1 rS boost and trivalent rS primary series/JN.1 rS boost, respectively, and the GM% of germinal center B cells were 0.88% and 0.78%, respectively.
In summary, the Omicron JN.1 rS induced antibody and cell-mediated immune responses in mice. The antibody responses induced by JN.1 rS neutralized pseudoviruses expressing the spike proteins of JN.1-lineage Omicron variants.

The JN.1 rS was further tested in rhesus macaques by injecting animals in Study 702-173 with the human dose of JN.1 rS (5 µg JN.1 rS with 50 µg Matrix-M) as a booster 5 months after the boost with XBB.1.5 rS (see Study 702-173 above). Serum samples obtained from macaques 14 days after the XBB.1.5 rS primary series, and at 6 months prior to the XBB.1.5 rS booster, as well as at 11 months prior to the JN.1 rS booster and 14 days after the JN.1 booster, were tested for antibody responses. The 50% GMT of pseudovirus neutralizing antibody titers (pVN_{50}) with 95% Confidence Interval (CI) in parenthesis are summarized in Table 48 (Adapted from Tables 35 – 39, document TR # 2022-44-702-173 v3.0, BLA 125817/0.42). High titers of neutralizing antibodies against pseudoviruses expressing the S protein of HV.1, and other JN.1lineage (JN.1.11.1, JN.1.7, and JN.1.13.1) were also measured in the Day 371 sera. In antigenic cartography analysis of the pseudovirus neutralizing antibody data, the antigenic distance of neutralizing antibody titers against pseudoviruses expressing the S protein of JN.1-lineage Omicron variants relative to the titer against JN.1 ranged from 0.55 to 1.47, suggesting a close antigenic space to JN.1, and the potential for cross-neutralization of JN.1-lineage variants.

Table 48. Neutralizing antibody **GMT (pVN_{50})** in macaques after JN.1 rS booster

Group Treatment (n = 5)	**Test serum collection day**	**XBB.1.5 S pV**	**JN.1 S pV**	**JN.1.16 S pV**	**KP.2 S pV**	**KQ.1 S pV**	**KP.1.1 S pV**	**KP.3 S pV**	**LA.2 S pV**
XBB.1.5 rS (Day 0, 21)	35	5157 [1732, 15353]	231.3 [28.2, 1897)]	ND	ND	ND	ND	ND	ND
XBB.1.5 rS (Day 0, 21)	204 (pre-XBB.1.5 rS boost)	307.3 [170.1, 555.2]	55.3 [41.9, 72.9]	ND	ND	ND	ND	ND	ND
XBB.1.5 rS (Day 0, 21, and 211),	225 (post-XBB.1.5 rS boost)	17720 [7316, 42919]	1865 [829.2 , 4195]	ND	2413 [1128 , 5159]	1372 [527. 5, 3569]	1283 [872.4, 1887]	ND	ND

XBB.1.5 rS (Day 0, 21, and 211),	357 (pre-JN.1 rS boost)	1880 [997.1, 3545]	125.8 [41.7, 379.5]	97.1 [36.5, 258.5]	168.1 [53.5, 528.3]	143.3 [48.9, 420.7]	116.8 [34.8, 392.3]	138.5 [35.7, 537.9]	91.9[39.5, 214.0]
XBB.1.5 rS (Day 0, 21, and 211) + JN.1 rS (Day-357)	371 (post-JN.1 rS boost)	13394 [7609, 23577]	5081 [2217, 11643]	2932 [1408, 6105]	2468 [1287, 4736]	3188 [1891, 5375]	1875 [1207, 2913[	1841 [867.7, 3904]	2931 [1903, 4513]

ND = No Data; S = SARS-CoV-2 Spike protein; PV = Pseudovirus; GMT = Geometric Mean Titer; CI = Confidence Interval.

Peripheral blood mononuclear cells collected before (Day 302) and after (Days 371 and 408) the administration of JN.1 rS booster were tested for cell-mediated immune responses by intracellular cytokine staining for $CD4^+$ cells expressing Th1 cytokines (IFN-γ, IL-2, TNF-α and triple-staining cells), and Th2 cytokines (IL5 and IL13) upon *in vitro* re-stimulation with different rS proteins. Consistent with data from the mouse study, irrespective of the rS antigen (Wuhan, XBB-lineage, or JN.1-lineage) used in re-stimulating PBMCs, all vaccination groups elicited a Th1-biased (IFN-γ^+, IL-2^+, and TNF-α^+) $CD4^+$ T-cell response, with comparable numbers of cells in each treatment group expressing different cytokines per million cells. For example, the average number of polyfunctional (triple-staining; IFN-γ^+/IL-2^+/TNF-α^+) $CD4^+$ T cells in the Day 408 PBMCs was 424, 383, 288, and 304 per million cells, upon re-stimulation with XBB.1.5 rS, JN.1 rS, JN.1.13.1 rS, and KP.2 rS, respectively. IL-5 and IL-13-expressing $CD4^+$ T cells (Th2 response) were also detected across the board, but at relatively lower cell numbers than $CD4^+$ cells expressing Th1 cytokines, suggesting the induction of a predominantly Th1 response.

4.2.2 Pharmacokinetics

Nonclinical pharmacokinetic studies of the SARS-CoV-2 rS antigen in animals were not conducted. However, the applicant assessed the biodistribution of Matrix-M adjuvant in mice. The Matrix-M biodistribution study is covered in (b) (6) review memo.

Reviewer's Overall Assessment of Relevant Sections of Module 4:

In the course of development of COVID-19 Vaccine, Adjuvanted, the applicant conducted a series of nonclinical studies aimed primarily at demonstrating the immunogenicity of recombinant SARS-CoV-2 spike proteins as candidate vaccines. In preliminary studies conducted in mice with the Wuhan rS, it is apparent that formulation of the candidate rS with Matrix-M adjuvant induced superior antibody responses, including neutralizing antibodies than unadjuvanted rS. The studies also showed a dose response to rS, with antibody responses increasing with increase in the amount of rS. In hamsters and non-human primates, the adjuvanted Wuhan rS protected animals from weight loss and virus clearance in immunized animals was more rapid compared to unimmunized (placebo-treated) control animals. In animals vaccinated with low doses (i.e., 0.01 µg, 0.1 µg or 1 µg) of Wuhan rS prior to challenge with SARS-CoV-2, no evidence of exacerbation of disease was found in necropsy and histopathology, thus alleviating concerns about potential occurrence of vaccine-induced enhanced disease in vaccine recipients upon subsequent exposure to SARS-CoV-2. As data show that the antibody response against Wuhan rS did not neutralize SARS-CoV-2 Omicron variants, recombinant S protein constructs based on the predominant contemporary SARS-CoV-2 variants were constructed and evaluated in animals. Thus, the XBB.1.5 rS, the active

ingredient the 2023-2024 Formula, and the JN.1 rS, the active ingredient in the 2024-2025 Formula were constructed and tested for immunogenicity in animals. Like the Wuhan rS, the XBB.1.5 rS and the JN.1 rS induced spike-specific IgG antibodies, ACE2 binding inhibiting antibodies, and neutralizing antibodies in animals. The induced antibody responses are strain-specific and/or lineage-specific. Thus, the antibody response induced by JN.1 rS neutralized most of the pseudoviruses expressing the spike proteins of SARS-CoV-2 Omicron variants of the JN.1 lineage but did not neutralize variants of the Omicron XBB.1.5 lineage, and vice versa. Splenocytes from vaccinated mice of PBMCs from non-human primates contain Th1 cytokine-producing CD4 cells detected by ELISpot or intracellular cytokine staining after in vitro re-stimulation with Wuhan rS or rS of various Omicron variants, and appear to be non-specific to the homologous immunizing rS.

The nonclinical pharmacology studies were not strictly compliant with good laboratory practice but were designed and executed with good scientific principles. In addition, the immunogenicity assays are not validated because of challenges in validating nonclinical immunogenicity assays (e.g., insufficient volume of serum samples from small animals). However, the SOPs for total IgG ELISA, ACE2 binding inhibition assay, and pseudovirus neuralization assay are similar to the clinical immunogenicity assays which are validated. Overall, data from studies in different animal species showed that the rS of SARS-CoV-2 (Wuhan, variants, and Omicron subvariants) formulated with Matrix-M adjuvant induce a broad spectrum of immune responses, including binding antibodies, ACE2-binding-inhibiting antibodies, and neutralizing antibodies, as well as T-cell responses with a dominant Th1-type $CD4^+$ T-cell responses, including polyfunctional effector T cells. While data from the non-clinical evaluation of candidate rS may not translate to a direct assessment of clinical benefits, they provide preliminary evidence of potential clinical benefits and allow for the initiation of clinical studies to evaluate potential clinical benefits of candidate COVID-19 vaccines in protecting against SARS-CoV-2 morbidity and mortality in humans.

5.3.1 Reports of Biopharmaceutic Studies

Clinical assays used to support the Clinical Efficacy Endpoints

The clinical assays align to the clinical data that was included in the clinical memo from studies 301 (Microneutralization (MN) Assay for Wuhan), 311 Part 1 (MN BA.1) and 2 (Pseudotype Virus Neutralization Assay (PVNA) for BA.5 and XBB1.5) and study 313 part 1 (PVNA for XBB.1.5) and 2 (PVNA for XBB.1.5) in support of the BLA approval.

All the assays used to support the approval of the BLA have been adequately validated and appropriate for their intended purpose. A summary of the assays used and the results from their validation reports is provided below.

A. Immunogenicity Assays to quantify SARS CoV-2 neutralizing antibodies (nAbs) in human sera following vaccination

1. Microneutralization (MN) Assay

The assay was assay was previously validated and reviewed to support the original EUA 28237, approved on July 13, 2022, and validation information was included in the CMC memo from (b) (6) uploaded in CBER connect on July 8, 2022. Additionally, two memos were uploaded for Novavax IND 22430 Amendment 90 and 127 that also

contain complete information on the assay validation data, comments to the applicant and their responses.

(b) (4)



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

(b) (4)



B. Diagnostic Assays to determine the serostatus and determine SARS CoV-2 infection

1. Abbott's SARS-CoV-2 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

The assay was previously validated and reviewed to support the original Emergency Use Authorization (EUA) 28237, approved on July 13, 2022. Complete assay validation information was included in the CMC memo from (b) (6) uploaded in CBER connect on July 8, 2022.

(b) (4)



(b) (4)

2. Roche's Elecsys Anti-SARS-CoV-2 N antibody assay

Similarly, the Roche's Elecsys antibody assay was previously validated and reviewed to support EUA 28237, approved on July 13, 2022. Validation information was included in the CMC memo from (b) (6) uploaded in CBER connect on July 8, 2022.

The assay is an EUA-approved antibody-based assay that targets the qualitative detection of antibody to the nucleocapsid (N) protein of SARS-CoV-2 in clinical serum and plasma samples and was used in determining the SARS-CoV-2 serostatus of study subjects. The kit is an electrochemiluminescence immunoassay that can be run on different cobas immunoassay analyzers (b) (4), 601, 602 and 801). This antibody assay was also employed at the (b) (4) for the diagnosis of SARS-CoV-2 infection to support the pivotal Phase 3 clinical study of the vaccine. A cutoff index (COI) of (b) (4) is considered non-reactive (i.e., sample is negative for SARS-CoV-2 antibody) and a COI (b) (4) is considered to be reactive (positive).

A validation/verification study report on the (b) (4) was submitted in IND 22430/122 and reviewed and found to be acceptable. During validation, assay Linearity, Range, Accuracy, Precision, and diagnostic sensitivity and specificity were assessed.

All of the pre-defined validation parameters were met and therefore the assay was deemed suitable to be used for its intended purpose of determining the serostatus of the participants in the Pivotal Phase 3 study.