Protocol Amendment 4

Study ID: 208109

Official Title of Study: PProtocol Title: A Phase I, Single-center, Randomized, Observer-blind, Placebo-controlled Study to Evaluate Safety, Reactogenicity and Immunogenicity of GSK's Clostridium difficile Investigational Vaccine Based on the F2 Antigen With or Without AS01B Adjuvant, When Administered Intramuscularly According to a 0, 1-month Schedule to Healthy Adults Aged Between 18-45 Years and Between 50-70 Years, Followed by an Additional Dose Administered in a Partial blind Manner Within an Interval of Approximately 15 Months After Dose 2, in a Subcohort of Subjects aged 50-70 Years

NCT number:

Date of Document: 06 Dec 2021

TITLE PAGE

Protocol Title: A Phase I, Single-center, Randomized, Observer-blind, Placebo-controlled Study to Evaluate Safety, Reactogenicity and Immunogenicity of GSK's *Clostridium difficile* Investigational Vaccine Based on the F2 Antigen With or Without $AS01_B$ Adjuvant, When Administered Intramuscularly According to a 0, 1-month Schedule to Healthy Adults Aged Between 18-45 Years and Between 50-70 Years, Followed by an Additional Dose Administered in a Partial blind Manner Within an Interval of Approximately 15 Months After Dose 2, in a Subcohort of Subjects aged 50-70 Years

Protocol Number: 208109 (CDIFF-004)

Amendment Number: Amendment 04

Product: GlaxoSmithKline Biologicals SA (GSK) *Clostridium difficile* investigational vaccine based on the F2 antigen (GSK2904545A)

Short Title: Safety and Immunogenicity Study of GSK's *Clostridium difficile* Vaccine 2904545A When Administered in Healthy Adults Aged 18-45 Years and 50-70 Years

Study Phase: I

Sponsor Name: GlaxoSmithKline Biologicals SA

Legal Registered Address: GlaxoSmithKline Biologicals SA, Rue de l'Institut, 89, 1330 Rixensart, Belgium

Regulatory Agency Identifying Number(s): EudraCT number: 2018-002304-14

Date of Protocol: 02 December 2021

Sponsor Signatory:

I have read this protocol in its entirety and agree to conduct the study accordingly:

Juan Pablo Yarzabal Rodriguez **Clinical and Epidemiology Project Lead (CEPL)**	**Date**

Medical Monitor name and contact information can be found in Appendix 3.

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

Table 1 Document History

Document	Date	Substantial	Region
Amendment 04	02 December 2021	No	Global
Amendment 03	18 May 2021	Yes	Global
Amendment 02	16 April 2020	Yes	Global
Amendment 01	12 December 2019	Yes	Global
Original Protocol	02 May 2019	-	-

Amendment 04 (02 December 2021)

This amendment is considered non-substantial based on the criteria defined in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union because it neither impacts the safety or physical/mental integrity of subjects nor the scientific value of the study.

Overall Rationale for the Current Amendment:

The CDIFF-004 (208109) study protocol was amended on 18 May 2021 (Protocol Amendment 03, approved by FAMHP on 25 August 2021) to introduce a third study vaccine dose, to be offered in a subcohort of subjects recruited in Step 4. Following approval of this amendment, the Sponsor would like to update accordingly one of the protocol exclusion criteria related to the lapse to consider, in case of administration of Shingrix. The update to be considered concerns the exclusion criterion 9 of the protocol which should state that the planned administration of GSK's Herpes Zoster vaccine marketed as Shingrix or an adjuvanted recombinant varicella zoster virus envelope gE subunit vaccine (HZ/su) should not occur within 180 days before the first dose and within 180 days after the last dose of the study vaccine (instead of the second dose, as written in Protocol Amendment 03).

The opportunity was also taken to do minor editorial corrections for typographical, grammar, and consistency errors.

Table 2 Description of Changes in Amendment 04

Section # and Name	Description of Change	Brief Rationale
5.2 Exclusion Criteria 9	It was clarified that the planned administration of GSK's Herpes Zoster vaccine marketed as Shingrix or an adjuvanted recombinant varicella zoster virus envelope gE subunit vaccine (HZ/su) within 180 days before the first dose and 180 days after the last dose was not allowed.	Updated the information to be in line with the additional third dose administration
Sponsor Signatory	Jeanne Marie Devaster replaced with Juan Pablo Yarzabal Rodriguez	To mention the name of current signatory

Amendment 03 (18 May 2021)

This amendment is considered substantial based on the criteria defined in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union because it significantly impacts the safety of subjects and/or the scientific value of the study.

Overall Rationale for the Current Amendment:

Results from a recent interim analysis on data from subjects immunized with 2 doses of the F2 antigen candidate (with or without the inclusion of an adjuvant), showed an acceptable safety profile for both formulations. Analysis on immunogenicity showed that an anti-toxin (A/B) neutralizing antibodies (TNA) response can be elicited by the F2 adjuvanted formulation. However, a lower immune response post second dose was observed for anti Tox B TNA in subjects who had no quantifiable antibodies at baseline, indicating that a third vaccine dose may be necessary to reach higher level of antibodies.

Published data from a Phase II study with another *Clostridium difficile* candidate[1], using full Toxin A and B aluminum-based toxoids, showed similar trend in toxin neutralization assay values post second dose, also evidencing that a third dose induced very high response in seronegative subjects.

The aim of this amendment is to offer a third dose of the vaccine to subjects recruited in the active groups of the last step of the study, to demonstrate if a similar kinetics in terms of anti-Toxin A/B TNA can be achieved with the F2 antigen formulation with or without an adjuvant. The purpose of this amendment is also to assess the safety and immunogenicity following the addition of a third dose in a subcohort of subjects aged 50-70 years, recruited in the Step 4 of the study.

The details of Clinical Laboratory Sciences, Marburg were deleted from the protocol, as it would no longer be used for the study purposes.

The COVID-19 guidance was updated to mention the most recent version applicable at the time of study conduct and the reporting period for pregnancies was updated to 24 hours from 2 weeks.

The opportunity was also taken to do minor editorial corrections for typographical, grammar, and consistency errors.

Table 3 Description of Changes in Amendment 03

Section # and Name	Description of Change	Brief Rationale
Title Page 1.1 Synopsis 1.2 Schema 4.1 Overall Design 6.1 Study Treatment(s) Administered 9.4.2.1 Primary Analyses	Added information about an additional third dose to be given to subjects on active treatment	To provide the information related to the proposed third dose
1.1 Synopsis 8.11 Study Procedures During Special Circumstances	Updated version of EMA Guidance on the Management of Clinical Trials during the Coronavirus pandemic.	To indicate the recent version, applicable to the study conduct
1.1 Synopsis 4.1 Overall Design 9.4.2.2 Internal Safety Review Committee Evaluations 9.7 Internal Safety Review Committee	Clarified that an ad hoc iSRC meeting may happen in case of any safety concerns during Dose 3	To protect subject's welfare and safety
1.1 Synopsis 6.6.3 Blinding 9.5 Interim Analyses 9.8 Sequence of Analysis	Added information about second interim analyses	Added information about an additional analysis related to the third dose
1.2 Schema 1.3 Schedule of Activities 3.0 Objectives and Endpoints 8.3 Adverse Events 8.8.2 Immunological Read outs Appendix 4	Added information about additional days when the study assessments will be performed.	Added information about additional assessments related to the third dose
4.1 Overall Design Appendix 2	Updated details of duration of study and Primary Completion Date Added definition of Study Conclusion for Individual Subjects	Updated the information to be in line with the additional study processes related to the third dose administration
4.1 Overall Design 4.4 End of Study Definition Appendix 2	Added definition of study conclusion for individual subjects.	Updated the information to be in line with the additional study processes related to the third dose administration
4.2.1 Rationale of Study Blinding 6.6.3 Blinding	Added details of partial blind.	Updated the information to be in line with the additional study processes

Section # and Name	Description of Change	Brief Rationale
Appendix 2		related to the third dose administration
4.2.2 Rationale for Randomization and the Use of Placebo	Added the rationale for excluding the subjects receiving placebo, from the third dose	Since the additional dose administration is related to the demonstration of kinetics in terms of anti-Toxin A/Toxin B, the third dose will not be given to the subjects receiving placebo
4.3 Justification of Dose	Added justification of third dose	Clarified why the additional dose is to be administered
5.2 Exclusion Criteria	Updated information for administration of a vaccine not foreseen by the study protocol	To protect subject's welfare and safety
5.4 Screen Failures	Updated the information for the screen failures related to Dose 3	To differentiate between the screen failures related to previous 2 doses and the third dose
6.8.1 Recording of Concomitant Medications/Products and Concomitant Vaccinations 6.8.2 Concomitant Medications/Products/Vaccines That may Lead to the Elimination of a Subject From Per protocol Analyses	Added details for concomitant medications during Dose 3	To protect subject's welfare and safety
9.3 Populations for Analyses	Added information about analysis sets related to the Dose 3	Updated the information to be in line with the additional study processes related to the third dose administration
Appendix 2	Updated definition of End of Study	For clarification
Appendix 5	Deleted the details of Clinical Laboratory Sciences, Marburg	This laboratory is no longer needed in the study
Appendix 6	Updated the time period for collecting and recording of adverse events	Updated the information to be in line with the additional study processes related to the third dose administration
Appendix 6 Appendix 7	Updated reporting period for pregnancies from 2 weeks to 24 hours	The reporting period was updated in lines of the recent version of the applicable guidelines

Amendment 02 (16 April 2020)

This amendment is considered substantial based on the criteria defined in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union because it significantly impacts the safety of subjects and/or the scientific value of the study.

Overall Rationale for the Current Amendment:

This protocol Amendment 2 outlines measures that may be applicable during special circumstances (eg, COVID-19 pandemic). The purpose of the amendment is to protect subject's welfare and safety, and as far as possible ensure the potential benefit to the subject and promote data integrity.

Table 4 Description of Changes in Amendment 02

Section # and Name	Description of Change	Brief Rationale
1.1 Synopsis	Added section on study procedures during special circumstances.	To protect subject's welfare and safety, and as far as possible ensure the potential benefit to the subject and promote data integrity.
1.2 Schema 1.3 Schedule of Activities 4.1 Overall Design 4.2.5 Rational for the Safety Monitoring Plan 6.6.2 Treatment Allocation to the Subject 8.0 Study Assessments and Procedures 9.2 Sample Size Determination	Added reference to Section 8.11 for study procedures to be considered during special circumstances.	To protect subject's welfare and safety, and as far as possible ensure the potential benefit to the subject and promote data integrity.
6.6.3 Blinding	Added toll-free number for GSK helpdesk.	Provided toll-free number to be dialed in case of emergency unblinding.
8.0 Study Assessments and Procedures	Added statement that the specific guidance from local public health and other competent authorities regarding the protection of individuals' welfare must be applied during special circumstances. For the duration of such special circumstances, certain study procedures may be implemented for enrolled subjects (refer to Section 8.11 for further details).	To protect subject's welfare and safety, and as far as possible ensure the potential benefit to the subject and promote data integrity.
8.3.8 Contact Information for Reporting of Serious Adverse Events, Adverse Events of Specific Interest,	Updated study contact for reporting of holding rules. Added CRO back-up study contact information.	To state that the Sponsor or Sponsor designee should be contacted for reporting of holding rules instead of the Local Medical Lead.

Section # and Name	Description of Change	Brief Rationale
Pregnancies, and Study Holding Rules		To provide additional back-up contact information for reporting of SAE/AESI and pregnancies.
8.11 Study Procedures During Special Circumstances	Added section on study procedures during special circumstances.	To protect subject's welfare and safety, and as far as possible ensure the potential benefit to the subject and promote data integrity.

AESI = adverse event of specific interest; CRO = Contract Research Organization; SAE = serious adverse event

Amendment 01 (12 December 2019)

Overall Rationale for the Amendment:

An optional blood sample will be added for subjects in Step 4 at Day 61 (Visit 5). To better and deeply characterize the antibody response, monoclonal antibodies produced by antigen specific B-cells will be isolated from a subset of subjects in Step 4 at Day 61 (Visit 5).

The selection of the subset will be based on the most pronounced response to the antigen of interest as determined by serological or cellular immunogenicity assessment.

The sequence of the monoclonal antibodies might be retrieved from the analysis of the complementary DNA generated from immunoglobulin messenger RNA. The sequence of the most interesting antibodies might be used to generate recombinant monoclonal antibodies to be further characterized for their properties in fighting the infectious agent.

This sequence may be used in the future for various possible applications, both for research and commercial purposes, to develop a new treatment that can help combat infectious diseases, such as generation of monoclonal antibodies that can be sold as therapeutic treatment.

The opportunity was also taken to add clarifications on possible ambiguities within the protocol surrounding the processes of sample collection and other assessments, simplification of assessments based on feedback from the investigator's site, and minor editorial corrections for typographical, grammar, and consistency errors.

Table 5 Description of Changes in Amendment 01

Section # and Name	Description of Change	Brief Rationale
1.1 Synopsis 9.5 Interim Analysis 9.8 Sequence of Analysis	Added that immunogenicity and reactogenicity data will also be used to perform the first analysis.	To clarify that safety, immunogenicity, and reactogenicity data will be used to perform the first analysis.
1.2 Schema 1.3 Schedule of Activities 8.8.1 Blood Sampling for Immunogenicity Response Assessment	An optional blood sample was added for subjects in Step 4 at Day 61 (Visit 5) for antibody determination.	To indicate that an additional optional blood sample will be drawn from subjects in Step 4 on Day 61. This sample will be optional. PBMC may be used for the generation of monoclonal antibodies.
6.6.2 Treatment Allocation to the Subject	Added details regarding the allocation of subjects to study groups.	To clarify how subjects of 2 different age groups will be sequentially enrolled into the study through 4 steps.
Appendix 5	Updated to indicate that the Neomed laboratory is now called Nexelis and is an outsourced laboratory.	Neomed laboratory has changed into Nexelis. Nexelis is not a GSK Laboratory, but an outsourced laboratory.
		Correction of typographical error.

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1.0 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: A Phase I, Single-center, Randomized, Observer-blind, Placebo-controlled Study to Evaluate Safety, Reactogenicity and Immunogenicity of GSK's *Clostridium difficile* Investigational Vaccine Based on the F2 Antigen With or Without $AS01_B$ Adjuvant, When Administered Intramuscularly According to a 0, 1-month Schedule to Healthy Adults Aged Between 18-45 Years and Between 50-70 Years, Followed by an Additional Dose Administered in a Partial blind Manner Within an Interval of Approximately 15 Months After Dose 2, in a Subcohort of Subjects aged 50-70 Years

Short Title: Safety and immunogenicity study of GSK's *Clostridium difficile* vaccine 2904545A when administered in healthy adults aged 18-45 years and 50-70 years

Rationale: The purpose of this study is to generate safety and immunogenicity data for the development of a candidate *Clostridium difficile* (*C. difficile*) vaccine that would protect against primary cases of *Clostridium difficile* infection (CDI) and CDI recurrence.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To evaluate the safety and reactogenicity profile of the *C. difficile* F2 fusion protein when administered with or without $AS01_B$ adjuvant as IM doses according to a 0, 1-month schedule, in healthy adults aged between 18-45 years and between 50-70 years followed by an additional dose administered approximately 15 months after Dose 2 in a subcohort of subjects aged 50-70 years, during the entire study period.	• Solicited local and general symptoms. – Occurrence of solicited local symptoms (any and Grade 3) within 7 days (Day 1 to Day 7) after each vaccination, in all subjects and in all groups. – Occurrence of solicited general symptoms (any, Grade 3, related, and Grade 3 related) within 7 days (Day 1 to Day 7) after each vaccination, in all subjects and in all groups. • Unsolicited AEs. – Occurrence of unsolicited AEs (any, Grade 3, related, Grade 3 related, and those resulting in a medically attended visit) within 30 days (Day 1 to Day 30) after each vaccination, according to the MedDRA classification, in all subjects and in all groups. • SAEs. – Occurrence of SAEs from Dose 1 (Day 1) up to study end, in all subjects and in all groups. • AEs of specific interest. – Occurrence of AEs of specific interest (pIMDs) from Dose 1 up to study end, in all subjects and in all groups.

Objectives	Endpoints
	• Laboratory parameters. – Occurrence of hematological (white blood cells, platelets count, and hemoglobin level) and biochemical (alanine aminotransferase, aspartate aminotransferase, creatinine, and uric acid) laboratory abnormalities at Screening, Days 8, 31, 38, 180, 390, 476, 491, 498, and 670 and urinary laboratory abnormalities (protein, glucose, and blood) at Screening. (Grades will be based on the local laboratory normal ranges and derived from the FDA Toxicity Grading Scale for laboratory abnormalities).
Secondary	
• To evaluate the humoral immunogenicity induced by IM doses of the *C. difficile* F2 fusion protein when administered with or without $AS01_B$ adjuvant according to a 0, 1-month schedule, in healthy adults aged between 18-45 years and between 50-70 years followed by an additional dose administered approximately 15 months after Dose 2 in a subcohort of subjects aged 50-70 years, up to study end.	• Humoral response to the investigational *C. difficile* F2 fusion protein when administered with or without $AS01_B$ adjuvant, at Days 1, 31, 61, 180, 390, 491, 521 and 670. – Serum neutralizing antibody titers anti-Toxin A and anti-Toxin B, as measured by TNA. – Serum antibody concentration anti-Toxin A and anti-Toxin B, as measured by ELISA.

AE = adverse event; $AS01_B$ = an adjuvant system containing MPL, QS-21 and liposome (50 µg MPL and 50 µg QS-21); *C. difficile* = *Clostridium difficile*; ELISA = enzyme-linked immunosorbent assay; FDA = Food and Drug Administration; GSK = GlaxoSmithKline Biologicals SA; IM = intramuscular; MedDRA = Medical Dictionary for Regulatory Activities; MPL = 3-O-desacyl-4'-monophosphoryl lipid A; pIMD = potential immune-mediated disease; QS-21 = *Quillaja saponaria* Molina, fraction 21 (Licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation); SAE = serious adverse event; TNA = toxin neutralization assay.

Overall Design:

This is a Phase I, first-in-human, single-center, randomized, observer-blind, placebo-controlled, study with 2 to 3 treatment groups per step in a 4-step staggered design in healthy subjects to generate safety, reactogenicity, and immunogenicity data for the development of a candidate *C. difficile* vaccine that would protect against primary cases of CDI and CDI recurrence.

A 4-step staggered design will be used to ensure maximum safety of the participating subjects:

- **Step 1**: Vaccination of approximately 20 subjects aged 18-45 years (approximately 10 subjects in the CDIFF antigen [Ag] group and approximately 10 subjects in the placebo group). An internal safety review committee (iSRC) will review all accumulating safety data after Dose 1 and then again after Dose 2. Vaccination in Step 2 will proceed in the absence of a safety concern detected by the iSRC on all accumulating safety data at the end of Step 1.
- **Step 2**: Vaccination of approximately 20 subjects aged 50-70 years (approximately 10 subjects in the CDIFF Ag group and approximately 10 subjects in the placebo group). An iSRC will review all accumulating safety data after Dose 1 and then again after Dose 2. Vaccination in Step 3 will proceed

in the absence of a safety concern detected by the iSRC on all accumulating safety data at the end of Step 2.

- **Step 3**: Vaccination of approximately 30 subjects aged 50-70 years (approximately 10 subjects in the CDIFF Ag group, approximately 10 subjects in the CDIFF Ag + an adjuvant system containing 3-O-desacyl-4'-monophosphoryl lipid A [MPL], QS-21 and liposome [50 µg MPL and 50 µg QS-21] [$AS01_B$] group, and approximately 10 subjects in the placebo group). An iSRC will review all accumulating safety data after Dose 1, and then again after Dose 2. Vaccination in Step 4 will proceed in the absence of a safety concern detected by the iSRC on all accumulating safety data at the end of Step 3.
- **Step 4**: Vaccination of approximately 70 subjects aged 50-70 years (approximately 25 subjects in the CDIFF Ag group, approximately 35 subjects in the CDIFF Ag + $AS01_B$ group, and approximately 10 subjects in the placebo group). An iSRC will review all accumulating safety data 3 weeks after the start of vaccination in Step 4 and then about every 3 weeks until all subjects have received Dose 1. In addition, the iSRC will review all safety data after Dose 2. A subcohort of subjects who have successfully completed all visits up to Visit 7, have received two doses on an active arm (ie, the subjects who received placebo will be excluded) do not meet any contraindication for subsequent vaccination, and still meet the eligibility criteria will receive a third dose after the iSRC has reviewed all the accumulating safety data at the end of Step 4 Dose 2. If there are any safety concerns observed during SRT reviews post Dose 3, an ad hoc iSRC meeting will take place to review the unblinded safety data.

During all 4 steps, all subjects should be closely observed for at least 60 minutes after vaccination.

For Steps 1, 2, and 3 only, all subjects should be vaccinated sequentially and at least 60 minutes apart to allow for monitoring of any acute events (eg, hypersensitivity reaction).

During vaccination days of Steps 1, 2, and 3 (ie, Day 1 and Day 31), vaccination will be limited to a maximum of 10 subjects per day. In Step 4, there is no limit related to the number of subjects to be vaccinated per day.

Number of Investigators and Study Centers:

One Principal Investigator and 1 study center are expected to participate in this study.

Number of Subjects:

A total of 120 subjects aged 50–70 years (30 receiving placebo, 45 receiving CDIFF Ag and 45 receiving CDIFF Ag + $AS01_B$) and 20 subjects aged 18-45 years (10 receiving placebo and 10 receiving CDIFF Ag) will be enrolled in this study.

Treatment Groups and Duration:

The duration of the study for each subject receiving 2 doses will be approximately 1 year (Visit 7) and each subject receiving 3 doses will be approximately 2 years (Visit 11).

Study treatment will be administered as indicated below:

Type of Contact and Timepoint	Study Group	Treatment Name	Volume to be Administered	Route	Injection Site	
					Location	Laterality[a]
Visit 1 (Day 1)	Placebo	Placebo	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag	CDIFF Ag	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag + $AS01_B$	CDIFF Ag + $AS01_B$	0.5 mL	IM	Deltoid	Nondominant
Visit 3 (Day 31)	Placebo	Placebo	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag	CDIFF Ag	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag + $AS01_B$	CDIFF Ag + $AS01_B$	0.5 mL	IM	Deltoid	Nondominant
Visit 8 for Step 4 (Day 491)	CDIFF Ag	CDIFF Ag	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag + $AS01_B$	CDIFF Ag + $AS01_B$	0.5 mL	IM	Deltoid	Nondominant

Ag = antigen; $AS01_B$ = an adjuvant system containing MPL, QS-21 and liposome (50 µg MPL and 50 µg QS-21); IM = intramuscular; MPL = 3-O-desacyl-4'-monophosphoryl lipid A.

[a] The nondominant arm is the preferred arm of injection. In case it is not possible to administer the vaccine in the nondominant arm, an injection in the dominant arm may be performed.

Study Procedures During Special Circumstances

During special circumstances (eg, COVID-19 pandemic), the specific guidance from local public health and other competent authorities regarding the protection of individuals' welfare must be applied. For the duration of such special circumstances, the following measures may be implemented for enrolled subjects:

- Safety follow-up may be made by a telephone call, other means of virtual contact, or home visit, if appropriate.
- Diary cards may be transmitted from and to the site by electronic means and/or conventional mail.
- Biological samples may be collected at a different location* other than the study site or at subject's home. Biological samples should not be collected if they cannot be processed in a timely manner or appropriately stored until the intended use.
- If despite best efforts it is not possible to collect blood samples within the interval predefined in the protocol, then the interval for blood sampling may be extended up to a maximum length of 30 days before the next blood sampling. Impact on the per protocol set for analysis of immunogenicity will be determined on a case-by-case basis.
- A limited number of subjects may be recruited in addition in the Step 4 in the active arms, should there be a high rate of missed visit and/or visit outside of planned interval and/or withdrawal due to the current exceptional and unpredictable circumstances.

*It is the Investigator's responsibility to identify an alternate location. The Investigator should ensure that this alternate location meets International Council for Harmonisation Good Clinical Practice requirements, such as adequate facilities to perform study procedures, appropriate training of the staff and documented delegation of responsibilities in this location. This alternate location should be covered by proper insurance for the conduct of study on subjects by Investigator and staff at a site other than the designated study site. Refer to EMA Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) pandemic (Version 4, 04 February 2021) for more details.

Statistical Methods:

The statistical analyses will be performed in 3 steps:

- The first interim analysis will be performed when all data up to 1 month after Dose 2, ie, Day 61 (ie, data that are as clean as possible) are available. The immunogenicity data available until Day 61, and the safety and reactogenicity data available at the time of this analysis will be described. At this point, the statistician will be unblinded for the analysis (ie, will have access to individual subject treatment assignments). The remaining study personnel will stay blinded (ie, will not have access to the individual subject treatment assignment) until study end. It is possible however, due to the limited sample size, that unblinding occurs for a few subjects having a specific adverse event (AE) or serious adverse event (SAE) (eg, an AE/SAE occurring only in a single group). Therefore, anyone having access to the analysis of Day 61 could become unblinded regarding those specific cases. The study will be considered as partial blind from this point onwards. Individual listings will be provided at the study end to the investigator. Subjects will be provided with information about the study arm, via the investigator at the study end.
- The second interim analysis will be performed when all data up to 1 month after Dose 3, ie, Day 521 (ie, data that are as clean as possible) are available. The immunogenicity data available until Day 521, and the safety and reactogenicity data available at the time of this analysis will be described.
- The final analysis will be performed when all data up to study end are available. An integrated clinical study report containing all data will be written and made available to the Principal Investigator at that time. In addition, all previous analyses will be re-produced based on cleaned data at this point. Individual listings will only be provided at this stage.

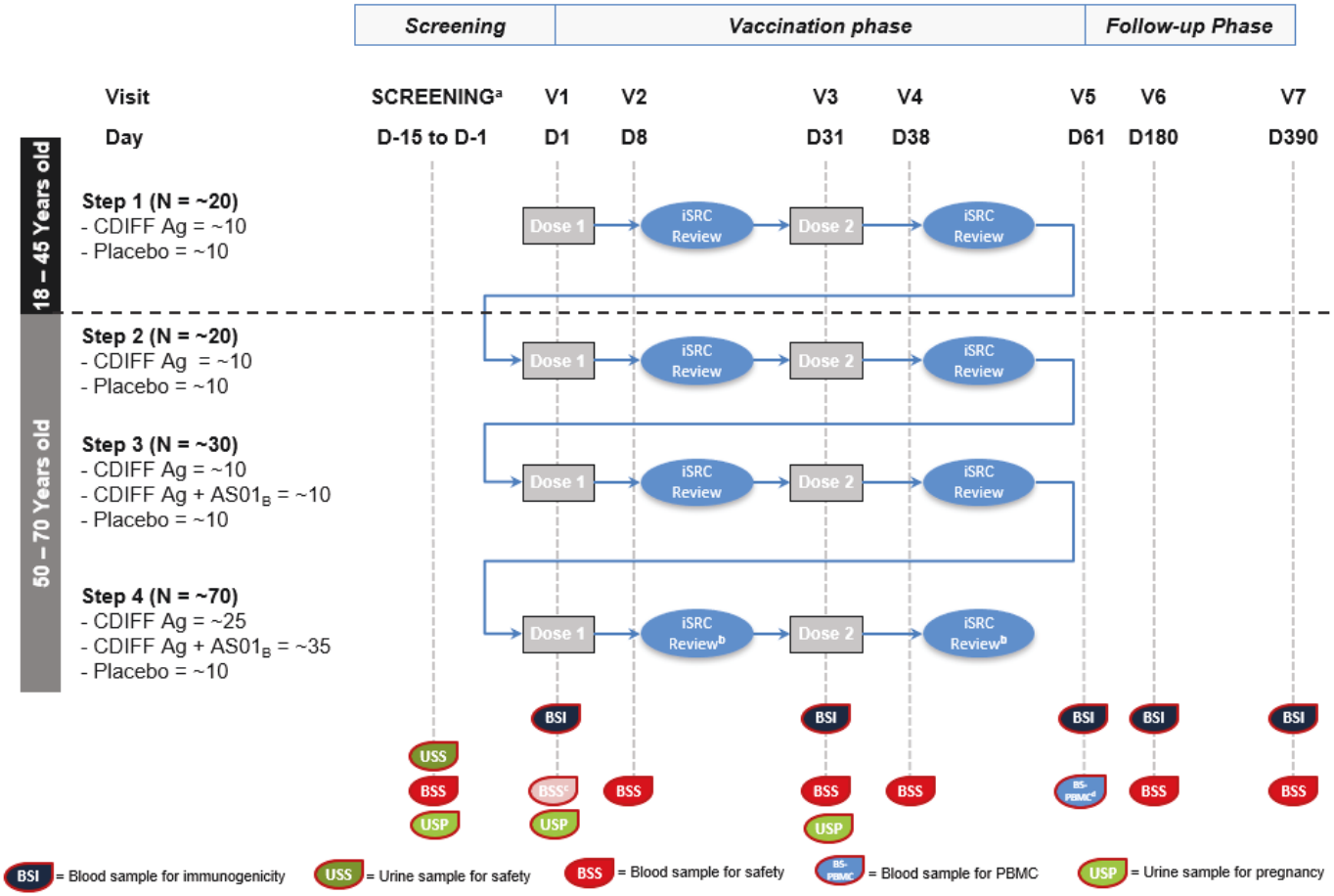
CCI [redacted]

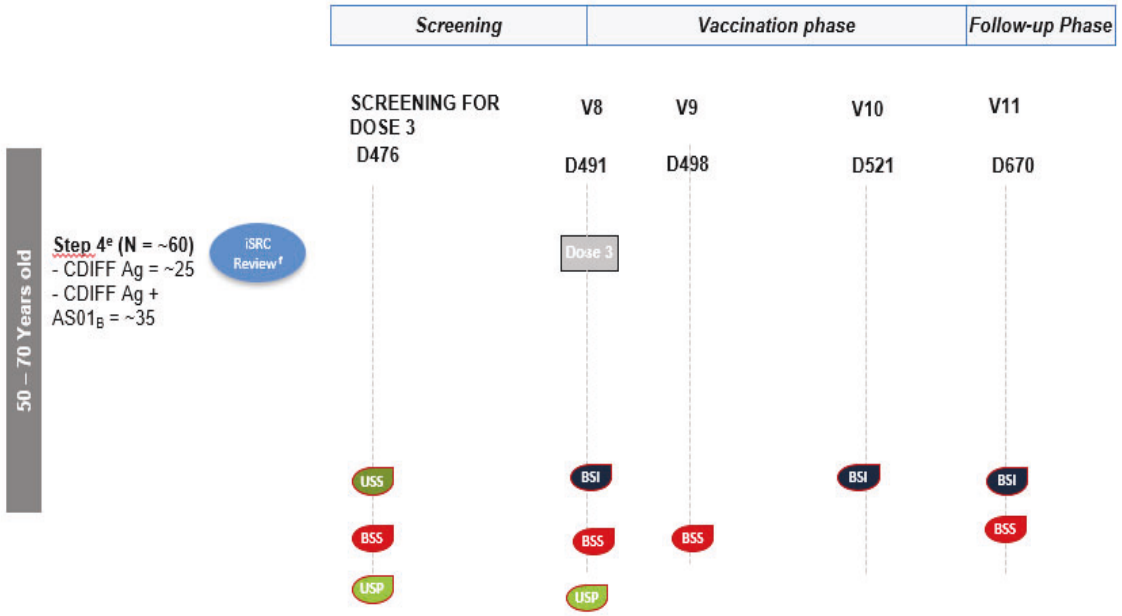
Internal Safety Review Committee: Yes

As the investigational vaccine formulation will be administered to human for the first time, an iSRC will be appointed in addition to the project's existing SRT and safety holding rules have been defined.

1.2 Schema

Figure 1 Study Design Overview (Refer to Section 8.11 for Study Procedures to be Considered During Special Circumstances)





Ag = antigen; $AS01_B$ = an adjuvant system containing MPL, QS-21 and liposome (50 µg MPL and 50 µg QS-21); D = day; iSRC = internal safety review committee; MPL = 3-O-desacyl-4'-monophosphoryl lipid A; N = number of subjects; PBMC = peripheral blood mononuclear cell; QS-21 = *Quillaja saponaria* Molina, fraction 21 (Licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation); V = visit

[a] As soon as the results from hematology and biochemistry analysis are available, Visit 1 (Day 1) should be scheduled. If the Investigator believes there is a reasonable justification to do so, screening procedures may be repeated (maximum 1 rescreening per subject is allowed). Only laboratory results from the Rescreening Visit, if it occurs, will be taken into consideration and recorded in the eCRF. Medical history, physical examination and review of inclusion/exclusion criteria must be repeated if a Rescreening Visit occurs. The subject can only be randomized once the Investigator receives the results and confirms the eligibility criteria

[b] The iSRC will review all accumulating safety data 3 weeks after the start of vaccination in Step 4 and then about every 3 weeks until all subjects have received Dose 1.

[c] Optional blood sampling for retesting, if deemed necessary by the Investigator.

[d] An additional blood sample of approximately 40 mL will be collected from subjects in Step 4 at Visit 5. This sample will be optional. PBMC may be used for the generation of monoclonal antibodies.

[e] A subcohort of subjects who have successfully completed all visits up to Visit 7, have received two doses on an active arm (ie, the subjects who received placebo will not be considered for Dose 3) do not meet any contraindication for subsequent vaccination, and still meet the eligibility criteria will receive an additional dose ie, Dose 3.

[f] The iSRC will review all the accumulating safety data at the end of Step 4 Dose 2. If there are any safety concerns observed during SRT reviews post Dose 3, an ad hoc iSRC meeting will take place to review the unblinded safety data.

1.3 Schedule of Activities

Table 6 Schedule of Activities (Refer to Section 8.11 for Study Procedures to be Considered During Special Circumstances)

Type of contact	Screening[a]	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Screening for Dose 3, Step 4	Visit 8 for Step 4	Visit 9 for Step 4	Visit 10 for Step 4	Visit 11 for Step 4
Timepoints	**Day -15**	**Day 1**	**Day 8**	**Day 31**	**Day 38**	**Day 61**	**Day 180**	**Day 390**	**Day 476**	**Day 491**	**Day 498**	**Day 521**	**Day 670**
Informed consent	●												
Informed consent addendum									●				
Check inclusion/exclusion criteria	●	●							●				
Collect demographic data[b]	●												
Medical history[c]	●												
Physical examination	●	●[e]	○[d]	●[e]	○[d]	○[d]	○[d]	○[d]	●	●[e]	○[d]	○[d]	○[d]
Vital signs (systolic/diastolic blood pressure and heart rate)	●	●[e]	○	●[e]	○	○	○	○	●	●[e]	○	○	○
Urine pregnancy test[f]	●	●[e]		●[e]					●	●[e]			
Measure/record height and weight	●												
Check contraindications to subsequent vaccination[g]				●[e]						●[e]			
Body temperature		●[e]		●[e]						●[e]			
Study group and treatment number allocation		○[e]											
Treatment number allocation for subsequent dose				○[e]						○[e]			

Table 6 Schedule of Activities (Refer to Section 8.11 for Study Procedures to be Considered During Special Circumstances)

Type of contact	Screening[a]	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Screening for Dose 3, Step 4	Visit 8 for Step 4	Visit 9 for Step 4	Visit 10 for Step 4	Visit 11 for Step 4
Timepoints	**Day -15**	**Day 1**	**Day 8**	**Day 31**	**Day 38**	**Day 61**	**Day 180**	**Day 390**	**Day 476**	**Day 491**	**Day 498**	**Day 521**	**Day 670**
Recording of administered treatment number		●		●						●			
Vaccine administration		●		●						●			
Blood sampling for antibody determination (humoral immunogenicity) (approximately 20 mL) – TNA and ELISA		●[e]		●[e]		●	●	●		●[e]		●	●
Optional blood sample for PBMC isolation (approximately 40 mL)						●[k]							
Blood sampling for hematology and biochemical analysis (approximately 5.5 mL)[h, i]	●	●[e, j]	●	●[e]	●		●	●	●	●[e]	●		●
Urine analysis (quantitative or semi-quantitative analysis)	●								●				
Record any concomitant medications/vaccinations	●	●[e]	●	●[e]	●	●	●	●	●	●[e]	●	●	●
Record any intercurrent medical conditions	●	●[e]	●	●[e]	●	●	●	●	●	●[e]	●	●	●
Distribution of the subject card	○												
Distribution of paper diary cards		○		○						○			

Table 6 Schedule of Activities (Refer to Section 8.11 for Study Procedures to be Considered During Special Circumstances)

Type of contact	Screening[a]	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Screening for Dose 3, Step 4	Visit 8 for Step 4	Visit 9 for Step 4	Visit 10 for Step 4	Visit 11 for Step 4
Timepoints	**Day -15**	**Day 1**	**Day 8**	**Day 31**	**Day 38**	**Day 61**	**Day 180**	**Day 390**	**Day 476**	**Day 491**	**Day 498**	**Day 521**	**Day 670**
Return of paper diary cards			○	○	○	○					○	○	
Diary card transcription by Investigator			●	●	●	●					●	●	
Recording of solicited AE (Day 1 to Day 7)		●	●	●	●					●	●		
Recording of unsolicited AE (Day 1 to Day 30)		●	●	●	●	●				●	●	●	
Recording of SAEs (all, fatal, related to study vaccine)		●	●	●	●	●	●	●	●	●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of AEs of specific interest (pIMDs)		●	●	●	●	●	●	●	●	●	●	●	●
Recording of pregnancies		●	●	●	●	●	●	●	●	●	●	●	●
Study conclusion for individual subjects								●[l]					●[m]

AE = adverse event; D = Day; ELISA = enzyme-linked immunosorbent assay; eCRF = electronic case report form; GSK = GlaxoSmithKline Biologicals SA; iSRC = internal safety review committee; PBMC = peripheral blood mononuclear cell; pIMD = potential immune-mediated disease; SAE = serious adverse event; TNA = toxin neutralization assay.

Note: The double-line border following Day 61 indicates the analyses which will be performed on all data (ie, data that are as clean as possible) obtained up to Day 61.

● Used to indicate a study procedure that requires documentation in the individual eCRF.

○ Used to indicate a study procedure that does not require documentation in the individual eCRF.

[a] If the Investigator believes there is a reasonable justification to do so, screening procedures may be repeated (maximum 1 rescreening per subject is allowed). Only laboratory results from the Rescreening Visit, if it occurs, will be taken into consideration and recorded in the eCRF. Medical history, physical examination and review of inclusion/exclusion criteria must be repeated if a Rescreening Visit occurs. The subject can only be randomized once the Investigator receives the results and confirms the eligibility criteria.

[b] Date of birth, sex, and race to be recorded in the subject's eCRF.

[c] Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded in the subject's eCRF.

[d] Physical examination, if deemed necessary by the Investigator or delegate.

[e] To be performed prior to vaccination.

[f] A urine pregnancy test will be performed only for women of childbearing potential.

[g] Contraindications to subsequent vaccinations are detailed in Section 6.2.

[h] The following hematological (white blood cells, platelets count and hemoglobin) and biochemical (alanine aminotransferase, aspartate aminotransferase, creatinine, and uric acid) parameters will be assessed at all timepoints specified above. C-reactive protein will be assessed **only** during the ***two*** screening visits.

[i] In case Grade 3 hematology or biochemistry parameter is observed, a retesting should be performed if deemed necessary by the Investigator, the iSRC, or the Sponsor.

[j] Optional blood sampling for retesting, if deemed necessary by the Investigator.

[k] An additional blood sample will be collected from subjects in Step 4 at Visit 5. This sample will be optional. PBMC may be used for the generation of monoclonal antibodies.

[l] The study conclusion for subjects receiving 2 doses will be on Visit 7.

[m] The study conclusion for subjects receiving 3 doses will be on Visit 11.

Table 7 Intervals Between Study Visits (Refer to Section 8.11 for Study Procedures to be Considered During Special Circumstances)

Interval	Optimal Length of Interval	Allowed Interval
Screening → Visit 1 (Day 1)	1 to 15 days	-
Visit 1 (Day 1) → Visit 2 (Day 8)	7 days	7 to 9 days
Visit 1 (Day 1) → Visit 3 (Day 31)	30 days	30 to 35 days
Visit 3 (Day 31) → Visit 4 (Day 38)	7 days	7 to 9 days
Visit 3 (Day 31) → Visit 5 (Day 61)	30 days	30 to 35 days

Visit 3 (Day 31) → Visit 6 (Day 180)	149 days	140 to 160 days
Visit 3 (Day 31) → Visit 7 (Day 390)	359 days	350 to 395 days
Visit 7 (Day 390) → Screening Dose 3 (Day 476)	20 to 150 days	-
Screening Dose 3 (Day 476)→ Visit 8 (Day 491)	1 to 15 days	-
Visit 8 (Day 491) → Visit 9 (Day 498)	7 days	7 to 9 days
Visit 8 (Day 491) → Visit 10 (Day 521)	30 days	30 to 35 days
Visit 8 (Day 491) → Visit 11 (Day 670)	179 days	170 to 190 days

2.0 INTRODUCTION

2.1 Study Rationale

The purpose of this study is to generate safety, reactogenicity, and immunogenicity data for the development of a candidate *Clostridium difficile* (*C. difficile*) vaccine that would protect against primary cases of *C. difficile* infection (CDI) and CDI recurrence.

2.2 Background

Clostridium difficile was characterized during the nineteen-seventies, but comprehensive epidemiological data were available since 2003 when large increases in incidence and mortality rates were observed in the United States of America (USA) and Europe. Since then there has been a steady increase in incidence of CDI, with subsequent increases in mortality, prolonged hospital stays with higher level care, and a substantial rise in healthcare costs.[2]

Clostridium difficile infection is a major cause of gastrointestinal illness, with approximately 500 000 infections and the leading cause of gastroenteritis associated death with 29 000 deaths annually in the USA. [2] The emergence of hypervirulent strains has contributed to increase the number and severity of CDI cases. In recent years, some countries (United Kingdom) have implemented hospital hygiene and bundle measures which resulted in significant reductions in the number of cases. The burden is, however, expected to remain significant until vaccination is available.

Currently, the CDI burden is known in North America and Europe (data are scarce from other regions of the world) and predominantly affects the older adult population, with increased severity of infection resulting in a 4% to 13% mortality rate depending on the age and underlying comorbidities. In the USA alone, there had been an estimated 453 000 incident cases of CDI and 29 300 deaths in 2011. [2] Treatment includes metronidazole and/or vancomycin and/or fidaxomicin, and complications such as toxic megacolon may require partial or full colectomy. Recurrences are reported in up to 25% of cases. *Clostridium difficile* is transmitted through ingestion of spores that germinate in the human intestine and become toxigenic. Virulent strains produce 2 toxins (Toxin A and Toxin B) with clear pathogenic role. Some newly emerging hypervirulent strains such as ribotype-027 or ribotype-078 produce greater quantities of Toxin A and Toxin B plus a binary toxin with a potentially important pathogenic role. [2]

In the US, from 1993 to 2009, hospital stays for patients with a primary CDI diagnosis have quadrupled. In 2009, CDI was associated with at least 1% of all hospital stays. The number of death certificates with enterocolitis due to *C. difficile* as the primary cause of death had multiplied by 10 from 1999 to 2008 in the USA. Similar trends were observed in Europe.

The rise in CDI-related hospitalizations worldwide has indicated that the affected population appears to have evolved. Indeed, there is a growing number of healthy adults without significant

medical comorbidities or prior antibiotic exposure who present with diarrheal symptoms and are diagnosed with CDI. In addition to that, emergence of community strains of *C. difficile* has been described.

Up to 40% of CDI cases may be community-acquired. Patients suffering from community-acquired CDI tend to be younger and healthier, without previously described risk factors.

The clinical burden associated with CDI is driven by morbidity and mortality in the hospitalized or institutionalized older adult population. Additional risk factors involve antibiotic use, comorbidities and to some extent, proton pump inhibitors. The economic burden is mainly driven by the increased length of stay and the cost of preventing dissemination within the institution (patient isolation and decontamination). Recently, the proportion of CDI occurring in patients outside the hospital setting has increased. Indeed, a large USA study demonstrated a community case incidence higher than previously reported. This supports the fact that the boundaries between hospital and community acquired CDI are becoming less distinct.[3,4]

First line treatments for CDI include metronidazole and vancomycin for primary and recurrent cases. However, 25% to 30% of cases result in recurrence of disease, with case fatality rates in the range of 6% to 30%.[2] A recently approved antibiotic (fidaxomicin) demonstrated noninferiority to vancomycin in initial clinical response rates but most importantly showed superiority in sustained response rate, ie, lower recurrence rate (15% versus 30% with vancomycin). Despite this, the uptake of fidaxomicin has been poor, due to concerns with its price as compared to vancomycin.

Please refer to the current Investigator Brochure (IB) for information regarding the preclinical studies and the epidemiological information of *C. difficile* investigational vaccine (GSK2904545A).

2.3 Benefit/Risk Assessment

Please refer to the current IB for the summary of potential risks and benefits of *C. difficile* investigational vaccine.

The Sponsor will immediately notify the Principal Investigator if any additional safety or toxicology information becomes available during the study.

This study will be performed in compliance with the protocol, International Council for Harmonisation (ICH) Good Clinical Practice (GCP) and applicable regulatory requirements. Aspects of the study concerned with the study treatment will meet the requirements of the European Union – Good Manufacturing Practice.

2.3.1 Risk Assessment

The risk assessment and mitigation strategy for this study protocol are outlined in Table 8.

Table 8 Risk Assessment

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Investigational vaccine: *Clostridium difficile* investigational vaccine		
Theoretical risk of acquiring a vaccine-induced autoimmune disease after vaccination.	Theoretical safety concerns have arisen from studies in which adjuvants have induced autoimmune diseases in various animal models and from literature reports that diverse compounds with "adjuvant" activity could be associated with autoimmunity.[5]	Close monitoring of pIMDs in clinical development programs using adjuvants systems. The potential risk of events of possible autoimmune etiology to occur is mentioned in the ICF.
Hypersensitivity reactions (including anaphylaxis).	Sudden potentially life-threatening allergic reactions to the vaccines are usually very rare. The first symptoms will usually occur shortly after the vaccination. In general, the symptoms can be treated successfully if the treatment is started quickly.	Subjects with known hypersensitivity to any of the components of the vaccine are excluded from enrollment. For Steps 1, 2, and 3 only, all subjects should be vaccinated sequentially and at least 60 minutes apart to allow monitoring of any acute events (eg, hypersensitivity reaction). During all 4 steps, all subjects should be closely observed for at least 60 minutes after vaccination. During vaccination days of Steps 1, 2, and 3 (ie, Day 1 and Day 31), vaccination will be limited to maximum 10 subjects per day.
Study Procedures		
Injection site hemorrhage in individuals with thrombocytopenia or any other coagulation disorder.	Injection site hemorrhage may occur at the injection site in populations at increased risk of hemorrhage, such as those with thrombocytopenia or acquired/hereditary coagulation disorders.	The Investigators are informed of possible injection site hemorrhage in individuals with thrombocytopenia or any coagulation disorder following study treatment administration by information included in product labels or IB based on the following language in the company Core Safety Information: "As with other vaccines administered

Table 8 Risk Assessment

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
		intramuscularly, all study vaccine should be given with caution to individuals with thrombocytopenia or any coagulation disorder since bleeding may occur following an IM administration to these subjects."
Risk from blood sampling.	Blood sampling-associated risk of discomfort, syncope, dizziness, and infection at the injection site after or during venipuncture.	Blood samples will be obtained by a trained professional and medical assistance will be available.

IB = Investigator's Brochure; ICF = informed consent form; IM = intramuscular; pIMDs = potential immune-mediated diseases

2.3.2 Benefit Assessment

Possible benefits include:

- Contribution to the process of developing a *C. difficile* vaccine that would potentially provide protection in the future.
- Medical assessment associated with this study (ie, physical examination, blood tests [hematology and biochemistry]) for the subjects enrolled.
- Access to medical care.
- Subjects receiving *C. difficile* investigational vaccine may potentially have the benefit of being protected against CDI. However, since the efficacy of the *C. difficile* investigational vaccine has not yet been assessed, it is not known whether it is effective in protecting humans.

2.3.3 Overall Benefit/Risk Conclusion

The *C. difficile* investigational vaccine is currently in a very early stage of clinical development and no vaccine efficacy has been demonstrated in humans. Taking into account the measures taken to minimize risk to subjects participating in this study, the potential risks identified in association with the *C. difficile* investigational vaccine are justified by the potential benefits linked to the development of the *C. difficile* investigational vaccine.

3.0 OBJECTIVES AND ENDPOINTS

Table 9 Study Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the safety and reactogenicity profile of the *C. difficile* F2 fusion protein when administered with or without $AS01_B$ adjuvant as IM doses according to a 0, 1-month schedule, in healthy adults aged between 18-45 years and between 50-70 years followed by an additional dose administered approximately 15 months after Dose 2 in a subcohort of subjects aged 50-70 years, during the entire study period.	• Solicited local and general symptoms. – Occurrence of solicited local symptoms (any and Grade 3) within 7 days (Day 1 to Day 7) after each vaccination, in all subjects and in all groups. – Occurrence of solicited general symptoms (any, Grade 3, related, and Grade 3 related) within 7 days (Day 1 to Day 7) after each vaccination, in all subjects and in all groups. • Unsolicited AEs. – Occurrence of unsolicited AEs (any, Grade 3, related, Grade 3 related, and those resulting in a medically attended visit) within 30 days (Day 1 to Day 30) after each vaccination, according to the MedDRA classification, in all subjects and in all groups. • SAEs. – Occurrence of SAEs from Dose 1 (Day 1) up to study end, in all subjects and in all groups. • AEs of specific interest. – Occurrence of AEs of specific interest (pIMDs) from Dose 1 up to study end, in all subjects and in all groups. • Laboratory parameters – Occurrence of hematological (white blood cells, platelets count, and hemoglobin level) and biochemical (alanine aminotransferase, aspartate aminotransferase, creatinine, and uric acid) laboratory abnormalities at Screening, Days 8, 31, 38, 180, 390, 476, 491, 498, and 670 and urinary laboratory abnormalities (protein, glucose, and blood) at Screening. (Grades will be based on the local laboratory normal ranges and derived from the FDA Toxicity Grading Scale for laboratory abnormalities).

Table 9 Study Objectives and Endpoints

Objectives	Endpoints
Secondary	
• To evaluate the humoral immunogenicity induced by IM doses of the *C. difficile* F2 fusion protein when administered with or without $AS01_B$ adjuvant according to a 0, 1-month schedule, in healthy adults aged between 18-45 years and between 50-70 years followed by an additional dose administered approximately 15 months after Dose 2 in a subcohort of subjects aged 50-70 years, up to study end.	• Humoral response to the investigational *C. difficile* F2 fusion protein when administered with or without $AS01_B$ adjuvant, at Days 1, 31, 61, 180, 390, 491, 521 and 670. – Serum neutralizing antibody titers anti-Toxin A and anti-Toxin B, as measured by TNA. – Serum antibody concentration anti-Toxin A and anti-Toxin B, as measured by ELISA.

AE = adverse event; $AS01_B$ = an adjuvant system containing MPL, QS-21 and liposome (50 µg MPL and 50 µg QS-21); *C. difficile* = *Clostridium difficile*; ELISA = enzyme-linked immunosorbent assay; FDA = Food and Drug Administration; GSK = GlaxoSmithKline Biologicals SA; IM = intramuscular; MedDRA = Medical Dictionary for Regulatory Activities; MPL = 3-O-desacyl-4'-monophosphoryl lipid A; pIMD = potential immune-mediated disease; QS-21 = *Quillaja saponaria* Molina, fraction 21 (Licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation); SAE = serious adverse event; TNA = toxin neutralization assay.

4.0 STUDY DESIGN

4.1 Overall Design

Protocol waivers or exemptions are not allowed unless necessary for the management of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline in the schedule of activities (Section 1.3), are essential and required for study conduct.

This is a Phase I, first-in-human, single-center, randomized, observer-blind, placebo-controlled, study with 2 to 3 treatment groups per step in a 4-step staggered design in healthy subjects to generate safety, reactogenicity, and immunogenicity data for the development of a candidate *C. difficile* vaccine that would protect against primary cases of CDI and CDI recurrence.

The duration of the study for each subject receiving 2 doses will be approximately 1 year and for each subject receiving 3 doses will be of approximately 2 years.

Primary completion date (PCD): Visit 11 (Day 670) (refer to Appendix 2 for the definition of PCD).

Study conclusion for individual subjects: The study conclusion for subjects receiving 2 doses will be Visit 7 (Day 390) and for subjects receiving 3 doses will be Visit 11 (Day 670) (refer to Appendix 2 for the definition of EoS).

A 4-step staggered design will be used to ensure maximum safety of the participating subjects (see Figure 1):

- **Step 1**: Vaccination of approximately 20 subjects aged 18-45 years (approximately 10 subjects in the CDIFF antigen [Ag] group and approximately 10 subjects in the placebo group). An internal safety review committee (iSRC) will review all accumulating safety data after Dose 1 and then again after Dose 2. Vaccination in Step 2 will proceed in the absence of a safety concern detected by the iSRC on all accumulating safety data at the end of Step 1.
- **Step 2**: Vaccination of approximately 20 subjects aged 50-70 years (approximately 10 subjects in the CDIFF Ag group and approximately 10 subjects in the placebo group). An iSRC will review all accumulating safety data after Dose 1 and then again after Dose 2. Vaccination in Step 3 will proceed in the absence of a safety concern detected by the iSRC on all accumulating safety data at the end of Step 2.
- **Step 3**: Vaccination of approximately 30 subjects aged 50-70 years (approximately 10 subjects in the CDIFF Ag group, approximately 10 subjects in the CDIFF Ag + an adjuvant system containing 3-O-desacyl-4'-monophosphoryl lipid A [MPL], QS-21 and liposome [50 µg MPL and 50 µg QS-21] $AS01_B$ group, and approximately 10 subjects in the placebo group). An iSRC will review all accumulating safety data after Dose 1, and then after Dose 2. Vaccination in Step 4 will proceed in the absence of a safety concern detected by the iSRC on all accumulating safety data at the end of Step 3.

- **Step 4**: Vaccination of approximately 70 subjects aged 50-70 years (approximately 25 subjects in the CDIFF Ag group, approximately 35 subjects in the CDIFF Ag + $AS01_B$ group, and approximately 10 subjects in the placebo group). An iSRC will review all accumulating safety data 3 weeks after the start of vaccination in Step 4 and then about every 3 weeks until all subjects have received Dose 1. In addition, the iSRC will review all safety data after Dose 2 (refer to Section 8.11 for study procedures to be considered during special circumstances). A subcohort of subjects who have successfully completed all visits up to Visit 7, have received two doses on an active arm (ie, the subjects who received placebo will be excluded), do not meet any contraindication for subsequent vaccination, and still meet the eligibility criteria will receive a third dose after the iSRC has reviewed all the accumulating safety data at the end of Step 4 Dose 2. If there are any safety concerns observed during SRT reviews post Dose 3, an ad hoc iSRC meeting will take place to review the unblinded safety data.

Refer to Section 9.6 for iSRC oversight.

During all 4 steps, all subjects should be closely observed for at least 60 minutes after vaccination.

For Steps 1, 2, and 3 only, all subjects should be vaccinated sequentially and at least 60 minutes apart to allow for monitoring of any acute events (eg, hypersensitivity reaction).

During vaccination days of Steps 1, 2, and 3 (ie, Day 1 and Day 31), vaccination will be limited to a maximum of 10 subjects per day. In Step 4, there is no limit related to the number of subjects to be vaccinated per day.

Table 10 Study Groups Foreseen in the Study *(Refer to Section 8.11 for Study Procedures to be Considered During Special Circumstances)*

Step	Study Groups	Number of Subjects	Age (Minimum/Maximum)
1	Placebo	10	18-45 years
	CDIFF Ag	10	18-45 years
2	Placebo	10	50-70 years
	CDIFF Ag	10	50-70 years
3	Placebo	10	50-70 years
	CDIFF Ag	10	50-70 years
	CDIFF Ag + $AS01_B$	10	50-70 years
4	Placebo	10	50-70 years
	CDIFF Ag	25	50-70 years
	CDIFF Ag + $AS01_B$	35	50-70 years

Ag = antigen; $AS01_B$ = an adjuvant system containing MPL, QS-21 and liposome (50 µg MPL and 50 µg QS-21); GSK = GlaxoSmithKline Biologicals SA; MPL = 3-O-desacyl-4'-monophosphoryl lipid A; QS-21 = *Quillaja saponaria* Molina, fraction 21 (Licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation)

The Investigator is not permitted to start the administration of the next dose until receipt of the favorable outcome of the safety evaluation, documented and provided in writing (scanned and emailed), authorizing the Investigator to proceed. Moreover, if the Investigator becomes aware of a holding rule being met, he/she will suspend vaccination and will inform GlaxoSmithKline Biologicals SA (GSK) immediately (see Table 11 and Table 12).

Refer to Section 4.6.1 for detailed description of holding rules and safety monitoring.

4.2 Scientific Rationale for Study Design

4.2.1 Rationale for Study Blinding

Given the different appearance of the *C. difficile* investigational vaccines and placebo, double blinding is not possible, and the study will be conducted in an observer-blind manner. In an observer-blind study, the subject, the Contract Research Organization personnel, and the study center and Sponsor personnel involved in the clinical evaluation of the subjects are blinded while other study personnel may be aware of the treatment assignment.

When all data up to Day 61 are available, a statistical analysis will be performed (first interim analysis). The immunogenicity data available until Day 61, and the safety and reactogenicity data available at the time of this analysis will be described. At this point, the statistician will be unblinded for the analysis (ie, will have access to individual subject treatment assignments). The remaining study personnel will stay blinded (ie, will not have access to the individual subject treatment assignment) until study end. It is possible however, due to the limited sample size, that unblinding occurs for a few subjects having a specific adverse event (AE) or serious adverse event (SAE) (eg, an AE/SAE occurring only in a single group). Therefore, anyone having access to the analysis of Day 61 could become unblinded regarding those specific cases. The study will be considered as partial blind from this point onwards.

The third dose will be administered to the Step 4 subjects who received the active components, in a partial blind manner. The Investigator and the subjects will not have access to the treatment allocation up to study end (Day 390). After Day 390, only the subjects will remain fully blinded, while the Investigator will be partially blinded, as no placebo subjects will be included for Dose 3.

Please refer to Appendix 2 for the definition of observer-blind and partial blind.

4.2.2 Rationale for Randomization and the Use of Placebo

Randomized, placebo-controlled studies are widely considered to be the most rigorous method for the efficacy evaluation of treatments or prevention interventions. To be ethical, clinical

research requires balancing rigorous science with the protection of subjects. The use of placebo controls in studies for conditions with no effective treatment is widely accepted.[6] Up to date, there is no established standard of care to prevent CDI.

Likewise, the Declaration of Helsinki allows placebo controls and states: "Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo will not be subjected to additional risks of serious or irreversible harm."[7]

Randomization is used to reduce bias of selection and the placebo group is included as a control for the reactogenicity, the safety and the immunogenicity assessments.

The third dose of the vaccine is being given to the subjects recruited in the active groups of the last step of the study, to demonstrate if a similar kinetics in terms of anti-Toxin A/Toxin B can be achieved with the F2 antigen formulation with or without an adjuvant. Therefore, the third dose will not be given to the subjects receiving placebo.

4.2.3 Rationale for the Use of F2 Fusion Protein Antigen

The F2 antigen is a fusion protein comprising the C-terminal portions of Toxin A and Toxin B. The rationale for selecting the C-terminal portions of Toxin A and Toxin B is based on the fact that these portions correspond to the binding domains of the toxins.

Substantial evidences suggest a protective role of antitoxin antibodies. In humans, antibodies against *C. difficile* Toxin A and Toxin B have been reported to protect against CDI[8,9,10,11] and recurrent episodes of CDI.[12,13,14] There is evidence suggesting that antibodies to the C-terminal part of Toxin B can drive protection while data remain unclear on the protective role conferred by antibodies induced by the C-terminal part of Toxin A.

4.2.4 Rationale for the Use of $AS01_B$ Adjuvant

As the *C. difficile* investigational vaccine is intended for subjects at risk for CDI who also may have an impaired immune system due to immunosenescence and/or comorbidities, the *C. difficile* investigational vaccine includes an adjuvant. Using an adjuvant might indeed help to induce a higher and longer-lasting immune response.

The *C. difficile* investigational vaccine used in the present study will be adjuvanted with $AS01_B$. $AS01_B$ is an adjuvant included in the GSK Biologicals' Herpes Zoster vaccine marketed as *Shingrix*, which has recently received regulatory approvals in the US, Canada, Europe, and Japan for the prevention of shingles in adults aged 50 years and above. $AS01_B$ contains 50 µg of MPL and 50 µg of QS-21 (a saponin molecule purified from the bark extract of *Quillaja saponaria* Molina, fraction 21 [Licensed by GSK from Antigenics Inc. a wholly owned subsidiary of Agenus Inc. a Delaware, USA corporation]).

4.2.5 Rationale for the Safety Monitoring Plan

The *C. difficile* investigational vaccine will be administered for the first time in humans therefore a 4-step staggered design will be used to allow evaluation of safety of the 2 formulations (with or without adjuvant) in a limited number of subjects before exposing a larger number of subjects to the *C. difficile* investigational vaccine (refer to Section 4.1). The *C. difficile* investigational vaccine without adjuvant will be first administered to subjects aged 18-45 years before administration to subjects aged 50-70 years (refer to Section 4.1 and to Figure 1).

The *C. difficile* investigational vaccine without adjuvant will be administered to approximately 55 subjects (approximately 10 subjects aged 18-45 years and approximately 45 subjects aged 50-70 years), the *C. difficile* investigational vaccine with $AS01_B$ adjuvant will be administered to approximately 45 subjects aged 50-70 years and placebo will be administered to approximately 40 subjects (approximately 10 subjects aged 18-45 years and approximately 30 subjects aged 50-70 years) (refer to Section 8.11 for study procedures to be considered during special circumstances).

For Steps 1, 2, and 3 only, vaccination will be limited to a maximum of 10 subjects per day. Subjects will be vaccinated sequentially, separated by a minimum interval of 60 minutes to allow for monitoring of any acute events (eg, hypersensitivity reaction). During all 4 steps, all subjects should be closely observed for at least 60 minutes after vaccination.

Specific holding rules are defined to ensure well-controlled exposure to the *C. difficile* investigational vaccine (see Section 4.6.1).

4.3 Justification for Dose

The F2 candidate antigen developed by GSK contains a portion of the amino acids sequence of the full-length *C. difficile* toxins. Another manufacturer developing a *C. difficile* vaccine based on the use of genetically detoxified full-length Toxin A and Toxin B, reported optimal immunogenicity data at a dose of 100 µg each (clinical trial: NCT01706367). The proposed dose of F2 antigen is defined to obtain an equivalent number of epitopes (included in F2 sequence) as reported in the Phase I study.

Moreover, findings from the repeated dose toxicology study indicate that the investigational vaccine F2/$AS01_B$ was well tolerated when administered three times at two-week intervals. No safety risks were identified. Please refer to the current IB for more information regarding the preclinical studies of *C. difficile* investigational vaccine (GSK2904545A).

In addition to these observations, pooled analyzes of AS01 adjuvanted vaccines in humans yielded that there is no safety concern for the administration of the same AS01 adjuvanted vaccine. Increase of reactogenicity is expected after dose 2 or dose 3 of the same adjuvanted vaccine.

Based on these data, it is recognized that CDIFF F2 $AS01_B$ has an acceptable safety profile (reactogenicity).

4.4 End of Study Definition

See Appendix 2 for the definition of EoS.

Study conclusion for individual subjects: A subject is considered to have completed the study if he/she returns for the concluding visit/is available for the concluding contact (Visit 7 [Day 390] for subjects receiving 2 doses and Visit 11 [Day 670] for subjects receiving 3 doses) as described in the protocol.

Global completion of the study is required in order to provide sufficient subjects as defined in Section 9.2.

4.5 Dose Escalation Criteria

Not applicable.

4.6 Study Holding Criteria

4.6.1 Study Holding Rules

The safety holding rules which will be assessed by the Investigator are defined in Table 11 and the safety holding rules which will be assessed by the iSRC are defined in Table 12. The toxicity grading scales for hematology, biochemistry, and urinalysis parameters applicable for this study are presented in Table 13.

Table 11 Holding Rules Assessed by the Investigator

Holding Rule	Event	Number of Subjects
1a	Death or any life-threatening SAE.	≥1
1b	Any withdrawal from the study following a Grade 3 AE that cannot reasonably be attributed to a cause other than vaccination.	≥1
1c	Any SAE or fever >40°C (104°F) that cannot reasonably be attributed to a cause other than vaccination, or visible necrosis at the injection site, within the 7-day (Day 1 to Day 7) period after vaccination.	≥1

AE = adverse event; SAE = serious adverse event.

Table 12 Holding Rules Assessed During Internal Safety Review Committee Evaluations

Holding Rule	Event	Percentage of Subjects
2a	Any Grade 3 solicited local AE (lasting 48 hours or more) in an investigational group, within the 7-day (Day 1 to Day 7) period after vaccination.	≥20% (per group)
2b	Any Grade 3 solicited general AE (lasting 48 hours or more) in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 7-day (Day 1 to Day 7) period after vaccination.	≥20% (per group)
2c	Any Grade 3 unsolicited AE in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 7-day (Day 1 to Day 7) period after vaccination.	≥20% (per group)
2d	Any Grade 3 abnormality in prespecified hematological or biochemical laboratory parameters in an investigational group within the 7-day (Day 1 to Day 7) period after vaccination (refer to Table 13).	≥20% (per group)

AE = adverse event.

Figure 2 illustrates that, with 10 subjects per group:

- For holding rules 1a, 1b, and 1c, using a cutoff of 1/10, there is more than 80% chance that the holding rule is met for a vaccine with a true incidence rate above 15% and there is more than 60% chance that the holding rule is not met for a vaccine with a true incidence rate below 5%.
  Since this holding rule will be assessed on a continuous basis by the Investigator, irrespective of the number of subjects enrolled, the statistical interpretation of this holding rule is less relevant.
- For holding rules 2a, 2b, 2c, and 2d, using a cutoff of 2/10, there is more than 80% chance that the holding rule is met for a vaccine with a true incidence rate above 27% and there is more than 80% chance that the holding rule is not met for a vaccine with a true incidence rate below 8%.

A similar figure is presented for 45 subjects per group (Figure 3).

Figure 2 Evaluations Based on 10 Enrolled Subjects - Risk Assessment Curves for 1 Formulation Based on the Proposed Safety Holding Rules

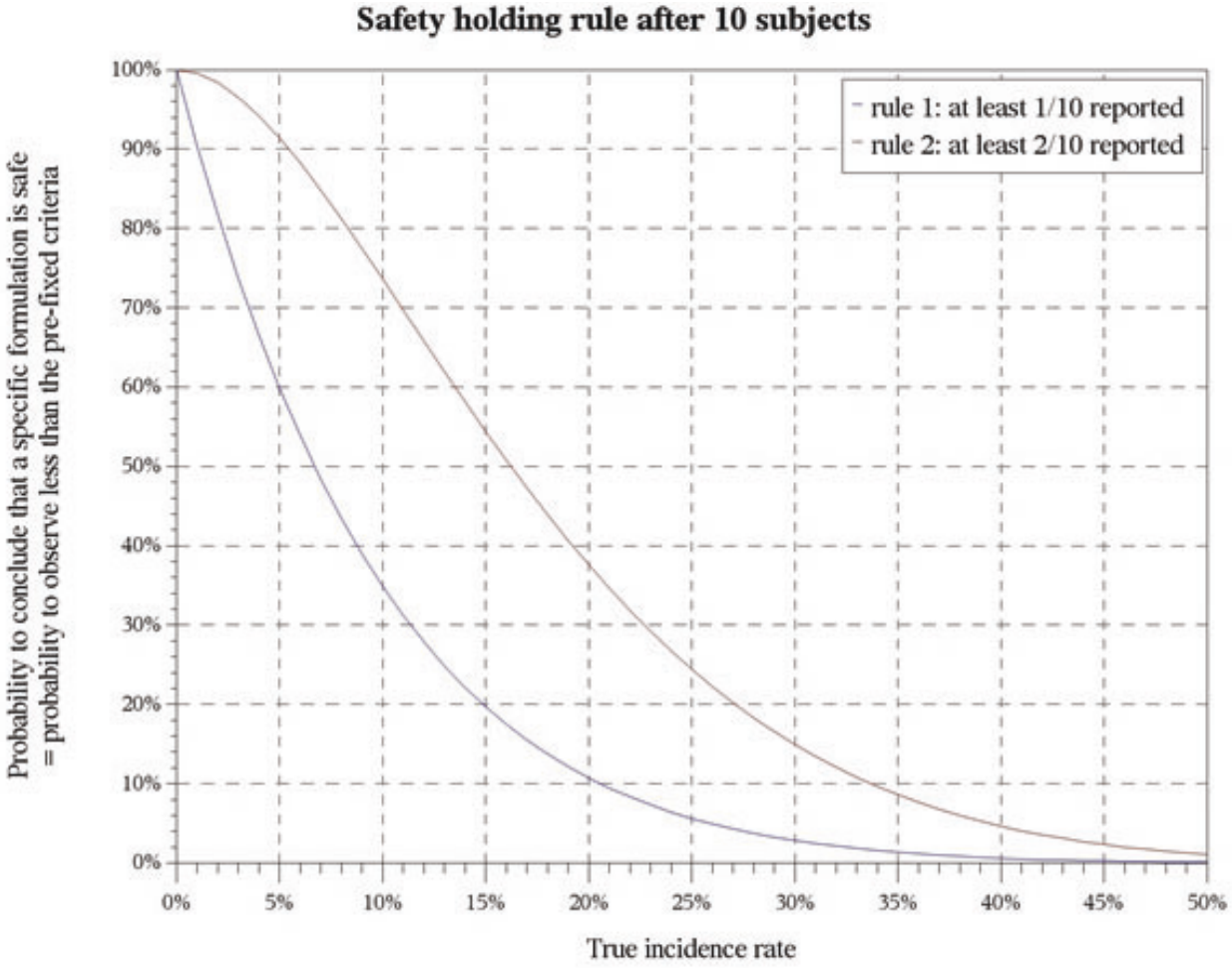


Figure 3 Evaluations Based on 45 Enrolled Subjects - Risk Assessment Curves for 1 Formulation Based on the Proposed Safety Holding Rules

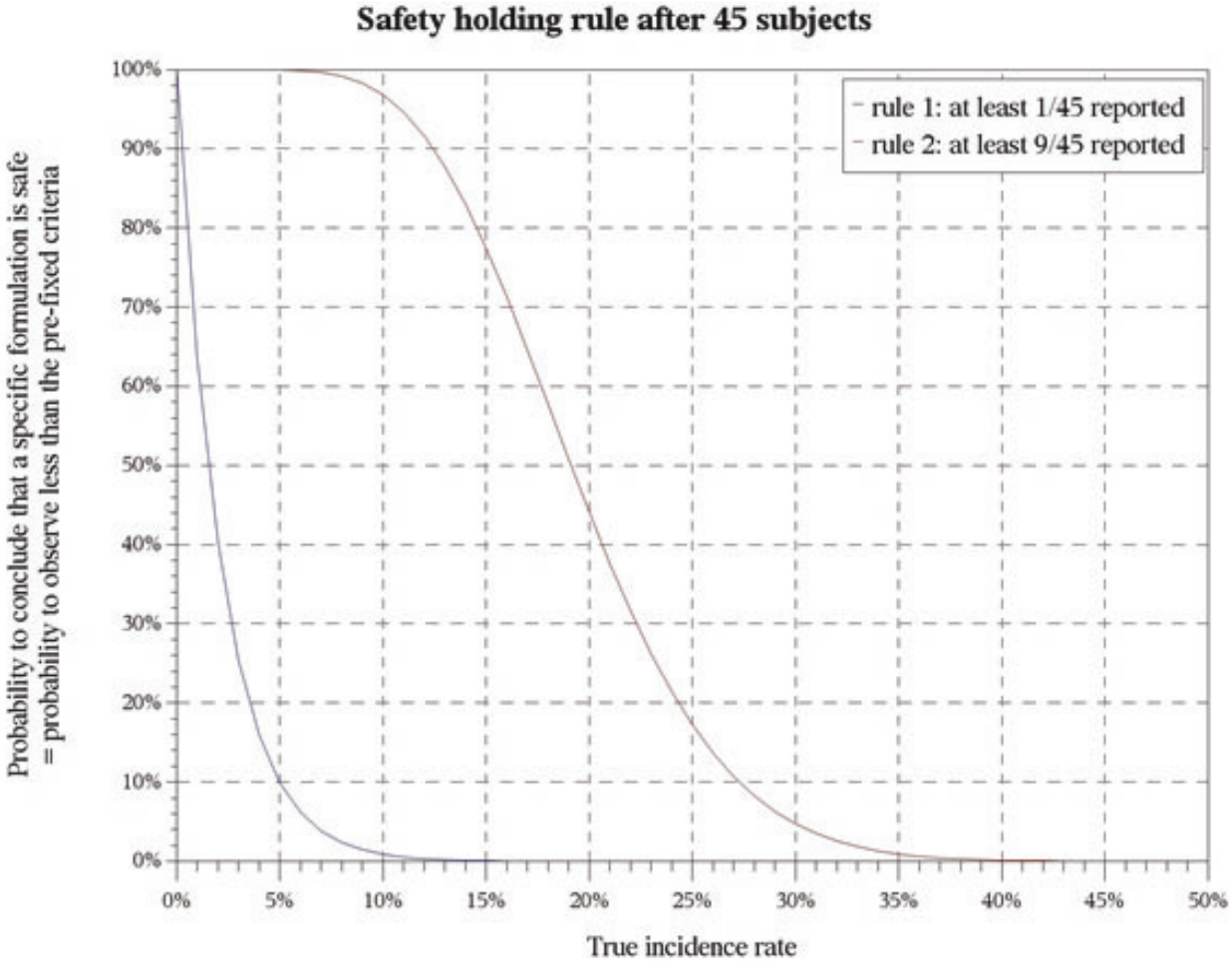


Table 13 Toxicity Grading Scales for Hematology, Biochemistry, and Urinalysis Parameters Applicable for this Study

Discipline (System)	Component	CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.
Hematology (whole blood)	Hemoglobin (gm/dL) - female	
	Hemoglobin (gm/dL) - male	
	Hemoglobin (gm/dL) - female change from baseline value	
	Hemoglobin (gm/dL) - male change from baseline value	
	White blood cells increase (cell/mm^3)	
	White blood cells decrease (cell/mm^3)	
	Platelets decrease (cell/mm^3)	
Biochemistry (serum)	Alanine aminotransferase (increase by factor)	
	Aspartate aminotransferase (increase by factor)	
	Creatinine (mg/dL)	
	Uric acid	
Urinalysis (urine)	Protein (mg/dL)	

CTCAE = Common Terminology Criteria for Adverse Events; FDA = Food and Drug Administration; iSRC = internal safety review committee; ULN = upper limit of the normal range

The laboratory values provided in these tables are taken from the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials", except for uric acid for which laboratory values are taken from CTCAE Version 4.[15,16]

[a] In case Grade 3 hematology or biochemistry parameter is observed, a re-testing should be performed if deemed necessary by the Investigator, the iSRC, or the Sponsor.

4.6.2 Procedure if the Study is Put on Hold

If the Investigator detects 1 of the holding rules, he/she will immediately put the enrollment or the vaccination on hold and he/she will immediately inform the Sponsor and enter the data in the electronic case report form (eCRF), but all other procedures relating to safety and immunology will continue. In case 1 of the holding rules (1a, 1b, or 1c) is met, the iSRC will meet urgently and review the unblinded data. During this time the study will be kept on hold. The iSRC will inform the study Clinical Research and Development Lead (CRDL) who will inform the relevant bodies of the Sponsor about the outcome of the review and the recommendation on whether to suspend, modify, or continue the conduct of the study on all groups or on selected groups. The decision regarding the further conduct of the study will be documented and provided in writing to the Investigators.

During this review period, the study will be temporarily stopped, while the decision is being made. In case of a decision to stop the study, a letter indicating the reason will be sent to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) by the Investigator and to the Regulatory Agency by the Sponsor.

5.0 STUDY POPULATION

5.1 Inclusion Criteria

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study, or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

1. Subjects who, in the opinion of the Investigator, can and will comply with the requirements of the protocol (eg, completion of the diary cards, return for Follow-up Visits).
2. Written or witnessed/thumb print informed consent obtained from the subject prior to performance of any study specific procedure.
3. **For Step 1 only:** A male or female between, and including, 18-45 years of age at the time of the first vaccination.
4. **For Steps 2, 3, and 4:** A male or female between, and including, 50-70 years of age at the time of the first vaccination.
5. Healthy subjects (ie, without progressive, unstable, or uncontrolled clinical conditions) as established by medical history (as verified by either a personal physician or medical practitioner, as appropriate) and clinical examination before entering into the study.
6. Subjects free of any uncontrolled chronic illnesses as established by medical history and clinical examination before entering into the study.
7. Female subjects of nonchildbearing potential may be enrolled in the study.

    – Nonchildbearing potential is defined as premenarche, current bilateral tubal ligation or occlusion, hysterectomy, bilateral ovariectomy, or postmenopause (refer to Appendix 7 for definitions of premenarche and postmenopause).

    Female subjects of childbearing potential may be enrolled in the study, if the subject (refer to Appendix 7 for definitions of woman of childbearing potential and adequate contraception):

    – Has practiced adequate contraception for 30 days prior to vaccination, and
    – Has a negative urine pregnancy test on the day of vaccination, and
    – Has agreed to continue adequate contraception during the entire treatment period and for 2 months after completion of the vaccination series.

5.2 Exclusion Criteria

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study, or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study.

1. Health condition that, in the opinion of the Investigator, may interfere with optimal participation in the study or place the volunteer at increased risk of adverse events (AEs). Study clinicians, in consultation with the Principal Investigator will use clinical judgment on a case-by-case basis to assess safety risks under this criterion. The Principal Investigator will consult with the Medical Monitor as appropriate.
2. Use of any investigational or nonregistered product (drug, vaccine, or medical device) other than the study vaccine(s) during the period starting 30 days before the first dose of study vaccine(s) (Day -29 to Day 1), or planned use during the study period.
3. Any medical condition that in the judgment of the Investigator would make intramuscular (IM) injection unsafe and subjects under treatment with anticoagulant therapy.
4. Chronic administration (defined as more than 14 consecutive days in total) of immunosuppressants or other immune-modifying drugs during the period starting 3 months prior to the first vaccination. For corticosteroids, this will mean prednisone ≥5 mg/day (for adult subjects), or equivalent. Inhaled and topical steroids are allowed.
5. Administration of long-acting immune-modifying drugs at any time during the study period (eg, infliximab).
6. Administration of immunosuppressive therapy, including chemotherapeutic agents used to treat cancer or other conditions, and treatments associated with organ or bone marrow transplantation or autoimmune disease.
7. Administration of immunoglobulins and/or any blood products during the period starting 3 months before the first vaccination of study treatment or planned administration during the study period.
8. Planned administration/administration of a vaccine not foreseen by the study protocol in the period starting 6 weeks before the first vaccination and ending 6 weeks after the last vaccination, with the exception of inactivated influenza vaccine which can be administered up to 14 days before or from 30 days after the last study vaccination.
   In case an emergency mass vaccination for an unforeseen public health threat (eg, a pandemic) is recommended and/or organized by public health authorities outside the routine immunization program, the time period described above can be reduced if, necessary for that vaccine, provided it is used according to the local governmental recommendations and that the Sponsor is notified accordingly.
9. Planned administration of GSK's Herpes Zoster vaccine marketed as *Shingrix* or an adjuvanted recombinant varicella zoster virus envelope gE subunit vaccine (HZ/su) within 180 days before the first dose and within 180 days after the last dose of the study vaccine.
10. Planned elective surgery during the study period.

11. Concurrently participating in another clinical study, at any time during the study period, in which the subject has been or will be exposed to an investigational or a non-investigational vaccine/product (pharmaceutical product or device).
12. Body mass index <19 kg/m^2 or ≥35 kg/m^2.
13. Clinically relevant physical examination abnormalities.
14. For subjects aged 18–45 years, Grade 2 or higher abnormal hematological, biochemical, and urinary parameters.
15. For subjects aged 50–70 years, Grade 3 or higher abnormal hematological, biochemical, and urinary parameters.
16. Documentation of current or prior episode of CDI.
17. Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination (no laboratory testing required).
18. Recurrent history or uncontrolled neurological disorders or seizures.
19. Family history of congenital or hereditary immunodeficiency.
20. History of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccines.
21. Acute disease and/or fever at the time of enrollment.

    - Fever is defined as temperature ≥38.0°C/100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.
    - Subjects with a minor illness (such as mild diarrhea, mild upper respiratory infection) without fever may, be enrolled at the discretion of the Investigator.

22. Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic, or renal functional abnormality, as determined by physical examination or laboratory screening tests.
23. Pregnant or lactating female.
24. History of intestinal bleeding or history of diverticular intestinal bleeding.
25. Surgery for gastrointestinal malignancy in the period starting 3 months prior to the first vaccination.
26. History of chronic alcohol consumption and/or drug abuse as deemed by the Investigator to render the potential subject unable/unlikely to provide accurate safety reports.
27. Female planning to become pregnant or planning to discontinue contraceptive precautions.
28. Documented human immunodeficiency virus-positive subject, known positivity for the surface Ag of the hepatitis B virus or known positive serologic test for the hepatitis C virus.
29. Involvement in the planning and/or conduct of the study (applies to both GSK personnel and/or personnel at the study center).

5.3 Lifestyle Considerations

No restrictions pertaining to lifestyle and/or diet beyond the details indicated in Section 5.2.

5.4 Screen Failures

Screen failures before first dose are defined as subjects who consent to participate in the clinical study but who are not subsequently randomly assigned to study treatment. Screen failures before third dose are defined as subjects who consent but did not receive the third dose. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information for screen failures before first dose includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAEs). Minimal information for screen failures before third dose includes screen failure details, eligibility criteria, and any SAEs.

Upon completion of all screening procedures (refer to Section 1.3), the Investigator will review the inclusion/exclusion criteria for each subject. Subjects meeting all eligibility criteria will be enrolled in the study. Their screening information will be recorded in the eCRF.

If the Investigator believes there is a reasonable justification to do so, screening procedures may be repeated (maximum 1 Rescreening per subject is allowed).

Only laboratory results from the Rescreening Visit, if it occurs, will be taken into consideration and recorded in the eCRF. Medical history, physical examination and review of inclusion/exclusion criteria must be repeated if a Rescreening Visit occurs. The subject can only be randomized once the Investigator receives the results and confirms the eligibility criteria.

6.0 STUDY TREATMENT

Study treatment is defined as a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject.

6.1 Study Treatment(s) Administered

Table 14 Description of Study Treatment

Treatment Name	Vaccine Name	Formulation	Presentation	Volume to be Administered	Number of Doses
Placebo	NaCl	NaCl = 150mM	Clear liquid for suspension	0.5 mL	2 doses
CDIFF Ag	F2	F2 antigen	Lyophilized	0.5 mL	2 ***or 3 doses****
	NaCl	NaCl = 150mM	Clear liquid for suspension		
CDIFF Ag + $AS01_B$	F2	F2 antigen	Lyophilized	0.5 mL	2 ***or 3 doses****
	$AS01_B$	MPL = 50 µg; QS-21 = 50 µg; Liposomes	Liquid in a monodose vial		

Ag = antigen; $AS01_B$ = an adjuvant system containing MPL, QS-21 and liposome (50 µg MPL and 50 µg QS-21); GSK = GlaxoSmithKline Biologicals SA; MPL = 3-O-desacyl-4'-monophosphoryl lipid A; QS-21 = *Quillaja saponaria* Molina, fraction 21 (Licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation)

**** a third dose will be administered to a subcohort of subjects in Step 4***

Table 15 Dosage and Administration of Study Treatment

Type of Contact and Timepoint	Study Group	Treatment Name	Volume to be Administered	Route	Injection Site	
					Location	Laterality[a]
Visit 1 (Day 1)	Placebo	Placebo	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag	CDIFF Ag	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag + $AS01_B$	CDIFF Ag + $AS01_B$	0.5 mL	IM	Deltoid	Nondominant
Visit 3 (Day 31)	Placebo	Placebo	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag	CDIFF Ag	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag + $AS01_B$	CDIFF Ag + $AS01_B$	0.5 mL	IM	Deltoid	Nondominant
Visit 8 (Day 491)	CDIFF Ag	CDIFF Ag	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag + $AS01_B$	CDIFF Ag + $AS01_B$	0.5 mL	IM	Deltoid	Nondominant

Ag = antigen; $AS01_B$ = an adjuvant system containing MPL, QS-21 and liposome (50 µg MPL and 50 µg QS-21); IM = intramuscular; MPL = 3-O-desacyl-4'-monophosphoryl lipid A.

[a] The nondominant arm is the preferred arm of injection. In case it is not possible to administer the vaccine in the nondominant arm, an injection in the dominant arm may be performed.

For each subject, the Investigator is not permitted to start the administration of the next dose until receipt of the favorable outcome of the safety evaluation, documented and provided in writing (scanned and emailed), authorizing the Investigator to proceed. Moreover, if the Investigator becomes aware of a holding rule being met, he/she will suspend vaccination and will inform GSK immediately (see Table 11 and Table 12).

Refer to Section 4.6.1 for detailed description of holding rules and safety monitoring.

The subjects will be observed closely for at least 60 minutes following the administration of the vaccine(s), with appropriate medical treatment readily available in case of anaphylaxis and syncope.

6.2 Contraindication to Subsequent Vaccination

Prior to receipt of additional study vaccination, subjects must be evaluated to confirm that they are eligible for subsequent vaccination.

If subjects meet any of the original exclusion criteria or the criteria listed below, they should not receive additional vaccinations. However, the subjects should be encouraged to continue other study procedures at the discretion of the Investigator (Section 8.3.3).

- Subjects who experience any SAE judged to be possibly or probably related to study vaccine or non-study vaccines, including hypersensitivity reactions.

- Subjects who develop any new condition which, in the opinion of the Investigator, may pose additional risk to the subject if he/she continues to participate in the study.
- Anaphylaxis following the administration of vaccines.
- Grade 3 or higher abnormal hematological, biochemical, and urinary parameters.
- Occurrence of a new potential immune-mediated disease (pIMD) or the exacerbation of an existing pIMD that, in the opinion of the Investigator, expose the subject to unacceptable risk from subsequent vaccination. In such cases, the Investigator should use his/her clinical judgment prior to administering the next dose of the vaccine(s)/product(s). Refer to Appendix 2 for the definition of pIMDs.

6.3 Criteria for Temporary Delay of Vaccination

Vaccination may be postponed within the allowed time interval until transient circumstances cited below have been resolved.

Acute disease and/or fever at the time of vaccination.

- Fever is defined as temperature ≥38.0°C/100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.
- Subjects with a minor illness (such as mild diarrhea, mild upper respiratory infection) without fever can be administered all vaccines.

6.4 Intercurrent Medical Condition That May Lead to Elimination of a Subject From the Per-protocol Analysis

At each study visit subsequent to the first vaccination/the vaccination visit, it must be verified if the subject has experienced or is experiencing any intercurrent medical condition that may lead to elimination from the per-protocol analysis. If it is the case, the condition(s) must be recorded in the eCRF.

Subjects may be eliminated from the Per-protocol Set for analysis of immunogenicity if, during the study, they incur a condition that has the capability of altering their immune response or are confirmed to have an alteration of their initial immune status.

6.5 Preparation/Handling/Storage/Accountability

The study vaccine(s) must be stored at the respective label storage temperature conditions in a safe and locked place. Access to the storage space should be limited to authorized study personnel. The storage conditions will be assessed during prestudy activities under the responsibility of the Sponsor study contact. The storage temperature should be continuously monitored with calibrated (if not validated) temperature monitoring device(s) and recorded. Refer to the Module on Clinical Trial Supplies in the Study Procedures Manual (SPM) for more details on storage of the study vaccine(s).

A temperature excursion is any temperature that is not in range of the label storage temperature conditions. Temperatures outside the range of label storage temperature conditions must be reported and/or documented. Temperature excursion impacting study vaccine(s) must be reported and/or documented.

In the frame of the reporting, the lack/absence of temperature monitoring documentation from a device meeting GSK requirements has to be considered as a temperature excursion.

Study vaccine(s) that are impacted by a temperature excursion may not be used and must be quarantined at label storage conditions until usage approval has been obtained from/via the local study contact (eg, study center monitor).

Refer to the Module on Clinical Trial Supplies in the SPM for details and instructions on the temperature excursion reporting and usage decision process, packaging and accountability of the study vaccine(s).

6.5.1 Replacement of Unusable Vaccinations

In addition to the vaccine doses provided for the planned number of subjects (including over-randomization when applicable), at least 20% additional vaccine doses will be supplied to replace those that are unusable.

The Investigator will use a System Built for Internet Randomization (SBIR) to obtain the replacement dose number. The replacement numbers will be allocated by component. The system will ensure, in a blind manner, that the replacement dose matches the formulation the subject was assigned to by randomization.

6.6 Measures to Minimize Bias: Randomization and Blinding

6.6.1 Subject Identification

Subject identification numbers will be assigned sequentially to the subjects who have consented to participate in the study, according to the range of subject identification numbers allocated to the study center.

6.6.2 Treatment Allocation to the Subject

Treatment numbers will be allocated by dose.

The enrollment will be performed to ensure equal distribution of the population within the age strata (subjects 18-45 years of age versus subjects 50-70 years of age).

Allocation of the subject to a study group at the study center will be performed using SBIR. As the study will enroll 2 age groups sequentially through 4 steps (Step 1: 18-45 years of age and Steps 2, 3, and 4: 50-70 years of age), the steps will be considered as stratification factors with a specific size and group ratio at each step (Step 1: 20 subjects with ratio 1:1 between CDIFF Ag

and placebo alone; Step 2: 20 subjects with ratio 1:1 between CDIFF Ag and placebo alone, Step 3: 30 subjects with ratio 1:1:1 between CDIFF Ag, CDIFF Ag + $AS01_B$ and placebo; Step 4: 70 subjects with ratio 5:7:2 between CDIFF Ag, CDIFF Ag + $AS01_B$ and placebo). Within the 18-45 years age group, the randomization algorithm will use a minimization procedure accounting for gender (female, male), while within the 50-70 years age group, the randomization algorithm will use a minimization procedure accounting for age (50-59 years of age, 60-70 years of age) and gender (female, male). Minimization factors will have equal weight in the minimization algorithm. A minimum of 20% of each minimization factor category will be required to allow enrolling all categories within each minimization factor (refer to Section 8.11 for study procedures to be considered during special circumstances).

After obtaining the signed or witnessed/thumb printed and dated informed consent form (ICF) from the subject and having checked the eligibility of the subject, the study staff in charge of the vaccine administration will access SBIR. Upon providing the age (subjects 18-45 years of age versus subjects 50-70 years of age), the gender, and the subject identification number, the randomization system will determine the study group and will provide the treatment number to be used for the first dose.

The number of each administered treatment must be recorded in the eCRF on the Vaccine Administration screen.

When SBIR is not available, please refer to the SBIR user guide or the SPM for specific instructions.

6.6.2.1 Treatment Number Allocation for Subsequent Doses

For each dose subsequent to the first dose, the study staff in charge of the vaccine administration will access SBIR, provide the subject identification number, and the system will provide a treatment number consistent with the allocated study group.

6.6.3 Blinding

Data will be collected in an observer-blind manner. By observer-blind, it is meant that during the course of the study, the vaccine recipient and those responsible for the evaluation of any study endpoint (eg, safety and reactogenicity) will all be unaware of which vaccine was administered. To do so, vaccine preparation and administration will be done by authorized medical personnel who will not participate in any of the study clinical evaluations. The Investigator and the subjects will not have access to the treatment allocation up to Day 390.

The statistical analyses will be performed in 3 steps. Refer to Section 9.8 for controlled unblinding for the first and second interim analysis.

The data for third dose will be collected in a partial blind manner.

After Day 390, only the subjects will remain fully blinded. Those responsible for the evaluation of any study endpoint (eg, safety and reactogenicity) will be partially blinded, as no placebo subjects will be included for Dose 3.

The laboratory in charge of the laboratory testing will be blinded to the treatment, and codes will be used to link the subject and study (without any link to the treatment attributed to the subject) to each sample.

6.6.3.1 Emergency Unblinding

Unblinding of a subject's individual treatment code should occur only in the case of a medical emergency when knowledge of the treatment is essential for the clinical management or welfare of the subject.

The emergency unblinding process consists of the automated internet-based system (SBIR) that allows the Investigator to have unrestricted, immediate and direct access to the subject's individual study treatment.

As back-up process, the Investigator has the option of contacting a GSK Helpdesk (refer to Table 16) if he/she needs support to perform the unblinding (ie, he/she cannot access SBIR).

Non-investigator physician (eg, physician from emergency room) or subject/care giver/family member can also request emergency unblinding either via the Investigator (preferred option) or via the GSK Helpdesk (back-up process). Contact details of Investigator and GSK Helpdesk are reported in the subject card.

Table 16 Contact Information for Emergency Unblinding

GSK Helpdesk 24-hour and 7-day availability
The Helpdesk is available by phone, fax and email Toll-free number: 00 800 4344 1111 Phone: +32(2)656 68 04 Fax: +32(2)401 25 75 email: rix.ugrdehelpdesk@gsk.com

A subject may continue in the study if that subject's treatment assignment is unblinded.

GSK Vaccines Clinical Safety and Pharmacovigilance staff may unblind the treatment assignment for any subject in case of Suspected Unexpected Serious Adverse Reaction (SUSAR) as well as in case of fatal or life-threatening cases. If the SAE requires that an expedited regulatory report be sent to 1 or more regulatory agencies, a copy of the report, identifying the subject's treatment assignment, may be sent to Investigators in accordance with local regulations and/or GSK policy.

6.7 Study Treatment Compliance

The prescribed dosage, timing, and mode of administration may not be changed.

All dose administrations will be performed in the study center under the supervision of appropriately trained staff.

6.7.1 Treatment Strategy

The study center staff is responsible for the ongoing safety and wellbeing of the subjects while they are in the study center. There is a paging system to alert the clinical staff to any area in the center where a subject may need medical attention. There is medical advice available by phone 24 hours a day. In addition, if necessary, the clinical staff can contact further on-call physicians or public emergency services in the event of a serious medical event.

6.7.2 Warnings and Precautions

As this is the first administration of study treatment to humans, all effects cannot be reliably predicted. The preclinical data suggest an acceptable safety margin. Facilities and staff for resuscitation and the treatment of other medical emergencies will be provided.

6.8 Concomitant Therapy

6.8.1 Recording of Concomitant Medications/Products and Concomitant Vaccinations

At each study visit, the Investigator or delegate should question the subject about any medications/products taken and vaccinations received by the subject.

The following concomitant medication(s)/product(s)/vaccine(s) must be recorded in the eCRF:

- All concomitant medications/products, except vitamins and dietary supplements, administered between Day 1 and Day 61.
- All concomitant medications/products, except vitamins and dietary supplements, administered between Day 491 and Day 521.
- Any concomitant vaccination administered in the period starting 6 weeks before the first dose of study treatment and ending at the last study visit (Day -42 to Day 390 for subjects receiving 2 doses/Day 670 for subjects receiving 3 doses).
- Prophylactic medication (ie, medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination).

An antipyretic is considered to be prophylactic when it is given in the absence of fever and any other symptom, to prevent fever from occurring (fever is defined as temperature ≥38.0°C/100.4°F regardless the location of measurement). The preferred location for measuring temperature in this study will be the oral cavity.

- Any concomitant medications/products/vaccines leading to the withdrawal or non-eligibility of the subject from the study (refer to Section 6.8.2).
- Any concomitant medications/products/vaccines relevant to an SAE/pIMD to be reported as per protocol or administered during the study period for the treatment of an SAE/pIMD. In addition, concomitant medications relevant to SAEs and pIMDs need to be recorded on the expedited AE report.

6.8.2 Concomitant Medications/Products/Vaccines That may Lead to the Elimination of a Subject From Per-protocol Analyses

The use of the following concomitant medications/products/vaccines will not require withdrawal of the subject from the study but may determine a subject's evaluability in the per-protocol analysis. See Section 9.3 for populations to be analyzed.

- Any investigational or nonregistered product (drug or vaccine) other than the study vaccine(s) used during the study period.
- Immunosuppressants or other immune-modifying drugs administered chronically (ie, more than 14 consecutive days in total) during the study period (until Day 390 for subjects receiving 2 doses and until Day 670 for subjects receiving 3 doses). For corticosteroids, this will mean prednisone ≥5 mg/day (for adult subjects), or equivalent. Inhaled and topical steroids are allowed.
- Long-acting immune-modifying drugs administered at any time during the study period (eg, infliximab).
- A vaccine not foreseen by the study protocol administered during the period starting the day of the first vaccination and ending 6 weeks after the last vaccination*, with the exception of inactivated influenza vaccine which can be administered up to 14 days before or from 30 days after the last study vaccination.
  * In case an emergency mass vaccination for an unforeseen public health threat (eg, a pandemic) is recommended and/or organized by public health authorities outside the routine immunization program, the time period described above can be reduced if, necessary for that vaccine, provided it is used according to the local governmental recommendations and that the Sponsor is notified accordingly.
- Immunoglobulins and/or any blood products administered during the study period.
- Drug and/or alcohol abuse.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.9 Dose Modification

Dose modifications are not planned or allowed in this study.

6.10 Treatment After the End of the Study

During the study conclusion visit, the Investigator will ask each subject if they are interested in participating in a booster study/long-term study. If a subject is not interested in participating in the booster study/long-term study the reason for refusal will be documented, when available, in the subject's eCRF.

The Sponsor will not provide any additional care to subjects after they completed the study.

7.0 DISCONTINUATION OF STUDY TREATMENT AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Vaccine

A "withdrawal" from the study vaccine(s) refers to any subject who does not receive the complete treatment, ie, when no further planned dose is administered from the date of withdrawal. A subject withdrawn from the study vaccine(s) may continue further study procedures (safety or immunogenicity) if planned in the study protocol, as deemed appropriate by the Investigator.

Primary reason relative to premature discontinuation of the study vaccine(s) will be documented on the Vaccine Administration screen of the eCRF. The Investigator will document whether the decision to discontinue further vaccination was made by the subject himself/herself or by the Investigator, as well as which of the following possible reasons was responsible for withdrawal:

- AEs requiring expedited reporting.
- Nonserious AE (specify).
- Unsolicited nonserious AE.
- Solicited AE.
- Not willing to be vaccinated.
- Other (specify).

7.2 Subject Discontinuation/Withdrawal From the Study

- A subject may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons.
- If the subject withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a subject withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the study center study records.
- See Section 1.3 for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed. Should a subject request or decide to withdraw from the study, all efforts must be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. Subjects withdrawing due to an AE should be followed up according to the Follow-up Visit.
- Subjects withdrawn due to an AE will not be replaced.

From an analysis perspective, a "withdrawal" from the study refers to any subject who did not come back for the concluding visit foreseen in the protocol.

All data and samples collected until the date of withdrawal/last contact of the subject will be used for the analysis.

A subject is considered a "withdrawal" from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected for this subject from the date of withdrawal/last contact.

Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up.

The primary reason for study withdrawal will be documented in the eCRF. The Investigator will document whether the decision to withdraw a subject from the study was made by the subject himself/herself or by the Investigator, as well as which of the following possible reasons was responsible for withdrawal:

- AEs requiring expedited reporting.
- Unsolicited nonserious AE.
- Solicited AE.
- Protocol deviation.
- Withdrawal by subject, not due to an AE.*
- Migrated/moved from the study area.
- Lost to follow-up.
- Sponsor study termination.
- Other (specify).

*In case a subject is withdrawn from the study because he/she has withdrawn consent, the Investigator will document the reason for withdrawal of consent, if specified by the subject, in the eCRF.

Subjects who are withdrawn from the study because of SAEs/AEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn from the study as result of an SAE/AE until resolution of the event (see Section 8.3.3).

7.3 Lost to Follow-up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study center.

The following actions must be taken if a subject fails to return to the study center for a required study visit:

- The study center must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit

schedule and ascertain whether or not the subject wishes to and/or should continue in the study.

- Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, he/she will be considered to have withdrawn from the study.

8.0 STUDY ASSESSMENTS AND PROCEDURES

Study procedures and their timing are summarized in Section 1.3.

Protocol waivers or exemptions are not allowed unless necessary for the management of immediate safety concerns.

Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the subject(s) should discontinue study treatment.

Adherence to the study design requirements, including those specified in Section 1.3, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The Investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the subject's routine clinical management (eg, blood count) and obtained before signing of ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the timeframe defined in Section 1.3.

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying SPM. The SPM provides the Investigator and the study center personnel with administrative and detailed technical information that does not impact the safety of the subjects.

The maximum amount of blood collected from each subject over the duration of the study, will not exceed 200 mL. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples (refer to Section 8.2.3 for blood sampling related to safety and to Section 8.8.1 for blood sampling related to immunogenicity assessment).

During special circumstances (eg, COVID-19 pandemic), the specific guidance from local public health and other competent authorities regarding the protection of individuals' welfare must be applied. For the duration of such special circumstances, certain study procedures may be implemented for enrolled subjects (refer to Section 8.11 for further details).

8.1 Efficacy Assessments

Not applicable.

8.2 Safety Assessments

Planned timepoints for all safety assessments are provided in Section 1.3.

8.2.1 Physical Examinations

- Physical examination of the subject, including assessment of oral body temperature.
- Collected information needs to be recorded in the eCRF.
- If the Investigator determines that the subject's health on the day of vaccination temporarily precludes vaccination, the visit will be rescheduled within the protocol visit window.
- Treatment of any abnormality observed during this examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

8.2.2 Vital Signs

- Resting vital signs: systolic/diastolic blood pressure and heart rate after at least 10 minutes of rest.
- Vital signs are to be taken before blood collection for laboratory tests and will consist of 1 pulse and 1 blood pressure measurement.
- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Collected information needs to be recorded in the eCRF.

8.2.3 Clinical Safety Laboratory Assessments

Approximately 5.5 mL of blood for hematology and biochemical analysis and approximately 10 mL of urine for urinalysis will be collected from all subjects at the timepoints specified in Section 1.3.

Please refer to Appendix 4 for a detailed description of the assays performed in the study. Please refer to Appendix 5 for the address of the clinical laboratories used for sample analysis. Hematology, biochemistry, and urine assessments will be performed in the Investigator's laboratory as per local practice using standardized and validated procedures (Appendix 4).

- The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with an underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition.
- All laboratory tests with values considered clinically significant abnormal during participation in the study or within 30 days after the last dose of study treatment should be repeated until the values return to normal or baseline or are no longer considered significantly abnormal by the Investigator or Medical Monitor. Refer to Appendix 6 for clinical laboratory abnormal assessments qualified as AEs or SAEs.
- If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, and the Sponsor notified.
- All protocol-required laboratory safety assessments, as defined in Appendix 4, must be conducted in accordance with the laboratory manual and Section 1.3.

8.3 Adverse Events

Please refer to Appendix 6 for safety definitions.

Adverse events will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative) or identified at the study visit.

The Investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the subject to discontinue the study treatment (see Section 7.0).

An overview of the protocol-required reporting periods for AEs, SAEs, AEs of specific interest, and pregnancies is given in Table 17.

Table 17 Reporting Periods for Collecting Safety Information

	Screening	Visit 1		Visit 2		Visit 3		Visit 4		Visit 5	Visit 6	Visit 7	Screening for Dose 3	Visit 8 for Step 4	Visit 9 for Step 4	Visit 10 for Step 4	Visit 11 for Step 4
		D1	D7	D8	D30	D31	D37	D38	D60	D61	D180	D390	D476	D491	D498	D521	D670
Solicited local and general AEs																	
Unsolicited AEs																	
SAEs (all, fatal, related to study vaccine)																	
SAEs related to study participation or concurrent GSK medication/vaccine																	
Pregnancies																	
AEs of specific interest (pIMDs)																	

AE = adverse event; D = day; GSK = GlaxoSmithKline Biologicals SA; pIMDs = potential immune-mediated diseases; SAE = serious adverse event

8.3.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information

All AEs and SAEs will be collected from the signing of the ICF until the Follow-up Visit at the timepoints specified in Section 1.3.

Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the eCRF, not the AE section.

All SAEs will be recorded and reported via Expedited AE Reporting Form to the Sponsor or designee immediately and under no circumstance should this exceed 24 hours after the Investigator became aware of it, as indicated in Appendix 6. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

A poststudy AE/SAE is defined as any event that occurs outside of the AE/SAE reporting period defined in Table 17. Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the Investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the study vaccine(s), the Investigator will promptly notify the Study Contact for Reporting SAEs.

Refer to Appendix 6 for local (injection site) and general solicited AEs to be collected.

At each vaccination visit, paper diary cards will be provided to the subject. The subject will be instructed to measure and record the oral body temperature, and any solicited local/general AEs (ie, on the day of vaccination and during the next 6 days) or any unsolicited AEs (ie, on the day of vaccination and during the next 30 days) occurring after vaccination. The subject will be instructed to return the completed diary card to the Investigator at the next study visit.

Adverse events of specific interest (AESIs) for safety monitoring include pIMDs (refer to Appendix 2 for definition of pIMD). Adverse events that need to be recorded and reported as pIMDs include those listed in Table 18.

Table 18 List of Potential Immune-mediated Diseases

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve neuropathy, including paralysis and paresis (eg, Bell's palsy). • Optic neuritis. • Multiple sclerosis. • Transverse myelitis. • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants. • Acute disseminated encephalomyelitis, including site specific variants, eg, noninfectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis. • Myasthenia gravis, including Lambert-Eaton myasthenic syndrome. • Demyelinating peripheral neuropathies including: - Chronic inflammatory demyelinating polyneuropathy. - Multifocal motor neuropathy. - Polyneuropathies associated with monoclonal gammopathy. • Narcolepsy.	• Systemic lupus erythematosus and associated conditions. • Systemic scleroderma (systemic sclerosis), including: - Diffuse scleroderma. - CREST syndrome. • Idiopathic inflammatory myopathies, including: - Dermatomyositis. - Polymyositis. • Antisynthetase syndrome. • Rheumatoid arthritis and associated conditions including: - Juvenile idiopathic arthritis. - Still's disease. • Polymyalgia rheumatica. • Spondyloarthropathies, including: - Ankylosing spondylitis. - Reactive arthritis (Reiter's syndrome). - Undifferentiated spondyloarthritis. - Psoriatic arthritis. - Enteropathic arthritis. • Relapsing polychondritis. • Mixed connective tissue disorder. • Gout.	• Psoriasis. • Vitiligo. • Erythema nodosum. • Autoimmune bullous skin diseases (including pemphigus, pemphigoid, and dermatitis herpetiformis). • Lichen planus. • Sweet's syndrome. • Localized scleroderma (morphea).
Vasculitis	**Blood disorders**	**Others**
• Large vessels vasculitis including: - Giant cell arteritis (temporal arteritis). - Takayasu's arteritis. • Medium sized and/or small vessels vasculitis including: - Polyarteritis nodosa. - Kawasaki's disease. - Microscopic polyangiitis. - Wegener's granulomatosis (granulomatosis with polyangiitis). - Churg–Strauss syndrome (allergic granulomatous	• Autoimmune hemolytic anemia. • Autoimmune thrombocytopenia. • Antiphospholipid syndrome. • Pernicious anemia. • Autoimmune aplastic anemia. • Autoimmune neutropenia. • Autoimmune pancytopenia.	• Autoimmune glomerulonephritis including: - IgA nephropathy. - Glomerulonephritis rapidly progressive. - Membranous glomerulonephritis. - Membranoproliferative glomerulonephritis. - Mesangioproliferative glomerulonephritis. - Tubulointerstitial nephritis and uveitis syndrome. • Ocular autoimmune diseases including: - Autoimmune uveitis.

Table 18 List of Potential Immune-mediated Diseases

angiitis or eosinophilic granulomatosis with polyangiitis). - Buerger's disease (thromboangiitis obliterans). - Necrotizing vasculitis (cutaneous or systemic). - Antineutrophil cytoplasmic antibody positive vasculitis (type unspecified). - Henoch-Schonlein purpura (IgA vasculitis). - Behcet's syndrome. - Leukocytoclastic vasculitis.		- Autoimmune retinitis. • Autoimmune myocarditis. • Sarcoidosis. • Stevens-Johnson syndrome. • Sjögren's syndrome. • Alopecia areata. • Idiopathic pulmonary fibrosis. • Goodpasture syndrome. • Raynaud's phenomenon.
Liver disorders	**Gastrointestinal disorders**	**Endocrine disorders**
• Autoimmune hepatitis. • Primary biliary cirrhosis. • Primary sclerosing cholangitis. • Autoimmune cholangitis.	• Inflammatory bowel disease, including: - Crohn's disease. - Ulcerative colitis. - Microscopic colitis. - Ulcerative proctitis. • Celiac disease. • Autoimmune pancreatitis.	• Autoimmune thyroiditis (Hashimoto thyroiditis). • Grave's or Basedow's disease. • Diabetes mellitus type 1. • Addison's disease. • Polyglandular autoimmune syndrome. • Autoimmune hypophysitis.

IgA = immunoglobulin A

8.3.2 Method of Detecting Adverse Events and Serious Adverse Events

The method of recording, evaluating, and assessing intensity, causality and outcome of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 6.

Care will be taken not to introduce bias when detecting AE and/or SAE. Open-ended and nonleading verbal questioning of the subjects is the preferred method to inquire about AE occurrence.

8.3.3 Follow-up of Adverse Events and Serious Adverse Events

After the initial AE/SAE report, the Investigator is required to proactively follow each subject at subsequent visits. All SAEs and AESIs will be followed until the event is resolved, stabilized, otherwise explained, or the subject is lost to follow-up. Further information on follow-up procedures is given in Appendix 6.

8.3.4 Treatment of Adverse Events

Treatment of any AE is at the sole discretion of the Investigator and according to current good medical practice. Any medication administered for the treatment of an SAE/AESIs should be recorded in Expedited Adverse Event Report of the subject's eCRF (refer to Section 6.8).

8.3.5 Regulatory Reporting Requirements for Serious Adverse Events

- Prompt notification of an SAE by the Investigator to the Sponsor is essential for meeting legal obligations and ethical responsibilities for the safety of subjects and the safety of a study treatment under clinical investigation.
- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC, and Investigators.
- Investigator safety reports must be prepared for SUSARs according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.
- An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAE) from the Sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.6 Pregnancy

Female subjects of childbearing potential are to have a urine pregnancy test prior to any study vaccine administration. The study vaccine(s) may only be administered if the pregnancy test is negative.

Note: Pregnancy test must be performed even if the subject is menstruating at the time of the study visit.

See Appendix 7 for collection of pregnancy information.

8.3.7 Subject Card

Study subjects must be provided with the address and telephone number of the main contact for information about the clinical study.

The Investigator (or designate) must therefore provide a "subject card" to each subject. In an emergency situation, this card serves to inform the responsible attending physician that the subject is in a clinical study and that relevant information may be obtained by contacting the Investigator.

Subjects must be instructed to keep subject cards in their possession at all times during the study duration.

8.3.8 Contact Information for Reporting of Serious Adverse Events, Adverse Events of Specific Interest, Pregnancies, and Study Holding Rules

Table 19 Contact Information for Reporting of Serious Adverse Events, Adverse Events of Specific Interest, Pregnancies, and Study Holding Rules

Study Contact for Reporting of SAEs, AESIs, and Pregnancies: Refer to the local study contact information document.
Study Contact for Reporting of Holding Rules: As soon as the Investigator is aware that a holding rule is met, he/she must immediately inform the Sponsor or Sponsor designee.
Back-up Study Contact for Reporting SAEs, AESIs, and Pregnancies: 24/24 hour and 7/7 day availability: **IQVIA** Email address: 208109safety@iqvia.com **GSK Clinical Safety & Pharmacovigilance** Fax: +32 2 656 51 16 or +32 2 656 80 09 Email address: Rix.CT-safety-vac@gsk.com

AESI = adverse event of specific interest; SAE = serious adverse event.

8.4 Treatment of Overdose

Not applicable.

8.5 Pharmacokinetics

Not applicable.

8.6 Pharmacodynamics

Not applicable.

8.7 Genetics

Genetics are not evaluated in this study.

8.8 Immunogenicity Assessment

Blood Sampling for Immunogenicity Response Assessments

All subjects from Steps 1, 2, 3, and 4

A volume of approximately 20 mL of whole blood should be drawn for analysis of humoral immune response at each predefined timepoint specified in Section 1.3. After centrifugation, serum samples should be kept at –20°C or below until shipment. Refer to the SPM for more details on sample storage conditions.

Subjects from Step 4 who consented for the additional sampling

For subjects who agreed to the optional blood sampling, a volume of approximately 40 mL of whole blood should be drawn for peripheral blood mononuclear cell (PBMC) isolation on Day 61 (Visit 5; refer to the schedule of activities in Section 1.3) and may be used for generation of monoclonal antibodies. The blood should be kept at the study center at a constant room temperature and it must not be centrifuged. Samples will be shipped at a constant room temperature (15 to 25°C) to the designated laboratory for PBMC separation to be performed within 18 hours.

Refer to the SPM for more details on sample storage conditions.

Monoclonal antibodies may be generated using the samples collected during the study and used for further research purposes as well as commercial use.

Information on further investigations and their rationale can be obtained from GSK.

Any sample testing will be done in line with the consent of the individual subject.

8.8.1 Laboratory Assays

Please refer to Appendix 5 for the address of the clinical laboratories used for sample analysis.

- Anti-Toxin A and anti-Toxin B neutralizing antibody titers will be measured by toxin neutralization assay (TNA) on serum samples, using different cell lines, at a GSK laboratory or in a laboratory designated by GSK using standardized and validated procedures (Table 20).
- Anti-Toxin A and anti-Toxin B antibodies concentrations will be determined by enzyme-linked immunosorbent assay on serum sample at a GSK laboratory or in a laboratory designated by GSK using standardized and validated procedures (Table 20).

Table 20 Humoral Immunity (Antibody Determination)

System	Component	Method	Laboratory
Serum	Anti-Toxin A neutralizing antibodies (different cell lines)	TNA	GSK[a] or designated laboratory
	Anti-Toxin B neutralizing antibodies (different cell lines)		
	Anti-Toxin A IgG	ELISA	
	Anti-Toxin B IgG		

ELISA = enzyme-linked immunosorbent assay; GSK = GlaxoSmithKline Biologicals SA; IgG = immunoglobulin G; TNA = toxin neutralization assay.

[a] GSK laboratory refers to the Clinical Laboratory Sciences in Rixensart, Belgium, and Wavre, Belgium.

Additional testing on the vaccine and/or on the disease under study may be performed within the framework of the study if deemed necessary for accurate interpretation of the data or should such assay(s) become available at GSK. These assays may not be represented in the objectives/endpoints of the study protocol.

The GSK clinical laboratories have established a Quality System supported by procedures. The activities of GSK clinical laboratories are audited regularly for quality assessment by an internal (Sponsor-dependent) but laboratory-independent Quality Department.

8.8.2 Immunological Read-outs

In case of insufficient blood sample volume to perform assays for all antibodies, the samples will be analyzed according to priority ranking provided in Table 21.

Table 21 Immunological Read-outs

Blood Sampling Timepoint Type of Contact and Timepoint	Number of Subjects	Component	Components Priority Rank
Visit 1 (Day 1)	~140	Anti-Toxin B neutralizing antibodies	1
		Anti-Toxin A neutralizing antibodies	2
		Anti-Toxin B immunoglobin G	3
		Anti-Toxin A immunoglobin G	4
Visit 3 (Day 31)	~140	Anti-Toxin B neutralizing antibodies	1
		Anti-Toxin A neutralizing antibodies	2
		Anti-Toxin B immunoglobin G	3
		Anti-Toxin A immunoglobin G	4
Visit 5 (Day 61)	~140	Anti-Toxin B neutralizing antibodies	1
		Anti-Toxin A neutralizing antibodies	2
		Anti-Toxin B immunoglobin G	3
		Anti-Toxin A immunoglobin G	4
Visit 6 (Day 180)	~140	Anti-Toxin B neutralizing antibodies	1
		Anti-Toxin A neutralizing antibodies	2
		Anti-Toxin B immunoglobin G	3
		Anti-Toxin A immunoglobin G	4
Visit 7 (Day 390)	~140	Anti-Toxin B neutralizing antibodies	1
		Anti-Toxin A neutralizing antibodies	2
		Anti-Toxin B immunoglobin G	3
		Anti-Toxin A immunoglobin G	4
Visit 8 (Day 491)	~60	Anti-Toxin B neutralizing antibodies	1
		Anti-Toxin A neutralizing antibodies	2
		Anti-Toxin B immunoglobin G	3
		Anti-Toxin A immunoglobin G	4
Visit 10 (Day 521)	~50	Anti-Toxin B neutralizing antibodies	1
		Anti-Toxin A neutralizing antibodies	2
		Anti-Toxin B immunoglobin G	3
		Anti-Toxin A immunoglobin G	4
Visit 11 (Day 670)	~50	Anti-Toxin B neutralizing antibodies	1
		Anti-Toxin A neutralizing antibodies	2
		Anti-Toxin B immunoglobin G	3
		Anti-Toxin A immunoglobin G	4

8.8.3 Immunological Correlates of Protection

No generally accepted immunological correlate of protection has been demonstrated so far for the Ag used in the *C. difficile* investigational vaccine.

8.9 Biomarkers

Biomarkers are not evaluated in this study.

8.10 Health Economics or Medical Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

8.11 Study Procedures During Special Circumstances

During special circumstances (eg, COVID-19 pandemic), the specific guidance from local public health and other competent authorities regarding the protection of individuals' welfare must be applied. For the duration of such special circumstances, the following measures may be implemented for enrolled subjects:

- Safety follow-up may be made by a telephone call, other means of virtual contact, or home visit, if appropriate.
- Diary cards may be transmitted from and to the site by electronic means and/or conventional mail.
- Biological samples may be collected at a different location* other than the study site or at subject's home. Biological samples should not be collected if they cannot be processed in a timely manner or appropriately stored until the intended use.
- If despite best efforts it is not possible to collect blood samples within the interval predefined in the protocol (see Table 7), then the interval for blood sampling may be extended up to a maximum length of 30 days before the next blood sampling. Impact on the per protocol set for analysis of immunogenicity will be determined on a case-by-case basis.
- A limited number of subjects may be recruited in addition in the Step 4 in the active arms, should there be a high rate of missed visit and/or visit outside of planned interval and/or withdrawal due to the current exceptional and unpredictable circumstances.

*It is the Investigator's responsibility to identify an alternate location. The Investigator should ensure that this alternate location meets ICH GCP requirements, such as adequate facilities to perform study procedures, appropriate training of the staff and documented delegation of responsibilities in this location. This alternate location should be covered by proper insurance for the conduct of study on subjects by Investigator and staff at a site other than the designated study site. Refer to EMA Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) pandemic (Version 4, 04 February 2021) for more details.

9.0 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

No formal statistical hypotheses are to be tested.

9.2 Sample Size Determination

This study is a Phase I safety study to get preliminary safety and immunogenicity assessments of vaccine formulations in adults.

The target will be to enroll a maximum of 140 healthy subjects (approximately 20 subjects aged 18-45 years in Step 1, approximately 20 subjects aged 50-70 years in Step 2, approximately 30 subjects aged 50-70 years in Step 3, and approximately 70 subjects aged 50-70 years in Step 4 [refer to Table 10 and Figure 1]) (refer to Section 8.11 for study procedures to be considered during special circumstances).

The sample size of 45 subjects aged 50-70 years in each active arm (CDIFF Ag or CDIFF Ag + $AS01_B$) would provide a probability of 80% or 90% to observe at least 1 specific AE, if the true AE rate is 3.5% or 5.0%, respectively, in that group.

Table 22 illustrates the precision on the percentage of subjects with symptoms following vaccination with 45 subjects vaccinated in each active arm.

Table 22 95% Confidence Interval for 1 Proportion with 45 Subjects

Number of Subjects with a Symptom	Percentage	Exact 95% Confidence Interval	
		Lower Limit	Upper Limit
0	0.0%	0.0%	7.9%
1	2.2%	0.1%	11.8%
2	4.4%	0.5%	15.1%
3	6.7%	1.4%	18.3%
4	8.9%	2.5%	21.2%
5	11.1%	3.7%	24.1%
6	13.3%	5.1%	26.8%
7	15.6%	6.5%	29.5%
8	17.8%	8.0%	32.1%
9	20.0%	9.6%	34.6%
10	22.2%	11.2%	37.1%
11	24.4%	12.9%	39.5%
12	26.7%	14.6%	41.9%

Table 22 95% Confidence Interval for 1 Proportion with 45 Subjects

Number of Subjects with a Symptom	Percentage	Exact 95% Confidence Interval	
		Lower Limit	Upper Limit
13	28.9%	16.4%	44.3%
14	31.1%	18.2%	46.6%
15	33.3%	20.0%	49.0%
16	35.6%	21.9%	51.2%
17	37.8%	23.8%	53.5%
18	40.0%	25.7%	55.7%
19	42.2%	27.7%	57.8%
20	44.4%	29.6%	60.0%
21	46.7%	31.7%	62.1%
22	48.9%	33.7%	64.2%
23	51.1%	35.8%	66.3%
24	53.3%	37.9%	68.3%
25	55.6%	40.0%	70.4%
26	57.8%	42.2%	72.3%
27	60.0%	44.3%	74.3%
28	62.2%	46.5%	76.2%
29	64.4%	48.8%	78.1%
30	66.7%	51.0%	80.0%
31	68.9%	53.4%	81.8%
32	71.1%	55.7%	83.6%
33	73.3%	58.1%	85.4%
34	75.6%	60.5%	87.1%
35	77.8%	62.9%	88.8%
36	80.0%	65.4%	90.4%
37	82.2%	67.9%	92.0%
38	84.4%	70.5%	93.5%
39	86.7%	73.2%	94.9%
40	88.9%	75.9%	96.3%
41	91.1%	78.8%	97.5%
42	93.3%	81.7%	98.6%
43	95.6%	84.9%	99.5%
44	97.8%	88.2%	99.9%

Table 22 95% Confidence Interval for 1 Proportion with 45 Subjects

Number of Subjects with a Symptom	Percentage	Exact 95% Confidence Interval	
		Lower Limit	Upper Limit
45	100.0%	92.1%	100.0%

Exact 95% confidence interval computed based on Clopper-Pearson formula.

Although key assumptions using GSK tests are not available at this stage, the variability values used in the statistical computations have been derived from the clinical data published by Sheldon (2016).[17] The standard deviation (SD) log10 value of 0.84 and 1.4 have been used in the statistical calculations of the precision around the geometric mean fold-rise (GMFR) from prevaccination to postvaccination, respectively, for Toxin A and Toxin B.[17]

Assuming 20% of nonevaluable subjects, 36 older adults in each active arm (CDIFF Ag or CDIFF Ag + $AS01_B$) will be evaluable and included in the Per-protocol Set for analysis of immunogenicity. Table 23 illustrates the 95% confidence interval (CI) for various values of GMFR for Toxin A and Toxin B.

Table 23 95% Confidence Intervals Based on Variability Assumptions of 0.84-fold for Anti-Toxin A and 1.4-fold for Anti-Toxin B and on a Sample Size Including 36 Evaluable Subjects

Observed GMFR value	Toxin A	Toxin B
10	[5.20; 19.23]	[3.36; 29.79]
15	[7.80; 28.85]	[5.04; 44.68]
20	[10.40; 38.46]	[6.715; 59.57]
30	[15.60; 57.69]	[10.07; 89.36]
40	[20.80; 76.92]	[13.43; 119.14]
50	[26.00; 96.15]	[16.79; 148.93]

GMFR = geometric mean fold-rise.

Thirty subjects aged 50-70 years will be enrolled in the placebo group as control for safety.

Ten additional subjects aged 18-45 years receiving CDIFF Ag and 10 subjects aged 18-45 years receiving placebo will be enrolled to evaluate safety and reactogenicity of the *C. difficile* F2 fusion protein in young and healthy adults during the Step 1 of the staggered design (refer to Figure 1).

A total of 120 subjects aged 50-70 years (30 receiving placebo, 45 receiving CDIFF Ag, and 45 receiving CDIFF Ag + $AS01_B$) and 20 subjects aged 18-45 years (10 receiving placebo and 10 receiving CDIFF Ag) will be enrolled in this study.

9.3 Populations for Analyses

For purposes of analysis, the analysis sets in Table 24 are defined.

Table 24 Analysis Sets

Analysis Set	Description
Enrolled Set	All subjects who signed the ICF.
Exposed Set	All subjects who have received at least 1 dose of the study vaccine. The allocation in a group is done in function of the administered treatment.
• **Unsolicited Safety Set**	All subjects who received at least 1 dose of study treatment (Exposed Set) that report unsolicited AEs/report not having unsolicited AEs.
• **Solicited Safety Set**	All subjects who received at least 1 dose of the study treatment (Exposed Set) who have solicited safety data.
• **Subcohort Exposed Set**	All subjects who have received the Dose 3 on an active arm.
Full analysis Set	All subjects who received at least 1 dose of the study treatment and have postvaccination immunogenicity data.
Per-protocol Set	All subjects who received at least 1 dose of the study treatment to which they are randomized and have postvaccination data (Full Analysis Set) minus subjects with protocol deviations that lead to exclusion. The protocol deviations leading to exclusion of the subjects will be detailed in the SAP. Three Per-protocol Sets for analysis of immunogenicity will be derived.
• **Per-protocol Set for analysis of immunogenicity at Day 61 (1-month post dose 2)**	All subjects included in the Per-protocol Set, with no protocol deviations leading to exclusion until Day 61 and with at least 1 immunogenicity data available at Day 61.
• **Per-protocol Set for analysis of immunogenicity at Day 180**	All subjects included in the Per-protocol Set, with no protocol deviations leading to exclusion until Day 180 and with at least 1 immunogenicity data available at Day 180
• **Per-protocol Set for analysis of immunogenicity at Day 390**	All subjects included in the Per-protocol Set, with no protocol deviations leading to exclusion until Day 390 and with at least 1 immunogenicity data available at Day 390.
Subcohort Per-protocol Set	
• **Subcohort Per-protocol Set for analysis of immunogenicity at Day 521 in the Subcohort Set**	All subjects included in the Per-protocol Set, who received the Dose 3 on an active arm, with no protocol deviations leading to exclusion until Day 521 and with at least 1 immunogenicity data available at Day 521.
• **Subcohort Per-protocol Set for analysis of immunogenicity at Day 670 in the Subcohort set**	All subjects included in the Per-protocol Set, who received the Dose 3 on an active arm, with no protocol deviations leading to exclusion until Day 670 and with at least 1 immunogenicity data available at Day 670.

AE = adverse event; ICF = informed consent form; SAP = statistical analysis plan

9.4 Statistical Analyses

9.4.1 Analysis of Demographics

Demographic characteristics (age at first study vaccination in years, gender, and ethnicity), cohort description, withdrawal status will be summarized by group using descriptive statistics:

- Frequency tables will be generated for categorical variable such as gender.
- Mean, median, and SD will be provided for continuous data such as age.

9.4.2 Safety Analyses

9.4.2.1 Primary Analyses

The primary analysis will be performed on the Exposed Set.

The percentage of subjects with at least 1 local AE (solicited and unsolicited), with at least 1 general AE (solicited and unsolicited) and with any AE during the 7-day or the 30-day follow-up period will be tabulated with exact 95% CI after each vaccination and overall. The percentage of doses followed by at least 1 local AE (solicited and unsolicited), by at least 1 general AE (solicited and unsolicited) and by any AE will be tabulated, overall vaccination course, with exact 95% CI. The same computations will be done for Grade 3, any AEs considered related to vaccination and any Grade 3 AEs considered related to vaccination.

The percentage of subjects/doses reporting each individual solicited local and general AE during the 7-day follow-up period will be tabulated with exact 95% CI as follows:

- Over the 2 or 3 doses, the percentage of subjects with the symptom and its exact 95% CI.
- Over the 2 or 3 doses, the percentage of doses with the symptom and its exact 95% CI.
- At each study dose (visit), the percentage of subjects with the symptom and its exact 95% CI.

Occurrence of fever will be reported per 0.5°C cumulative increments as from ≥38°C as well as the occurrence of fever >40°C/104°F with causal relationship to the study treatment.

The verbatim reports of unsolicited symptoms will be reviewed by a physician and the signs and symptoms will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). Every verbatim term will be matched with the appropriate preferred term. The percentage of subjects with unsolicited AEs within 30 days after any doses with its exact 95% CI will be tabulated by group and by MedDRA preferred term. Similar tabulation will be done for Grade 3 unsolicited AEs, for unsolicited AEs that resulted in a medically attended visit, for Grade 3 and causally related unsolicited AEs and for unsolicited AEs causally related to vaccination.

The number of subjects who experienced at least 1 SAE or any AE of specific interest (pIMD) during the entire study period will be reported.

For each group and for each hematology, biochemistry, and urinalysis parameter:

- The percentage of subjects having hematology, biochemistry, and urinalysis results below or above the local laboratory normal ranges will be tabulated by timepoint.
- The maximum grading from Screening up to Visit 5 (Day 61)/Visit 7 (Day 390) for subjects receiving 2 doses/Visit 11 (Day 670) for subjects receiving a third dose will be tabulated (grades will be based on local laboratory normal ranges and derived from Food and Drug Administration [FDA] Guidance to Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" [refer to Table 13]). Those laboratory parameters not included in the FDA grading scale will not be graded).

The percentage of subjects using concomitant medication (any medication, any antipyretic and any antipyretic taken prophylactically, respectively) during the 7-day follow-up period and during the 30-day follow-up period will be summarized by group after each vaccination and overall.

Separate safety summaries for Subcohort Set may be generated as appropriate.

9.4.2.2 Internal Safety Review Committee Evaluations

At each iSRC timepoint, a blinded review will be done by the GSK Safety Review Team (SRT) followed by an unblinded review of safety data by the iSRC. No individual clinical study report (CSR) will be written as a result of these safety evaluations.

If there are any safety concerns observed during SRT reviews post Dose 3, an ad hoc iSRC meeting will take place to review the unblinded safety data.

9.4.3 Analysis of Immunogenicity

The primary analysis will be based on the Per-protocol Set for analysis of immunogenicity. If, in any study group, the percentage of vaccinated subjects with serological results excluded from the Per-protocol Set for analysis of immunogenicity is 10% or more, a second analysis based on the Full Analysis Set will be performed.

9.4.3.1 Within Group Assessment

For each study group, at each timepoint that blood samples are collected for humoral immune response and for each assay:

- Percentage of subjects above the assay cutoff with their exact 95% CI will be tabulated.
- Geometric mean concentrations/geometric mean titers and their 95% CI will be calculated.
- Geometric mean fold-rise with their exact 95% CI will be tabulated.

For each study group, at each blood sampling timepoint for humoral immune response after vaccination, and for each assay:

- Antibody concentrations/titers will be investigated using Reverse Cumulative Curves.

9.4.3.2 Between Groups Assessment

CCI [redacted]

9.4.4 Missing Data

Data from subjects who withdraw from the study, including AEs and any follow-up, will be included in the analyses of primary and secondary outcomes. Missing data will not be imputed.

9.5 Interim Analyses

A first interim analysis will be performed when all data up to 1 month after Dose 2, ie, Day 61 (ie, data that are as clean as possible) are available. The immunogenicity data available until Day 61, and the safety and reactogenicity data available at the time of this analysis will be described.

Second interim analysis will be performed when all data up to 1 month after Dose 3, ie, Day 521 (ie, data that are as clean as possible) are available. The immunogenicity data available until Day 521, and the safety and reactogenicity data available at the time of this analysis will be described.

All analyses are descriptive and with the aim to characterize the safety, reactogenicity, and immunogenicity data and therefore no statistical adjustment for interim analysis is required (refer to Section 9.8).

9.6 Safety Review Team

The project's SRT includes as core members the GSK Biologicals' Central Safety Leader, the CRDL, Epidemiologist, Global Regulatory Lead and a Biostatistician of the project. The SRT is responsible for ongoing safety monitoring of the entire project and meets on a regular basis. The SRT will inform the iSRC about any potential safety concern relevant to the study (in a blinded way).

Before each iSRC safety evaluation in this study (see below), the SRT will review the same safety data, but in a blinded manner in order to keep all people involved in the conduct, cleaning and final analysis of the study blinded.

9.7 Internal Safety Review Committee

As the investigational vaccine formulation will be administered to human for the first time, an iSRC will be appointed in addition to the project's existing SRT and safety holding rules have been defined.

This study will be overseen by an iSRC operating under a charter. Core members of the iSRC will include a GSK safety physician, a CRDL/CEPL, and a biostatistician who are not otherwise involved in the conduct of the project. The iSRC safety reviews will be conducted using unblinded data. Upon request, the iSRC has access to the subject randomization and reviews unblinded data.

In Steps 1, 2, and 3, the iSRC will review all accumulating safety data after Dose 1 and then again after Dose 2, and in Step 4 the iSRC will review all accumulating safety data 3 weeks after the start of vaccination in Step 4 and then about every 3 weeks until all subjects have received Dose 1. If there are any safety concerns observed during SRT reviews post Dose 3, an ad hoc iSRC meeting will take place to review the unblinded safety data.

The iSRC members will determine whether any of the predefined holding rules are met (refer to Section 4.6.1) or if there is any other safety signal. In this case, administration of the second vaccination will be immediately put on hold (refer to Section 4.6.2). If no safety signal is observed, the second vaccination will be administered to the subjects. At the end of each step, the iSRC will review all safety data from the entire step. The iSRC members will determine whether any of the predefined holding rules are met (refer to Section 4.6.1) or if there is any other safety signal. In this case, vaccination in the next step will be put on hold (refer to Section 4.6.2). If no safety signal is observed, vaccination in the next step will be performed (refer to Figure 1).

9.8 Sequence of Analysis

The statistical analyses will be performed in 3 steps:

- A first interim analysis will be performed when all data up to 1 month after Dose 2, ie, Day 61 (ie, data that are as clean as possible) are available. The immunogenicity data available until Day 61, and the safety and reactogenicity data available at the time of this analysis will be described. At this point, the statistician will be unblinded for the analysis (ie, will have access to individual subject treatment assignments). The remaining study personnel will stay blinded (ie, will not have access to the individual subject treatment assignment) until study end. It is possible however, due to the limited sample size, that unblinding occurs

for a few subjects having a specific AE or SAE (eg, an AE/SAE occurring only in a single group). Therefore, anyone having access to the analysis of Day 61 could become unblinded regarding those specific cases. The study will be considered as partial blind from this point onwards. Individual listings will be provided at the study end to the investigator. Subjects will be provided with information about the study arm, via the investigator at the study end.

- The second interim analysis will be performed when all data up to 1 month after Dose 3, ie, Day 521 (ie, data that are as clean as possible) are available. The immunogenicity data available until Day 521, and the safety and reactogenicity data available at the time of this analysis will be described. No individual listings will be provided.
- The final analysis will be performed when all data up to study end are available. An integrated CSR containing all data will be written and made available to the Principal Investigator at that time. In addition, all previous analyses will be re-produced based on cleaned data at this point. Individual listings will only be provided at this stage.

CCI [redacted]

The final CSR will contain at least the final analyses of all primary and secondary endpoints.

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11.0 APPENDICES

Appendix 1 Abbreviations

Abbreviation	Definition
AE	Adverse event
AESI	Adverse event of specific interest
Ag	Antigen
ANCOVA	Analysis of Covariance
$AS01_B$	An adjuvant system containing MPL, QS-21 and liposome (50 µg MPL and 50 µg QS-21)
CDI	*Clostridium difficile* infection
C. difficile	*Clostridium difficile*
CEPL	Clinical and Epidemiology Project Lead
CFR	Code of Federal Regulations
CI	Confidence interval
CRDL	Clinical Research and Development Lead
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CTA	Clinical Trial Application
eCRF	Electronic case report form
ELISA	Enzyme-linked immunosorbent assay
EoS	End of Study
FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
GMFR	Geometric mean fold-rise
GSK	GlaxoSmithKline Biologicals SA
HRT	Hormone replacement therapy
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IM	Intramuscular

Abbreviation	Definition
IRB	Institutional Review Board
iSRC	Internal safety review committee
MedDRA	Medical Dictionary for Regulatory Activities
MPL	3-O-desacyl-4'-monophosphoryl lipid A
PBMC	Peripheral blood mononuclear cell
PCD	Primary completion date
pIMD	Potential immune-mediated disease
QS-21	*Quillaja saponaria* Molina, fraction 21 (Licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation)
SAE	Serious adverse event
SAP	Statistical analysis plan
SBIR	System Built for Internet Randomization
SD	Standard deviation
SPM	Study Procedures Manual
SRT	Safety Review Team
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNA	Toxin Neutralization Assay
ULN	Upper limit of normal
USA	United States of America

Appendix 2 Glossary of Terms

Adverse Event:	Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.
Blinding:	A procedure in which 1 or more parties to the study are kept unaware of the treatment assignment in order to reduce the risk of biased study outcomes. The level of blinding is maintained throughout the conduct of the study, and only when the data are cleaned to an acceptable level of quality will appropriate personnel be unblinded or when required in case of a serious adverse event. In a partial-blind study, the subject, the investigator, or anyone assessing the outcome is aware of the assignment in at least one treatment group but unaware of the treatment assignment(s) in one or more other treatment groups. In an observer-blind study, the subject and the study center and Sponsor personnel involved in the clinical evaluation of the subjects are blinded while other study personnel may be aware of the treatment assignment (see Section 6.6.3 for details on observer-blinded studies).
Certified Copy:	A copy (irrespective of the type of media used) of the original record that has been verified (ie, by a dated signature or by generation through a validated process) to have the same information, including data that describe the context, content, and structure, as the original.
Eligible:	Qualified for enrollment into the study based upon strict adherence to inclusion/exclusion criteria.
End of Study: **(Synonym of End of Trial):**	For studies with collection of human biological samples, the End of Study is defined as last testing results released of samples collected at Visit 11 (Day 670)*. *In this case End of Study must be achieved no later than 8 months after last subject last visit.

Essential Documents:	Documents which individually and collectively permit evaluation of the conduct of a study and the quality of the data produced.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the Per-protocol analysis (see Section 9.3 for details on criteria for evaluability).
Immunological Correlate of Protection:	The defined immune response above which there is a high likelihood of protection in the absence of any host factors that might increase susceptibility to the infectious agent.
Investigational Vaccine: (Synonym of Investigational Medicinal Product)	A pharmaceutical form of an active ingredient being tested in a clinical study, including a product with a marketing authorization when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.
Investigator:	A person responsible for the conduct of the clinical study at a study center. If a study is conducted by a team of individuals at a study center, the Investigator is the responsible leader of the team and may be called the Principal Investigator. The Investigator can delegate study-related duties and functions conducted at the study center to qualified individual or party to perform those study-related duties and functions.
Potential Immune-mediated Disease:	Potential immune-mediated diseases are a subset of adverse events that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune aetiology.
Primary Completion Date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for all primary outcomes, whether the clinical study was concluded according to the prespecified protocol or was terminated.
Protocol Amendment:	The International Council on Harmonisation defines a protocol amendment as: “A written description of a change(s) to or formal clarification of a protocol.” GSK further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.
Randomization:	Process of random attribution of treatment to subjects in order to reduce bias of selection.

Solicited Adverse Event:	Adverse events to be recorded as endpoints in the clinical study. The presence/occurrence/intensity of these events is actively solicited from the subject or an observer during a specified postvaccination follow-up period.
Source Data:	All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents (original records or certified copies).
Source Documents:	Original documents, data, and records (eg, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical study).
Study Vaccine:	Any investigational vaccine being tested and/or any authorized use of a vaccine/placebo as a reference or administered concomitantly, in a clinical study that evaluates the use of an investigational vaccine.
Study Conclusion for Individual Subjects:	The study conclusion for subjects receiving 2 doses will be Visit 7 (Day 390) and for subjects receiving 3 doses will be Visit 11 (Day 670).
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the vaccine(s) or as a control.
Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject.
Treatment Number:	A number identifying a treatment to a subject, according to the treatment allocation.
Unsolicited Adverse Event:	Any adverse event reported in addition to those solicited during the clinical study. Also, any "solicited" symptom with onset outside the specified period of follow-up for solicited symptoms will be reported as an unsolicited adverse event.

Appendix 3 Regulatory, Ethical, and Study Oversight Considerations

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines.
  - Applicable ICH GCP Guidelines.
  - Applicable laws and regulations.
- The protocol, protocol amendments, ICF or informed assent form, IB, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the Investigator for review and approval. These documents will be signed and dated by the Investigator before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to subjects.
- GSK will provide full details of the above procedures to the Investigator, either verbally, in writing, or both.
- The Investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.
  - Providing oversight of the conduct of the study at the study center and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.
- After reading the protocol, each Investigator will sign the protocol signature page and send a copy of the signed page to the Sponsor or representative Appendix 8 The study will not start at any study center at which the Investigator has not signed the protocol.

Adequate Resources

The Investigator is responsible for supervising any individual or party to whom the Investigator delegates study-related duties and functions conducted at the study center.

If the Investigator/institution retains the services of any individual or party to perform study related duties and functions, the Investigator/institution should ensure this individual or party is

qualified to perform those study-related duties and functions and should implement procedures to ensure the integrity of the study-related duties and functions performed and any data generated.

Financial Disclosure

Investigators and Sub-investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interest prior initiation of the study center and at the end of the study. Investigators are responsible for providing an update of Financial Disclosure if their financial interest changes at any point during their participation in a study and for 1 year after completion of the study.

Insurance

The Sponsor has obtained liability insurance, which covers this study as required by local law and/or national regulations and/or ICH guidelines, whichever is applicable. The terms of the insurance will be kept in the study files.

Informed Consent Process

- The Investigator or his/her representative will explain the nature of the study to the subject or his/her legally authorized representative and answer all questions regarding the study.
- Subjects must be informed that their participation is voluntary.
- Freely given and written or witnessed/thumb printed informed consent must be obtained from each subject, as appropriate, prior to participation in the study.
- The content of ICF must meet the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the subject was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Subjects must be reconsented to the most current version of the ICF(s) or an ICF addendum during their participation in the study.
- A copy of the ICF(s) must be provided to the subject.
- Subjects who are rescreened outside of the protocol screening interval are required to sign a new ICF.

Data Protection

- Subjects will be assigned a unique identifier by the Sponsor. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.

- The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law.
- The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.
- GSK will also ensure the protection of personal data of Investigator and the study center staff which will be collected within the frame and for the purpose of the study.

Administrative Structure

See Section 9.6 for information regarding the iSRC.

Medical Monitor

PPD

Phone: PPD

Medical Oversight from GSK

PPD, up to End of March 2021 and
PPD, as of April 2021.

Publication Policy

GSK aims to publish the results of this study in searchable, peer reviewed scientific literature. GSK will target to submit within 18 months from last subject last visit for interventional studies and from the completion of the analysis for noninterventional studies and follows the guidance from the International Committee of Medical Journal Editors.

Dissemination of Clinical Study Data

The key design elements of this protocol will be posted on the GSK Clinical Study Register and on publicly accessible registers including ClinicalTrials.gov. Where required, protocol summaries will also be posted on national or regional clinical trial registers or databases (eg, EudraCT database) in compliance with the applicable regulations.

GSK also assures that results will be submitted to ClinicalTrials.gov within the required timeframe, in compliance with the current regulations mentioned in the table below.

At the time of study results posting, the full study protocol and SAP will also be posted on ClinicalTrials.gov.

In addition, for studies that are in scope of the EU Clinical Trial Directive, summaries of the results of GSK interventional studies (Phase I to IV) in adult population will be posted within defined timelines on the publicly available EU Clinical Trial Register.

If it is not possible to submit a summary of the results within the required timelines in the concerned EU member state, the summary of results shall be submitted as soon as it is available. In this case, the protocol shall specify when the results are going to be submitted, together with a justification.

	Clinicaltrial.gov	**EU**
Protocol summary	Before enrollment of subjects	As per CTA submission/before enrollment of subjects.
Results summary	Within 12 months of PCD (primary and safety endpoint results)/Within 12 months of last subject last visit[a] (for secondary endpoint results)	Within 6 months (for pediatric population studies)/within 12 months (for adult population studies) of EoS[a].

CTA = Clinical Trial Applications; EoS End of Study; PCD = primary completion date.

[a] As defined in the study protocol.

Under the framework of the SHARE initiative, anonymized patient-level data from GSK sponsored interventional studies that evaluate products will be made available within 6 months of this publication to independent researchers whose research proposals have been approved by an independent panel. Requests for access may be made through www.clinicalstudydatarequest.com.

Where required by applicable regulatory requirements, an Investigator signatory will be identified for the approval of the CSR, provided reasonable access to statistical tables, figures, and relevant reports. GSK will also provide the Investigator with the full summary of the study results. The Investigator is encouraged to share the summary results with the study subjects, as appropriate.

Data Quality Assurance

The Investigator should maintain a record of the location(s) of their respective essential documents including source documents. The storage system used during the study and for archiving (irrespective of the type of media used) should provide for document identification, version history, search, and retrieval.

Essential documents for the study may be added or reduced where justified (in advance of study initiation) based on the importance and relevance to the study. When a copy is used to replace an original document (eg, source documents, eCRF), the copy should fulfil the requirements for certified copies.

All subject data relating to the study will be recorded on printed or eCRF unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the eCRF.

The Investigator must maintain adequate and accurate source documents and study records that include all pertinent observations on each of the study center's subjects that supports the information entered in the eCRF.

The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source documents or certified copies.

The Sponsor or designee is responsible for the data management of this study including quality checking of the source data.

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized study center personnel are attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (eg, via an audit trail). Safety and rights of subjects must be protected and study be conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Study records and source documents, including signed ICF, pertaining to the conduct of this study must be retained by the Investigator for 25 years from the issue of the final CSR/equivalent summary unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

Source Documents

Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Investigator should maintain a record of the location(s) of their source documents.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Definition of what constitutes source data and source documents can be found in Appendix 2.

Study and Study Center Closure

GSK or its designee reserves the right to close the study center or terminate the study at any time for any reason at the sole discretion of GSK, provided there is sufficient notice given to account for patient's safe exit from study participation. Study center regular closure will be upon study completion. A study center is considered closed when all required data/documents and study supplies have been collected and a study center closure visit has been performed.

The Investigator may initiate study center closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study center by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of subjects by the Investigator.
- Discontinuation of further study treatment development.

Appendix 4 Clinical Safety Laboratory Tests

The tests detailed in Table 25 will be performed by the local laboratory. Refer to Appendix 5 for GSK laboratories.

Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5.0 of the protocol.

The Investigator is not allowed to do extra testing on samples outside of what has been agreed upon by the ethics committees.

Table 25 Protocol-required Safety Laboratory Assessments

System	Discipline	Component	Scale	Timepoints	Method	Laboratory
Whole blood	Hematology	Leukocytes (white blood cells) Platelet count Hemoglobin	Quantitative	Screening Visit 2 (Day 8) Visit 3 (Day 31) Visit 4 (Day 38) Visit 6 (Day 180) Visit 7 (Day 390) Screening for Dose 3 (Day 476) Visit 8 (Day 491) Visit 9 (Day 498) Visit 11 (Day 670)	As per local practice	Local laboratory
Serum	Biochemistry	Alanine aminotransferase Aspartate aminotransferase Creatinine Urate/uric acid C-reactive protein	Quantitative	Screening Screening for Dose 3 (Day 476) Visit 8 (Day 491) Visit 9 (Day 498) Visit 11 (Day 670)		
Urine	Urinalysis	Erythrocytes (red blood cells) Protein Glucose	Quantitative/ semi-quantitative	Screening Screening for Dose 3 (Day 476)		

Appendix 5 Clinical Laboratories

GlaxoSmithKline Biologicals SA laboratories:

Laboratory	**Address**
GSK Biological's Clinical Laboratory Sciences, Rixensart	Biospecimen Reception-B7/44 Rue de l'Institut, 89 B-1330 Rixensart Belgium
GSK Biological's Clinical Laboratory Sciences, Wavre-Nord Noir Epine	Avenue Fleming, 20 B-1300 Wavre Belgium

Outsourced Laboratory:

Laboratory	**Address**
Nexelis	525, Cartier Ouest Laval Quebec Canada H7V 3S8 Nexelis

Investigator's Local Laboratory:

Laboratory	**Address**
CEVAC Core Laboratory	University of Ghent 185 De Pintelaan, GENT Oost-Vlaanderen 9000 Belgium

Appendix 6 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of Adverse Event

Adverse Event Definition
• An AE is any untoward medical occurrence in a clinical study subject, temporally associated with the use of a study treatment, whether or not considered related to the study treatment. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

Events Meeting the Adverse Event Definition
• Significant or unexpected worsening or exacerbation of the condition/indication under study. • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study vaccine administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study vaccine or a concomitant medication (overdose per se will not be reported as an AE/SAE). • Signs, symptoms temporally associated with study vaccine(s) administration. • Pre or posttreatment events that occur as a result of protocol-mandated procedures (ie, invasive procedures, modification of subject's previous therapeutic regimen). • Medically attended visits related to AEs (eg, hospital stays, physician visits and emergency room visits). Adverse events to be recorded as endpoints (solicited AEs) are described below under "Solicited Adverse Events." All other AEs will be recorded as UNSOLICITED AEs.

Events NOT Meeting the Adverse Event Definition
• Situations where an untoward medical occurrence did not occur (eg, social and/or convenience admission to a hospital, admission for routine examination). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen. • Pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination. These events will be recorded in the Medical History section of the eCRF.

Definition of Serious Adverse Event

An SAE is any untoward medical occurrence that:
a) Results in death
b) Is life-threatening

The term “life-threatening” in the definition of “serious” refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c) Requires hospitalization or prolongation of existing hospitalization Note: In general, hospitalization signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician’s office or in an outpatient setting. Complications that occur during hospitalization are also considered AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether “hospitalization” occurred, or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an AE.
d) Results in disability/incapacity The term disability means a substantial disruption of a person’s ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e) Is a congenital anomaly/birth defect in the offspring of a study subject
Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent 1 of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

Solicited Adverse Events

a) Solicited local (injection site) AEs. • Pain at injection site. • Redness at injection site. • Swelling at injection site.
b) Solicited general AEs. • Fatigue. • Fever. • Gastrointestinal symptoms.* • Headache. • Myalgia. • Shivering. • Arthralgia. *Gastrointestinal symptoms include nausea, vomiting, diarrhea, and/or abdominal pain. Note: Subjects will be instructed to measure and record the oral body temperature in the evening. Should additional temperature measurements be performed at other times of day, subjects will be instructed to record the highest temperature in the diary card.

Unsolicited Adverse Events

An unsolicited AE is an AE that was not solicited using a Subject Diary and that was spontaneously communicated by subjects who has signed the informed consent.

Potential unsolicited AEs may be medically attended (defined as symptoms or illnesses requiring hospitalization, or emergency room visit, or visit to/by a health care provider) or were of concern to the subjects. In case of such events, subjects will be instructed to contact the study center as soon as possible to report the event(s). The detailed information about the reported unsolicited AEs will be collected by the qualified study center personnel during the interview and will be documented in the subject's records.

Unsolicited AEs that are not medically attended nor perceived as a concern by subjects will be collected during interview with the subjects and by review of available medical records at the next visit.

Adverse Events of Specific Interest

Potential immune-mediated Diseases

- Potential immune-mediated diseases are a subset of AESIs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology (refer to Table 18 for a list of pIMDs).
- However, the Investigator will exercise his/her medical and scientific judgment in deciding whether other diseases have an autoimmune origin (ie, pathophysiology involving systemic or organ-specific pathogenic autoantibodies) and should also be recorded as a pIMD.

When there is enough evidence to make any of the above diagnoses, the AE must be reported as AESI. Symptoms, signs or conditions which might (or might not) represent the above diagnoses, should be recorded and reported as AEs but not as AESI until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

Clinical Laboratory Parameters and Other Abnormal Assessments Qualifying as Adverse Events or Serious Adverse Events

In absence of diagnosis, abnormal laboratory findings (eg, clinical chemistry, hematology, urinalysis) or other abnormal assessments that are judged by the Investigator to be clinically significant will be recorded as AE or SAE if they meet the definition of an AE or SAE. Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the Investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, will not be reported as AEs or SAEs.

The Investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

In case of invalid or missing results or clinically significant abnormal laboratory findings that cannot be reasonably explained (eg, due to a pre-existing or current medical condition), the Investigator will be recommended to recall the subject in a timely manner (preferably within 7 days after Investigator's awareness/assessment of the abnormal findings, if applicable) for a repeat test to confirm the result.

Pregnancy

Female subjects who are pregnant or lactating at the time of vaccination must not receive additional doses of study vaccine(s) but may continue other study procedures at the discretion of the Investigator.

While pregnancy is not considered an AE or SAE, any adverse pregnancy outcome or complication or elective termination of a pregnancy for medical reasons will be recorded and reported as an AE or a SAE.

Note: The pregnancy should always be recorded on an electronic pregnancy report.

The following should always be considered as SAE and will be reported as described in "Prompt Reporting of Serious Adverse Events, Pregnancies, and Other Events" and "Completion and Transmission of Pregnancy Reports to GSK":

- Spontaneous pregnancy loss, including:
  - spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation).
  - ectopic and molar pregnancy.
  - stillbirth (intrauterine death of fetus after 22 weeks of gestation).

Note: the 22 weeks' cutoff in gestational age is based on World Health Organization-International Classification of Diseases 10 noted in the European Medicines Agency Guideline on pregnancy exposure [EMA, 2006]. It is recognized that national regulations might be different.

- Any early neonatal death (ie, death of a live born infant occurring within the first 7 days of life).
- Any congenital anomaly or birth defect identified in the offspring of a study subject (either during pregnancy, at birth or later) regardless of whether the fetus is delivered dead or alive. This includes anomalies identified by prenatal ultrasound, amniocentesis or examination of the products of conception after elective or spontaneous abortion.

Furthermore, any SAE occurring as a result of a poststudy pregnancy AND considered by the Investigator to be reasonably related to the study vaccine(s) will be reported to GSK as described in "Reporting of Serious Adverse Events, Pregnancies, and Other Events." While the Investigator is not obligated to actively seek this information from former subjects, he/she may learn of a pregnancy through spontaneous reporting.

Detecting and Recording Adverse Events, Serious Adverse Events and Pregnancies

A paper diary hereafter referred to as Subject Diary will be used in this study to capture solicited AEs. The subject should be trained on how and when to complete each field of the Subject Diary.

The subjects will be instructed to contact the Investigator immediately should the subjects manifest any signs or symptoms they perceive as serious.

Subject Diary training should be directed at the individual(s) who will perform the measurements of AEs and who will enter the information into the Subject Diary. This individual may not be the subject, but if a person other than the subject enters information into the Subject Diary, this person's identity must be documented in the Subject Diary/subject's source record. Any individual that makes entries into the Subject Diary must receive training on completion of the Subject Diary at the time of the visit when Subject Diary is dispensed. This training must be documented in the subject's source record.

- Collect and verify completed diary cards during discussion with the subject on Visit 7.

- Any unreturned diary cards will be sought from the subject through telephone call(s) or any other convenient procedure.

The Investigator will transcribe the collected information into the eCRF in English.

Time Period for Detecting and Recording Adverse Events, Serious Adverse Events, and Pregnancies
• All solicited AEs during 7 days following administration of each dose of study vaccine(s) (Day 1 to Day 7) must be recorded in the eCRF, irrespective of intensity or whether or not they are considered vaccination-related. • All other AEs during 30 days following administration of each dose of study vaccine(s) (Day 1 to Day 30) must be recorded in the appropriate section of the eCRF, irrespective of intensity or whether or not they are considered vaccination-related. • The time period for collecting and recording SAEs will begin at the first receipt of study vaccine(s) and will end 359 days following administration of the last dose of study vaccine(s) for each subject receiving 2 doses and 6 months following administration of the last dose of study vaccine(s) for each subject receiving 3 doses. See "Reporting of Serious Adverse Events, Pregnancies, and Other Events" for instructions on reporting of SAEs. • All AEs/SAEs leading to withdrawal from the study will be collected and recorded from the time of the first receipt of study vaccine. • In addition to the above-mentioned reporting requirements and in order to fulfil international reporting obligations, SAEs that are related to study participation (ie, protocol-mandated procedures, invasive tests, a change from existing therapy) or are related to a concurrent GSK medication/vaccine will be collected and recorded from the time the subject consents to participate in the study until she/he is discharged from the study. • The time period for collecting and recording pregnancies will begin at the first receipt of study vaccine(s) and will end 359 days following administration of the last dose of study vaccine(s) each subject receiving 2 doses and 6 months following administration of the last dose of study vaccine(s) for each subject receiving 3 doses. See "Reporting of Serious Adverse Events, Pregnancies, and Other Events" for instructions on reporting of pregnancies. • The time period for collecting and recording of AESIs will begin at the first receipt of study vaccine(s) and will end 359 days following administration of the last dose of study vaccine(s) each subject receiving 2 doses and 6 months following administration of the last dose of study vaccine(s) for each subject receiving 3 doses. See "Reporting of Adverse Events of Specific Interest to GSK" for instructions on reporting of AESIs.
Evaluation of Adverse Events and Serious Adverse Events
Active questioning to detect AEs and SAEs: • As a consistent method of collecting AEs, the subject should be asked nonleading questions such as: "Have you felt different in any way since receiving the vaccine(s) or since the previous visit?" • When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (eg, hospital progress notes, laboratory and diagnostics reports) relative to the event. The Investigator will then record all relevant information regarding an AE/SAE in the eCRF. The Investigator is not allowed to send photocopies of the subject's medical records to GSK instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK.

- The Investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

Assessment of Adverse Events:

Assessment of Intensity

The intensity of the following solicited AEs will be assessed as described in the table below:

Adverse Event	Intensity grade	Parameter
CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.		

CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.

[a] Fever is defined as temperature ≥38.0°C/100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.

The Investigator will assess the maximum intensity that occurred over the duration of the event for all unsolicited AEs (including SAEs) recorded during the study. The assessment will be based on the Investigator's clinical judgment.

The intensity should be assigned to 1 of the following categories:

CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.

CCI An event is defined as "serious" when it meets 1 of the predefined outcomes as described in "Definition of Serious Adverse Events."

Assessment of Causality

The Investigator is obligated to assess the relationship between study vaccine(s) and the occurrence of each AE/SAE using clinical judgment. In case of concomitant administration of multiple vaccines, if possible, the Investigator should specify if the AE could be causally related to a specific vaccine administered (ie, investigational, control/placebo or co-administered vaccine). When causal relationship to a specific vaccine(s) cannot be determined, the Investigator should indicate the AE to be related to all products.

Alternative plausible causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study vaccine(s) will be considered and investigated. The Investigator will also consult the IB and/or Summary of Product Characteristics and/or Prescribing Information for marketed products to determine his/her assessment.

There may be situations when an SAE has occurred and the Investigator has minimal information to include in the initial report to GSK. However, it is very important that the Investigator always makes an assessment of causality for every event prior to submission of the Expedited Adverse Events Report to GSK. The Investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly. The causality assessment is 1 of the criteria used when determining regulatory reporting requirements.

All solicited local (injection site) reactions will be considered causally related to vaccination. Causality of all other AEs should be assessed by the Investigator using the following question:

Is there a reasonable possibility that the AE may have been caused by the study vaccine?

YES: There is a reasonable possibility that the study vaccine(s) contributed to the AE.
NO: There is no reasonable possibility that the AE is causally related to the administration of the study vaccine(s). There are other, more likely causes and administration of the study vaccine(s) is not suspected to have contributed to the AE.

If an event meets the criteria to be determined as "serious" (see "Definition of Serious Adverse Events"), additional examinations/tests will be performed by the Investigator in order to determine ALL possible contributing factors for each SAE.

Possible contributing factors include:

- Medical history.
- Other medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Lack of efficacy of the vaccine, if applicable.
- Erroneous administration.
- Other cause (specify).

Assessment of Outcome

The Investigator will assess the outcome of all unsolicited AEs (including SAEs) recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

Medically Attended Visits

For each solicited and unsolicited symptom the subject experiences, the subject will be asked if he/she received medical attention defined as hospitalization, or an otherwise unscheduled visit to or from medical personnel for any reason, including emergency room visits. This information will be recorded in the eCRF.

Reporting of Serious Adverse Events, Pregnancies, and Other Events

Prompt Reporting of Serious Adverse Events, Pregnancies, and Other Events to GSK

- SAEs that occur in the time period defined in "Detecting and Recording Adverse Events, Serious Adverse Events, and Pregnancies" will be reported promptly to GSK within the timeframes described in the table below, once the Investigator determines that the event meets the protocol definition of an SAE.
- Pregnancies that occur in the time period defined in "Detecting and Recording Adverse Events, Serious Adverse Events, and Pregnancies" will be reported promptly to GSK within the timeframes described in the table below, once the Investigator becomes aware of the pregnancy.
- AESIs that occur in the time period defined in "Detecting and Recording Adverse Events, Serious Adverse Events, and Pregnancies" will be reported promptly to GSK within the timeframes described

in the table below, once the Investigator determines that the event meets the protocol definition of a AESIs.

Timeframes for submitting SAEs, pregnancy, and other events reports to GSK:

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	**Documents**	**Timeframe**	**Documents**
SAEs	24 hours[a,c]	Electronic Expedited Adverse Events Report	24 hours[a]	Electronic Expedited Adverse Events Report
Pregnancies	24 hours[a]	Electronic pregnancy report	24 hours[a]	Electronic pregnancy report
AESIs (pIMDs)	24 hours[b,c]	Electronic Expedited Adverse Events Report	24 hours[a]	Electronic Expedited Adverse Events Report

AESI = adverse event of specific interest; pIMD = potential immune-mediated disease; SAE = serious adverse event

a Timeframe allowed after receipt or awareness of the information.

b Timeframe allowed once the Investigator determines that the event meets the protocol definition of an AESI.

c The Investigator will be required to confirm review of the SAE/AESI causality by ticking the "reviewed" box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE/AESI.

Serious Adverse Events Requiring Expedited Reporting to GSK

- Once an Investigator becomes aware that an SAE has occurred in a study subject, the Investigator (or designate) must complete the information in the electronic Expedited Adverse Events Report WITHIN 24 HOURS. The report will always be completed as thoroughly as possible with all available details of the event. Even if the Investigator does not have all information regarding an SAE, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS.
- The Investigator will always provide an assessment of causality at the time of the initial report. The Investigator will be required to confirm the review of the SAE causality by ticking the "reviewed" box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE.

Back-up System in Case the Electronic Reporting System Does Not Work

- If the electronic reporting system does not work, the Investigator (or designate) must complete, then date and sign a paper Expedited Adverse Events Report and fax it to the Study Contact for Reporting SAEs (refer to the Sponsor Information) or to GSK Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic reporting system is not working and NOT if the system is slow. As soon as the electronic reporting system is working again, the Investigator (or designate) must complete the electronic Expedited Adverse Events Report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

Completion and Transmission of Pregnancy Reports to GSK

- Once the Investigator becomes aware that a subject is pregnant, the Investigator (or designate) must complete the required information onto the electronic pregnancy report WITHIN 24 hours.

  Note: Conventionally, the estimated gestational age of a pregnancy is dated from the first day of the last menstrual period of the cycle in which a woman conceives. If the last menstrual period is uncertain or unknown, dating of estimated gestational age and the estimated date of delivery should be estimated by ultrasound examination and recorded in the pregnancy report.

Reporting of AESI's to GSK
• Once an AESI is diagnosed (serious or nonserious) in a study subject, the Investigator (or designate) must complete the information in the electronic Expedited Adverse Events Report WITHIN 24 HOURS after he/she becomes aware of the diagnosis. The report allows specify that the event is an AESI and whether it is serious or nonserious. The report will always be completed as thoroughly as possible with all available details of the event, in accordance with the AESIs standard questionnaire provided. Even if the Investigator does not have all information regarding an AESI, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS. • The Investigator will always provide an assessment of causality at the time of the initial report. The Investigator will be required to confirm the review of the AESI causality by ticking the "reviewed" box in the electronic Expedited Adverse Events Report within 72 hours of submission of the AESI.

Updating of Serious Adverse Events, Pregnancy, and Adverse Events of Specific Interest Information After Removal of Write Access to the Subject's Electronic Case Report Form

When additional SAE, pregnancy, or AESI information is received after removal of the write access to the subject's eCRF, new or updated information should be recorded on the appropriate paper report, with all changes signed and dated by the Investigator. The updated report should be faxed to the Study Contact for Reporting SAEs (refer to the Sponsor Information) or to GSK Clinical Safety and Pharmacovigilance department within the designated reporting timeframes specified in "Timeframes for submitting SAEs, pregnancy, and other events reports to GSK".

Follow-up of Adverse Events, Serious Adverse Events, and Pregnancies

Follow-up of Adverse Events, Serious Adverse Events During the Study
• After the initial AE/SAE report, the Investigator is required to proactively follow each subject and provide additional relevant information on the subject's condition to GSK (within 24 hours for SAEs; refer to: Timeframes for submitting SAEs, pregnancy, and other events reports to GSK). • All SAEs and AESIs (serious or nonserious) documented at a previous visit and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits until the last visit of the subject. • All AEs documented at a previous visit and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until 30 days after the last vaccination.
Follow-up After the Subject is Discharged from the Study
The Investigator will follow subjects: • With SAEs, AESIs (serious or nonserious), or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up. If the Investigator receives additional relevant information on a previously reported SAE, he/she will provide this information to GSK using an electronic Expedited Adverse Events Report and/or pregnancy report as applicable. GSK may request that the Investigator performs or arranges the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The Investigator is obliged to assist. If a subject dies during participation in the study or during a

recognized follow-up period, GSK will be provided with any available postmortem findings, including histopathology.
Follow-up of Pregnancies
• Pregnant subjects will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether full-term or premature, information on the status of the mother and child will be forwarded to GSK using the electronic pregnancy report and the Expedited Adverse Events Report if applicable. Generally, the follow-up period doesn't need to be longer than 6 to 8 weeks after the estimated date of delivery. • Regardless of the reporting period for SAEs for this study, if the pregnancy outcome is an SAE, it should always be reported as SAE.

Appendix 7 Contraceptive Guidance and Collection of Pregnancy Information

Definitions:

Woman of Childbearing Potential

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories are not considered women of childbearing potential

- Premenarchal
  Menarche is the onset of menses for the first time in a young female and is preceded by several changes associated with puberty including breast development and pubic hair growth. Menarche usually occurs within 1 to 2 years of breast development, thelarche. However, a young female can become pregnant before her first menses. Thus, a conservative definition of nonchildbearing potential in a premenarcheal female is a young female who has not yet entered puberty as evidenced by lack of breast development (palpable glandular breast tissue).
- Premenopausal female with 1 of the following:
  - Documented hysterectomy.
  - Documented bilateral salpingectomy.
  - Documented bilateral oophorectomy.
    Note: Documentation can come from the study center personnel's review of the subject's medical records, medical examination, or medical history interview.
- Postmenopausal female
  A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormone replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
- Females on HRT and whose menopausal status is in doubt will be required to use 1 of the nonhormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance

- Female subjects of childbearing potential are eligible to participate if they agree to use an adequate contraception consistently and correctly according to the methods listed in GSK list of highly effective contraceptive methods provided below:

Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*
Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[b] • Oral. • Intravaginal. • Transdermal.
Progestogen only hormonal contraception associated with inhibition of ovulation[b] • Injectable.
Highly Effective Methods That Are User Independent [a]
• Implantable progestogen-only hormonal contraception associated with inhibition of ovulation[b]. • Intrauterine device. • Intrauterine hormone-releasing system. • Bilateral tubal occlusion.
Vasectomized Partner *A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.*
Male partner sterilization prior to the female subject's entry into the study, and this male is the sole partner for that subject (*the information on the male sterility can come from the study center personnel's review of the subject's medical records; medical examination and/or semen analysis, or medical history interview provided by her or her partner*).
Sexual Abstinence *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.*
NOTES: [a] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for subjects in clinical studies. [b] Hormonal contraception may be susceptible to interaction with the study treatment, which may reduce the efficacy of the contraceptive method. In this case, 2 highly effective methods of contraception should be utilized during the treatment period and for at least 2 months after the last dose of study treatment.

Collection of Pregnancy Information

Female subjects who become pregnant

- Investigator will collect pregnancy information on any female subject, who becomes pregnant while participating in this study.
- Information will be recorded on the appropriate form and submitted to GSK within 24 hours of learning of a subject's pregnancy.
- Subject will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on subject and neonate, which will be forwarded to GSK. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date.
- Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE.
- A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any SAE occurring as a result of a poststudy pregnancy which is considered reasonably related to the study treatment by the Investigator, will be reported to GSK as described in "Reporting of Serious Adverse Events, Pregnancies, and Other Events." While the Investigator is not obligated to actively seek this information in former study subjects, he or she may learn of an SAE through spontaneous reporting.

Any female subject who becomes pregnant while participating will discontinue study treatment.

Appendix 8 Signature of Investigator

PROTOCOL TITLE: A Phase I, Single-center, Randomized, Observer-blind, Placebo-controlled Study to Evaluate Safety, Reactogenicity and Immunogenicity of GSK's *Clostridium difficile* Investigational Vaccine Based on the F2 Antigen With or Without $AS01_B$ Adjuvant, When Administered Intramuscularly According to a 0, 1-month Schedule to Healthy Adults Aged Between 18-45 Years and Between 50-70 Years, Followed by an Additional Dose Administered in a Partial blind Manner Within an Interval of Approximately 15 Months After Dose 2, in a Subcohort of Subjects aged 50-70 Years

PROTOCOL NO: 208109 (CDIFF-004)

VERSION: Amendment 04

This protocol is a confidential communication of GlaxoSmithKline Biologicals SA. I confirm that I have read this protocol, I understand it, and I will work according to this protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices and the applicable laws and regulations. Acceptance of this document constitutes my agreement that no unpublished information contained herein will be published or disclosed without prior written approval from the Sponsor.

Instructions to the Investigator: Please SIGN and DATE this signature page. PRINT your name, title, and the name of the study center in which the study will be conducted. Return the signed copy to Sponsor or CRO.

I have read this protocol in its entirety and agree to conduct the study accordingly:

Signature of Investigator: _____ Date: _____

Printed Name: _____

Investigator Title: _____

Name/Address of Center: _____

_____

_____

Protocol Amendment (Study Level) - 4 - Final Deliverable including Sponsor Signature - 06-Dec-2021

Electronic Signature Manifestation

This page is a manifestation of the electronic signature(s) used in compliance with the organization's electronic signature policies and procedures.

Signer Full Name	Meaning of Signature	Date and Time
PPD	Document Approval (I certify that I have the education, training and experience to perform this task)	06 Dec 2021 08:31:17 UTC