Clinical Study Protocol
Sponsor:
GlaxoSmithKline Biologicals SA
Rue de l'Institut, 89
1330 Rixensart, Belgium

Primary study vaccine and number	• GlaxoSmithKline (GSK) Biologicals' investigational Supra-seasonal Universal Influenza Vaccine (SUIV) - inactivated (GSK3816302A)
Other study vaccines/products	• GSK Biologicals' quadrivalent split virion influenza vaccine *Fluarix Quadrivalent* • Phosphate Buffered Saline (PBS)
eTrack study number and Abbreviated Title	207543 (FLU D-SUIV-ADJ-001)
EudraCT number	2017-001584-20
Investigational New Drug (IND) number	17602
Date of protocol	Final Version 1: 15 May 2017
Date of protocol amendment	Amendment 1 Final: 24 October 2017 Amendment 2 Final: 16 March 2018 Amendment 3 Final: 07 December 2018 Amendment 4 Final: 11 July 2019
Title	Reactogenicity, safety and immunogenicity study of GlaxoSmithKline (GSK) Biologicals' investigational supra-seasonal universal influenza vaccines - inactivated (SUIVs) (GSK3816302A) in healthy adults.
Detailed Title	A Phase I/II, randomized, controlled, observer-blind, multi-center study to assess the reactogenicity, safety and immunogenicity of three GlaxoSmithKline (GSK) Biologicals' investigational supra-seasonal universal influenza vaccines (SUIVs) (unadjuvanted or adjuvanted with AS03 or AS01) administered as a 1 or 2-dose priming schedule followed by a booster dose 12 months post-primary vaccination in 18 to 39 year-old healthy subjects.
Coordinating author (Amended: 11 July 2019)	• PPD Scientific Writer (***Modis Life Sciences*** for GSK Biologicals)

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Contributing authors **(Amended: 11 July 2019)**	• PPD, PPD, PPD, Clinical Research & Development Leads • PPD, PPD, Lead Statisticians • PPD ***, Expert Statistician*** • PPD, Stat Manager • PPD, PPD **,** Study Delivery Lead***s*** • PPD, PPD, Clinical Trial Supply Managers • PPD, Clinical Read-out Team Lead • PPD, Safety representative • PPD, Oversight Data Manager • PPD, PPD, Global Regulatory Affairs representative***s*** • PPD, PPD, Global Patent representatives • PPD, PPD, Pre-clinical representatives • PPD, PPD, Clinical and Epidemiology Project Leads

GSK Biologicals' Protocol DS v 15.0

Protocol Amendment 4 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	207543 (FLU D-SUIV-ADJ-001)
EudraCT number	2017-001584-20
IND number	17602
Date of protocol amendment	Amendment 4 Final: 11 July 2019
Detailed Title	A Phase I/II, randomized, controlled, observer-blind, multi-center study to assess the reactogenicity, safety and immunogenicity of three GlaxoSmithKline (GSK) Biologicals' investigational supra-seasonal universal influenza vaccines (SUIVs) (unadjuvanted or adjuvanted with AS03 or AS01) administered as a 1 or 2-dose priming schedule followed by a booster dose 12 months post-primary vaccination in 18 to 39 year-old healthy subjects.
Sponsor signatory	Frank Struyf Clinical and Epidemiology Project Lead, Supra-seasonal Influenza Vaccine
Signature	_____
Date	_____

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Protocol Amendment 4 Rationale

Amendment number: Amendment 4

Rationale/background for changes:

Following review of the Day 85 interim analysis and the additional interim analysis performed on available immunogenicity data from Phase I subjects who had completed their Visit 10 (Month 14 + 28 days), the Sponsor made the decision not to pursue the clinical development of the investigational supra-seasonal influenza vaccine. Based on these interim analyses results, some assays will not be performed or developed as initially planned, either because limited or no response is expected to be observed, or because the assay results are no longer relevant in light of the decision not to pursue the development of the investigational supra-seasonal influenza vaccine based on the chimeric hemagglutinin technology.

The changes are as follows:

- The cell-mediated immune response will not be assessed at Month 14 and later timepoints. The blood sampling for cell-mediated immunity at Visit 12 (Month 26) has been removed.
- The hemagglutination inhibition assay (HI) will not be performed on the inactivated influenza quadrivalent vaccine H1N1 component, cH11/1N1, cH6/1N5, H5N8 and H1N1 swine flu strains. For cH5/1N1 and cH8/1N1 the HI assay will only be performed until Visit 6 (Day 85).
- The micro-neutralisation (MN) assay will only be performed for cH6/1N5 and H1N1 until Visit 6 (Day 85). MN assay will not be performed for H5N8.
- The anti-group 2 hemagglutinin (HA) stalk response by ELISA will not be performed.
- The immune response in terms of anti-neuraminidase antibodies will not be assessed.
- The passive transfer experiment in mice will not be performed for the Month 14 and Month 26 timepoints. The blood collection at Month 26 for passive transfer has been removed.
- The anti-H9 full length HA ELISA will not be performed.
- Anti-stalk antibody functionality will not be further investigated, except for the antibody-dependent cell-mediated cytotoxicity until the interim analysis at Day 85.
- Occurrence of RT-PCR-confirmed influenza cases endpoint was removed.

In case samples have already been taken but not tested yet, they will be stored for future research.

In addition, the list of contributing authors has been updated.

Protocol Amendment 4 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any future protocol amendments or protocol administrative changes, with the terms of the clinical trial agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the GSK Biologicals' study vaccines/products and other study-related duties and functions as described in the protocol.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no clinical samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally acceptable representative.
- To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor or the investigational vaccines/products, and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

- Agree to supply GSK Biologicals with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- Agree to promptly update this information if any relevant changes occur during the course of the study and for one year following completion of the study.
- Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.

eTrack study number and Abbreviated Title	207543 (FLU D-SUIV-ADJ-001)
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Investigator name	_____
Signature	_____
Date	_____

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Sponsor Information

1. Sponsor

GlaxoSmithKline Biologicals
Rue de l'Institut, 89
1330 Rixensart, Belgium

2. Sponsor Medical Expert for the Study

Refer to the local study contact information document.

3. Sponsor Study Monitor

Refer to the local study contact information document.

4. Sponsor Study Contact for Reporting of a Serious Adverse Event

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section 8.4.2.

SYNOPSIS

Detailed Title	A Phase I/II, randomized, controlled, observer-blind, multi-center study to assess the reactogenicity, safety and immunogenicity of three GlaxoSmithKline (GSK) Biologicals' investigational supra-seasonal universal influenza vaccines (SUIVs) (unadjuvanted or adjuvanted with AS03 or AS01) administered as a 1 or 2-dose priming schedule followed by a booster dose 12 months post-primary vaccination in 18 to 39 year-old healthy subjects.
Indication	Active immunization for the prevention of disease caused by influenza virus.
Rationale for the study and study design	• **Rationale for the study** Current seasonal influenza vaccines show good efficacy when they are well-matched with the circulating virus strains. However, influenza viruses constantly change their surface glycoproteins that are the targets of most immune responses, allowing them to escape pre-existing immunity, a process called antigenic drift. Therefore, seasonal influenza vaccines have to be reformulated and re-administered on an annual basis. In addition, novel viruses can appear at irregular intervals and cause influenza virus pandemics that can claim millions of lives. Influenza A hemagglutinins (HAs) are phylogenetically divided into influenza A group 1 (H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18) and influenza A group 2 (H3, H4, H7, H10, H14, and H15) based on sequence similarities. Protection from influenza viruses is usually correlated with antibodies that bind to the membrane distal head domain of the HA molecule, thereby blocking the virus from attaching to host cell receptors. However, the head domain has high plasticity and is the main site of antigenic drift. The membrane proximal stalk domain of the HA is more conserved than the head domain and antibodies that target this domain have been shown to broadly neutralize influenza viruses across several subtypes. The stalk domain is immuno-subdominant compared to the head domain and is therefore usually not targeted by the immune system following exposure to influenza virus vaccines [Krammer, 2016].

The proposed approach of the GlaxoSmithKline (GSK) supra-seasonal universal influenza vaccine (SUIV) is to circumvent the immunodominance of the head domain by sequential exposure of the immune system to chimeric HAs (cHAs) that pair the globular head region of an exotic (for example, avian) HA with the HA stalk of a currently circulating seasonal influenza virus. The purpose of this approach is to boost pre-existing cross-reactive memory responses to the HA stalk without further boosting strain-specific responses against the head region of HA. This approach has previously provided good protection in mice [Goff, 2013a; Krammer, 2014a; Nachbagauer, 2016] and ferrets [Krammer, 2014b]. The use of adjuvants enhanced the induction of stalk-reactive antibodies in mice [Krammer, 2014a; Goff, 2013b]. Although the final formulation of SUIV is planned to be a trivalent vaccine containing three inactivated split virion cHA components (i.e. one from influenza A group 1 (H1), one from influenza A group 2 (H3) and one from influenza B), the Company will first assess monovalent SUIVs, each containing one inactivated split virion cHA antigen from influenza A group 1. It is expected that achieving the optimal immune response will require improvement of the immunogenic properties of the chimeric HAs. This will be assessed in this clinical study by comparing two adjuvanted formulations (i.e. a formulation adjuvanted with AS03 and a formulation adjuvanted with AS01) with the immunogenicity of a non-adjuvanted formulation. These adjuvants were taken into consideration for inclusion in the investigational SUIVs as they are the most commonly used adjuvant systems in other GSK Biologicals' vaccines (such as in the pandemic influenza vaccine, *Pandemrix*, which is adjuvanted with $AS03_A$; and the malaria vaccine, *Mosquirix,* which is adjuvanted with $AS01_E$).

An informative study performed by Nachbagauer *et al.,* [Nachbagauer, 2014] demonstrated that human sera from subjects who had received an inactivated H5N1 vaccine were able to protect mice against challenge with an influenza virus expressing an unmatched HA head, but a similar HA stalk. This study supports the concept of a chimeric HA universal influenza vaccine based on re-focusing the immune response to the conserved stalk domain by sequential exposure to HAs with divergent globular head domains, but conserved stalk domains. It has also been demonstrated in mice that a chimeric HA-based vaccination regimen induced higher stalk antibody titers than the seasonal influenza vaccine.

The stalk antibody responses were long lasting, cross-reactive to distantly related HAs and provided protection *in vivo* in a serum passive transfer / challenge model, which further support the development of a universal influenza virus vaccine candidate built on the chimeric HA technology platform [Nachbagauer, 2016].

- **Rationale for the study design**

Approximately 470 subjects 18-39 years of age will be equally randomized in 10 different treatment groups to receive 2 or 3 doses of a monovalent influenza A group 1 investigational SUIV or to receive an annual quadrivalent inactivated seasonal influenza vaccine. Each SUIV contains a split inactivated influenza virus expressing a chimeric HA with the same stalk domain (H1 stalk) at each dose, but a different exotic influenza A group 1 head. The antigen dose of 15 μg in each vaccine in this study is based on the standard antigen dose for inactivated seasonal influenza vaccines.

Although it is assumed that a minimum of two priming doses followed by at least one booster dose may be required to induce long-term protection, the use of the adjuvant could significantly improve the anti-stalk immune response and induce adequate response with only one dose, especially in adults that have been previously exposed to the conserved HA stalk domain by natural exposure or vaccination. In a recent study it has been shown that, in an adult population, anti-stalk antibodies are present at high titers pre-vaccination and that they are boosted after a single dose of *Pandemrix* (AS03-adjuvanted pandemic H1N1) [Tete, 2016]. In this study, it was also demonstrated that these H1 HA stalk-specific antibodies had neutralizing activity and they were detected already at baseline (i.e. prior to vaccination). Vaccination resulted in a significant increase in HA stalk-specific neutralizing antibodies in the absence of hemagglutination inhibition (HI) activity. It was also shown that the avidity of the anti-H1 stalk response was 3-fold greater relative to the avidity of the anti-HA head response to *Pandemrix*. Knowing that the H1N1pdm virus contained a novel HA head domain that was different from the pre-pandemic seasonal H1 virus, this paper provides rationale for testing, in adults who are likely to be already "primed", a single SUIV dose, followed by a booster dose 14 months later with a SUIV containing a different HA construct.

Therefore, the vaccine regimen in the SUIV groups will consist of sequential primary intramuscular immunization with one dose (Day 1) or 2 doses (Day 1 and Day 57) followed by a booster dose at Month 14 of a vaccine containing split inactivated influenza virus expressing a chimeric HA with at each dose the same stalk domain (H1 stalk) but a different exotic head. The SUIV vaccine will be adjuvanted with $AS03_A$ or $AS01_E$ or non-adjuvanted. The interval of two months between the two priming doses was selected to ensure optimal priming in a setting where the vaccine can be administered the whole year round without time pressure for completion of vaccination in contrast with a seasonal or pandemic influenza vaccination setting. The booster dose at Month 14 is anticipated to be necessary to obtain an adequate and persisting anti-stalk antibody response. In the control group, subjects will be administered one dose of *Fluarix Quadrivalent* inactivated influenza vaccine (IIV4) on Day 1 and then re-vaccinated at Month 14 with the next year's formulation.

The main purpose of this study will be to assess the safety and the reactogenicity of each SUIV compared to IIV4. This study will also evaluate the adjuvant effect of $AS03_A$ and $AS01_E$ on the immune response when compared to the non-adjuvanted formulation. In addition, the immune response after 1 priming dose and after 2 priming doses will be evaluated, as well as the immune response after a booster dose given 14 or 12 months after a one dose priming schedule or a 2-dose priming schedule, respectively. Since the vaccine sequence of the priming dose and the booster dose in the one dose-priming schedule groups varies, the effect of the chimeric HA vaccine sequence on the humoral immune response will also be assessed. Finally, the cell-mediated immune response and the protective effect *in vivo* of the anti-stalk antibodies will be explored. Passive surveillance will be put in place in order to capture the occurrence of ***influenza-like illnesses*** during the entire study period. **(Amended: 11 July 2019)**

Objectives

Primary

- To assess the reactogenicity and safety of each vaccine dose throughout the entire study period, in all study groups.
- To describe the anti-H1 stalk humoral immune response 28 days after each priming dose (1 or 2 dose(s)) in all study groups.

Secondary

- To evaluate the adjuvant effect of AS03 and AS01 on the humoral immune response after 1 and 2 priming dose(s) of investigational SUIVs when compared to the non-adjuvanted formulations.
- To describe the persistence of the anti-H1 stalk humoral immune response after each priming dose (1 or 2 dose(s)) in all study groups up to Month 14.
- To describe the humoral immune response after a booster dose at Month 14.
- To describe the breadth of the humoral immune response after each vaccination in all study groups.
- To describe the effect of the chimeric HA vaccination-sequence on the humoral immune response.

Tertiary (Amended: 11 July 2019)

- To explore the cell-mediated immune responses (B-cells and T-cells).
- To explore the immune response against the HA head of cH5/1N1 ***and*** cH8/1N1 strain by hemagglutination inhibition (HI) assay.
- To explore the protective effect of the stalk-reactive antibodies induced by vaccination in a passive transfer challenge experiment in mice.
- To develop assays for evaluation/characterization of the humoral and cellular immune responses to the investigational vaccines.
- To explore anti-stalk antibody functionality, e.g., antibody-dependent cell-mediated cytotoxicity (ADCC).

Study design

- **Experimental design**: Phase I/II, observer-blind, randomized, controlled, multi-centric study with 10 parallel groups.
- **Duration of the study**:
  - Epoch 001: Screening (Day -28 to -2) – only for Phase I subjects (refer to the Enrolment section below).
  - Epoch 002: Primary starting at Visit 1 (Day 1) and ending at Visit 7 (Month 8).
  - Epoch 003: Booster starting at Visit 8 (Month 14) and ending at Visit 12 (Month 26).
- **Primary Completion Date (PCD)**: Visit 12 (Month 26).
- **End of Study (EoS)**: Last testing results released of samples collected at Visit 12 (Month 26).
- **Study groups**: The study groups and epochs foreseen in this study are provided in Synopsis Table 1.

Synopsis Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min - Max)	Epochs	
			Epoch 002	Epoch 003
cH8/P/cH5-AS03	47	18 years – 39 years	x	x
cH5/P/cH8-AS03	47	18 years – 39 years	x	x
cH8/5/11-AS03	47	18 years – 39 years	x	x
cH8/P/cH5-AS01	47	18 years – 39 years	x	x
cH5/P/cH8-AS01	47	18 years – 39 years	x	x
cH8/5/11-AS01	47	18 years – 39 years	x	x
cH8/P/cH5	47	18 years – 39 years	x	x
cH5/P/cH8	47	18 years – 39 years	x	x
cH8/5/11	47	18 years – 39 years	x	x
IIV4	47	18 years – 39 years	x	x

Synopsis Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/Product name	Study groups									
		cH8/P/cH5-AS03	cH5/P/cH8-AS03	cH8/5/11-AS03	cH8/P/cH5-AS01	cH5/P/cH8-AS01	cH8/5/11-AS01	cH8/P/cH5	cH5/P/cH8	cH8/5/11	IIV4
cH8/1N1+AS03$_A$-like*	cH8/1N1	x	x	x							
	AS03	x	x	x							
	PBS	x	x	x							
cH5/1N1+AS03$_A$-like*	cH5/1N1	x	x	x							
	AS03	x	x	x							
	PBS	x	x	x							
cH11/1N1+AS03$_A$-like*	cH11/1N1			x							
	AS03			x							
	PBS			x							
cH8/1N1+AS01$_E$-like#	cH8/1N1				x	x	x				
	AS01$_B$				x	x	x				
	PBS				x	x	x				
cH5/1N1+AS01$_E$-like#	cH5/1N1				x	x	x				
	AS01$_B$				x	x	x				
	PBS				x	x	x				
cH11/1N1+AS01$_E$-like#	cH11/1N1						x				
	AS01$_B$						x				
	PBS						x				
cH8/1N1	cH8/1N1							x	x	x	
	PBS							x	x	x	
cH5/1N1	cH5/1N1							x	x	x	
	PBS							x	x	x	
cH11/1N1	cH11/1N1									x	
	PBS									x	
Fluarix Quadrivalent	FLU D-QIV										x
PBS	PBS	x	x		x	x		x	x		x

*AS03$_A$-like is obtained by dilution of AS03 with PBS
AS01$_E$-like is obtained by dilution of AS01$_B$ with PBS

- **Control**: active control (Fluarix Quadrivalent).
- **Vaccination schedule:**
  - Two primary doses at Visit 1 (Day 1) and Visit 4 (Day 57).
  - A booster dose at Visit 8 (Month 14).

  Phase I subjects will be vaccinated one at the time, at least 60 minutes apart, with a maximum of 10 subjects a day. This is applicable for Dose 1 (Day 1), Dose 2 (Day 57) and booster dose (Month 14). Dose 2 can only be provided to Phase I subjects upon favorable outcome of the 7-day post-Dose 1 safety data review by the IDMC of at least 60 Phase I subjects.
- **Treatment allocation**: randomized (1:1:1:1:1:1:1:1:1:1:1 ratio) using GSK Biologicals' Randomization System on Internet (SBIR).
- **Blinding:** see Synopsis Table 3.

Synopsis Table 3 Blinding of study epochs

Study Epochs	Blinding
Epoch 001	Not applicable
Epoch 002	Observer-blind
Epoch 003	Observer-blind

- **Sampling schedule: (Amended: 11 July 2019)**
  - Blood samples for safety assessment will be drawn from all subjects at all visits: Screening*, Days 1, 8, 29, 57, 64, 85, Month 8, Month 14, Month 14 + 7 days, Month 14 + 28 days, Month 20 and Month 26.

    *Only for subjects enrolled in Phase I (refer to the Enrolment section below).
  - Blood samples for serology testing will be drawn from all subjects at Days 1 (Visit 1), 29 (Visit 3), 85 (Visit 6), Month 8 (Visit 7), Month 14 (Visit 8), Month 14 + 28 days (Visit 10), Month 20 (Visit 11) and Month 26 (Visit 12).
  - Blood samples for passive transfer experiment in animals will be drawn from all subjects at Days 1 (Visit 1), 85 (Visit 6), Month 14 (Visit 8)*.

- Blood samples for cell-mediated immunity (CMI) assessment will be drawn from a sub-cohort of ~225 subjects at Days 1 (Visit 1), 8 (Visit 2), 29 (Visit 3), 64 (Visit 5), 85 (Visit 6), Month 14 (Visit 8)*****, Month 14 + 7 days (Visit 9)*****, ***and*** Month 14 + 28 days (Visit 10)*****. The sub-cohort will consist of the first Phase II subjects enrolled in pre-specified centers.
- During the entire study period, nasal and throat swabs will be collected as soon as possible (preferably within 24 hours, but not later than 7 days) after the onset of an influenza-like illness (ILI) to test for influenza and/or other respiratory pathogens by RT-PCR ***if deemed necessary, or stored for future research.***

  ****Note that samples already collected for these timepoints by the time of Protocol Amendment 4 implementation at site will not be tested and will be stored, unless deemed necessary based on medical review of the cases.***

- **ILI surveillance:** ILI is defined as at least one of these systemic symptoms:
  - Temperature (oral) ≥ 37.8°C/98.6°F and/or,
  - Myalgia (widespread muscle ache);

  AND at least one of these respiratory symptoms:
  - Cough and/or,
  - Sore throat.

  Passive surveillance will be carried out from Visit 1 (after Dose 1) until the end of the study (Visit 12). Subjects will be instructed to contact the investigator/study staff as soon as they experience ILI symptoms.
- **Type of study**: self-contained.
- **Data collection**: electronic Case Report Form (eCRF).
- **Safety monitoring**: an Independent Data Monitoring Committee (IDMC) consisting of clinical experts and a biostatistician, independent from the Sponsor, will review unblinded safety data (including laboratory assessment) at a regular frequency to monitor the safety of the subjects throughout the study.

- **Enrolment**: the study will follow a staggered enrolment with 2 steps; the first being Phase I (N = ~80) and the second being Phase II (N = ~390):
  - Phase I: During the Phase I enrolment, subjects will be vaccinated one at a time, at least 60 minutes apart, with a maximum of 10 subjects per day until ~80 subjects are enrolled (i.e. to obtain treatment groups of at least 8 subjects/per group). If no safety issue is identified by the IDMC upon review of the 7-day post-dose 1 safety data (Days 1-7) of all Phase I subjects (N = ~80), Phase II enrolment will be allowed to start.
  - Phase II: Subjects will be enrolled and vaccinated without limitation on the number of vaccinees per day or time between consecutive subjects.

Number of subjects

A total of 470 subjects (47 per group) are planned to be enrolled in this study, in order to have 430 evaluable subjects for the primary immunogenicity endpoints.

Endpoints

Primary

Reactogenicity and safety

- Occurrence of solicited local and general AEs after each vaccination:
  - Occurrence of solicited local AEs during a 7-day follow-up period (i.e. on the day of vaccination and 6 subsequent days) after each vaccine dose, in all vaccine groups.
  - Occurrence of solicited general AEs during a 7-day follow-up period (i.e. on the day of vaccination and 6 subsequent days) after each vaccine dose, in all vaccine groups.
- Occurrence of unsolicited AEs after each vaccination:
  - Occurrence of unsolicited AEs during a 28-day follow-up period (i.e. on the day of vaccination and 27 subsequent days) after each vaccine dose, in all vaccine groups.

- Occurrence of hematological and biochemical laboratory abnormalities after each vaccination:
  - Any hematological (red blood cells, white blood cells and differential count, platelets count and hemoglobin level) or biochemical (alanine aminotransferase, aspartate aminotransferase, creatinine, blood urea nitrogen [BUN] and BUN-to-creatinine ratio) laboratory abnormality at each visit subsequent to Day 1, in all vaccine groups.
- Occurrence of MAEs, pIMDs and SAEs:
  - Occurrence of MAEs, pIMDs and SAEs throughout the entire study period, in all vaccine groups.

Immunogenicity

Anti-H1 stalk immune response measured by ELISA and by MN assay 28 days after each priming dose:

- Levels of anti-H1 stalk antibody titers by ELISA and by MN assay.

  The following aggregate variables will be calculated for the above parameters with 95% confidence interval (CI):
  - Seropositivity rates and geometric mean titers (GMTs) at Days 1, 29 and 85.
  - Percentage of subjects with a ≥ 4-fold increase from Day 1 to Days 29 and 85.
  - Percentage of subjects with a ≥ 10-fold increase from Day 1 to Days 29 and 85.
  - Mean geometric increase (MGI) from Day 1 to Days 29 and 85.

Secondary (Amended: 11 July 2019)

Immunogenicity

Adjuvant effect on the anti-stalk immune response in terms of:

- GMT ratio for anti-stalk ELISA titer SUIV+AS03 or AS01/SUIV non-adjuvanted, 28 days post vaccination (i.e. at Day 29 to evaluate the adjuvant effect post-dose 1 and at Day 85 to evaluate the adjuvant effect post-dose 2).

Anti-H1 stalk immune response measured by ELISA and by MN assay:

- Levels of anti-H1 stalk antibody titers by ELISA post-each vaccination.

  The following aggregate variables will be calculated for the above parameters with 95% CI:

  - Seropositivity rates and GMTs at Days 1, 29, 85, Month 8, Month 14, Month 14 + 28 days, Month 20 and Month 26.
  - Percentage of subjects with a ≥ 4-fold increase in antibody titers by ELISA from Day 1 to each subsequent timepoint listed above.
  - Percentage of subjects with a ≥ 10-fold increase in antibody titers by ELISA from Day 1 to each subsequent timepoint listed above.
  - MGI in antibody titers by ELISA from Day 1 to each subsequent timepoint listed above.

- Levels of anti-H1 stalk antibody titers by MN assay.

  The following aggregate variables will be calculated for the above parameters with 95% CI:

  - Seropositivity rates and GMTs at Days 1, 29 ***and*** 85.
  - Percentage of subjects with a ≥ 4-fold increase in antibody titers by MN assay from Day 1 to each subsequent timepoint listed above.
  - Percentage of subjects with a ≥ 10-fold increase in antibody titers by MN assay from Day 1 to each subsequent timepoint listed above.
  - MGI in antibody titers by MN assay from Day 1 to each subsequent timepoint listed above.

Breadth of the immune response:

- Levels of anti-H2 and anti-H18 antibody titers by ELISA.

  The following aggregate variables will be calculated for the above parameters with 95% CI:

  - Anti-H2 and anti-H18 seropositivity rates and GMTs at Days 1, 29, 85, Month 8, Month 14, Month 14 + 28 days, Month 20 and Month 26.

  - Percentage of subjects with a ≥ 4-fold increase in anti-H2 and anti-H18 antibody titers from Day 1 to each subsequent timepoint listed above.
  - Percentage of subjects with a ≥ 10-fold increase in anti-H2 and anti-H18 antibody titers from Day 1 to each subsequent timepoint listed above.
  - MGI in anti-H2 and anti-H18 antibody titers from Day 1 to each subsequent timepoint listed above.

- Levels of antibody titers by MN assay for H1N1 swine influenza and IIV4 H1N1 vaccine strains.

  The following aggregate variables will be calculated for the above parameters with 95% CI:

  - Seropositivity rates and GMTs at Days 1, 29 ***and*** 85.
  - Percentage of subjects with a ≥ 4-fold increase in antibody titers from Day 1 to each subsequent timepoint listed above.
  - Percentage of subjects with a ≥ 10-fold increase in antibody titers from Day 1 to each subsequent timepoint listed above.
  - MGI in antibody titers from Day 1 to each subsequent timepoint listed above.

Tertiary (Amended: 11 July 2019)

- Evaluation of CMI parameters in terms of frequencies of:
  - Antigen-specific CD4+/CD8+ T-cells identified as producing at least two markers among CD40L, IL-2, TNF-α and IFN-γ upon *in vitro* stimulation at Days 1, 29 ***and*** 85.
  - B-memory cells reactive with the challenge antigen(s) at Days 1, 8, 29, 64 ***and*** 85.
  - Plasmablasts reactive with the challenge antigens at Days 1, 8 ***and*** 64.

- Levels of HI antibody to chimeric vaccine strains ***cH5/1N1 and cH8/1N1***:

  The following aggregate variables will be calculated with 95% CI:

  – Seropositivity rates and GMTs at Days 1, 29 ***and*** 85.

  – Seroprotection rate (SPR) at each timepoint listed above.

  – Seroconversion rate (SCR) at Days 29 ***and*** 85.

  – MGI from Day 1 to each subsequent timepoint listed above.

- Assessment of the *in vivo* protective effect of the anti-stalk antibodies when transferring Day 1 ***and*** Day 85 pooled serum from all evaluable subjects of each vaccine groups to mice that will be subsequently challenged with cH6/1N5* or with H1N1 contained in the IIV4, using the following endpoints [refer to APPENDIX D]:

  – Survival over 14 days post-challenge (day of death/euthanasia for weight loss > 25% baseline body weight) in groups of 35 mice**/serum pool/vaccine group/timepoint.

  – Weight loss (change from baseline over 14 days post-challenge) in groups of 35 mice**/serum pool/vaccine group/timepoint.

  – Lung virus titer in $TCID_{50}$/mg (log_{10} fold change [Day 1 minus Day 85]), within challenge group.

  – Pre- and post-transfer titer of human IgG to cH6/1N5* by ELISA or HI.

  – Pre- and post-transfer titer of human IgG to H1N1 by ELISA or HI.

  – Pre- and post-transfer titer of human IgG to recombinant HA protein by ELISA.

  *Or an alternative challenge virus with similar attributes but more fit for purpose.

  **If sufficient serum volumes are not available, and depending on the challenge virus pathogenicity, the number of mice can be reduced to as low as 10 mice per timepoint and virus challenge.

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LIST OF ABBREVIATIONS

AE:	Adverse Event
ALT:	Alanine aminotransferase
ANOVA:	Analysis of Variance
AS01:	Adjuvant System 01
AS03:	Adjuvant System 03
AST:	Aspartate aminotransferase
BUN:	Blood Urea Nitrogen
CD4/8/40L:	Cluster of Differentiation 4/8/40 Ligand
cHA:	Chimeric Hemagglutinin
CI:	Confidence Interval
CLS:	Clinical Laboratory Sciences
CMI:	Cell-Mediated Immunity
DIL:	Dilution
eCRF:	electronic Case Report Form
EDD:	Estimated Date of Delivery
EGA:	Estimated Gestational Age
ELISA:	Enzyme-linked immunosorbent assay
EoS:	End of Study
ES:	Exposed Set
eTDF:	electronic Temperature excursion Decision Form
EU/mL:	ELISA units per milliliter
FCB:	Flow Cytometry B-cells
FDA:	Food and Drug Administration, United States
GCP:	Good Clinical Practice

GMT:	Geometric Mean Titer
GSK:	GlaxoSmithKline
HA:	Hemagglutinin
HI:	Hemagglutination Inhibition
IB:	Investigator's Brochure
ICF:	Informed Consent Form
ICH:	International Conference on Harmonization
ICS:	Intracellular Cytokine Staining
IDMC:	Independent Data Monitoring Committee
IEC:	Independent Ethics Committee
IFN-γ:	Interferon-gamma
IgG:	Immunoglobulin G
IIV4:	Quadrivalent inactivated influenza vaccine
IL-2:	Interleukin-2
ILI:	Influenza-Like Illness
IM:	Intramuscular
IMP:	Investigational Medicinal Product
IND:	Investigational New Drug
IRB:	Institutional Review Board
LMP:	Last Menstrual Period
MAE:	Medically Attended Event
MedDRA:	Medical Dictionary for Regulatory Activities
MGI:	Mean Geometric Increase
MN:	Microneutralization
NA:	Neuraminidase

PBMC:	Peripheral Blood Mononuclear Cell
PBS:	Phosphate Buffered Saline
PCD:	Primary Completion Date
pIMD:	Potential Immune-Mediated Disease
PT:	Preferred Term
RNA:	Ribonucleic Acid
RT-PCR:	Reverse Transcription-Polymerase Chain Reaction
SAE:	Serious Adverse Event
SBIR:	Randomization System on Internet
SCR:	Seroconversion Rate
SD:	Standard Deviation
SDV:	Source Document Verification
SmPC:	Summary of Product Characteristics
SPM:	Study Procedures Manual
SPR:	Seroprotection rate
SUIV:	Supra-seasonal Universal Influenza Vaccine
TBD:	To Be Determined
TNF-α:	Tumor Necrosis Factor-alpha
VAERD:	Vaccine-Associated Enhanced Respiratory Disease

GLOSSARY OF TERMS

Adequate contraception: Adequate contraception is defined as a contraceptive method with failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label for example:

- Abstinence from penile-vaginal intercourse, when this is their preferred and usual lifestyle,
- Combined estrogen and progesterone oral contraceptives,
- Injectable progestogen,
- Implants of etenogestrel or levonorgestrel,
- Contraceptive vaginal ring,
- Percutaneous contraceptive patches,
- Intrauterine device or intrauterine system,
- 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 site personnel's review of the subject's medical records; or interview with the subject on her medical history.
- Male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository), and/or progesterone alone oral contraceptive.

Adequate contraception does not apply to subjects of child bearing potential with same sex partners, or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis, when this is their preferred and usual lifestyle.

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 adverse event (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. For marketed medicinal products, this also

	includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.
Blinding:	A procedure in which one or more parties to the trial 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 trial, 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 an observer-blind study, the subject and the site 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 5.3 for details on observer-blinded studies).
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
End of Study (EoS): (Synonym of End of Trial)	For studies with collection of Human Biologicals Samples or imaging data, EoS is defined as the date of the last testing/reading released of the Human Biological Samples or imaging data, related to primary and secondary endpoints. EoS must be achieved no later than 8 months after Last Subject Last Visit.
Epoch:	An epoch is a set of consecutive timepoints or a single timepoint from a single protocol. Epochs are defined to support a main purpose which is either to draw conclusions on subject participation or to draw a complete conclusion to define or precise the targeted label of the product. Supporting means that data collected at the timepoints included in an epoch must be sufficient to fulfil the purpose of the epoch. Typical examples of epochs are screening, primary vaccinations, boosters, yearly immunogenicity follow-ups, and surveillance periods for efficacy or safety.
eTrack:	GSK's tracking tool for clinical trials.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the per-protocol analysis (see Sections 6.7.2 and 10.5 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.
Influenza-like illness (ILI):	ILI is defined as at least one of these systemic symptoms: • Temperature (oral) ≥ 37.8°C/98.6°F and/or, • Myalgia (widespread muscle ache); AND at least one of these respiratory symptoms: • Cough and/or, • Sore throat.
Investigational vaccine/product: (Synonym of Investigational Medicinal Product [IMP])	A pharmaceutical form of an active ingredient being tested in a clinical trial, 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.
Mean Geometric Increase:	Geometric mean of the fold increase in serum HI titers post-vaccination compared to Day 1.
Medically attended event:	An event for which the subject received medical attention defined as hospitalization, an emergency room visit or a visit to or from medical personnel (medical doctor) for any reason.
Menarche:	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 1-2 years of breast development, thelarche. However, a young female can become pregnant before her first menses. Thus, a conservative definition of non-childbearing potential in a pre-menarcheal female is a young female who has not yet entered puberty as evidenced by lack of breast development (palpable glandular breast tissue).
Menopause:	Menopause is the age associated with complete cessation of menstrual cycles, menses, and implies the loss of reproductive potential by ovarian failure. A practical definition accepts menopause after 1 year

	without menses with an appropriate clinical profile at the appropriate age e.g. > 45 years.
Potential Immune-Mediated Disease:	Potential immune-mediated diseases (pIMDs) are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology.
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 trial was concluded according to the pre-specified protocol or was terminated.
Protocol amendment:	The International Conference on Harmonisation (ICH) defines a protocol amendment as: "A written description of (a) change(s) to or formal clarification of a protocol". GSK Biologicals 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.
Self-contained study:	Study with objectives not linked to the data of another study.
Seroconversion rate:	The percentage of vaccinees with either a pre-vaccination HI titer < 1:10 and a post-vaccination HI titer ≥ 1:40 or a pre-vaccination HI titer ≥ 1:10 and at least 4-fold increase in post-vaccination HI titer.
Seroprotection rate:	The percentage of vaccinees with serum HI titer ≥ 1:40; usually accepted as indicating protection in at least 50% of the vaccinees.
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical studies at one or more investigational sites.
Solicited adverse event:	AEs 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 post-vaccination follow-up period.
Study vaccine/product:	Any investigational vaccine/product being tested and/or any authorized use of a vaccine/product/placebo as a

	reference or administered concomitantly, in a clinical trial that evaluates the use of an investigational vaccine/product.
Sub-cohort:	A group of subjects for whom specific study procedures are planned as compared to other subjects or a group of subjects who share a common characteristic (e.g. ages, vaccination schedule,…) at the time of enrolment.
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)/product(s) or as a control.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
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 treatment allocation.
Unsolicited adverse event:	Any AE 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 AE.

TRADEMARKS

The following trademarks are used in the present protocol.

Note: In the body of the protocol (including the synopsis), the names of the vaccines/products will be written without the superscript symbol ™ or ® and in *italics*.

Trademarks of the GlaxoSmithKline group of companies	Generic description
**Arepanrix*	GSK's licensed H1N1 AS03-adjuvanted pandemic vaccine
Fluarix	Seasonal influenza vaccine
Mosquirix	Malaria vaccine
***Pandemrix*	GSK's licensed H1N1 AS03-adjuvanted pandemic vaccine

*Arepanrix (H1N1) license expired in the European Union in December 2010
**Pandemrix (H1N1) license expired in the European Union in August 2015.

1. INTRODUCTION

1.1. Background

Influenza viruses are enveloped negative-sense RNA viruses with a segmented genome belonging to the *Orthomyxoviridae* family. They are classified on the basis of their core proteins into three distinct types, A, B, and C. Influenza A and B viruses are primarily responsible for human disease, with type A being the most pathogenic. The main antigenic determinants of influenza A and B viruses are two surface glycoproteins: neuraminidase (NA) and hemagglutinin (HA), both capable of eliciting immune response in human beings [Cox, 1998].

Influenza viruses are a serious public health problem, and cause variable but often high rates of seasonal disease in the human population, with consequent significant morbidity and mortality. Uncomplicated influenza disease is characterized by the abrupt onset of constitutional and respiratory symptoms which usually resolve within a week. However, in vulnerable populations such as the elderly and children, influenza can aggravate existing medical conditions and potentially lead to life-threatening complications. During seasonal epidemics, 5-15% of the population is typically infected, resulting in 3-5 million cases of severe illness and a quarter to half a million excess deaths worldwide annually. Most deaths associated with influenza in industrialized countries occur among people age 65 years or older [WHO, 2016], although infection is most common in children [O'Brien, 2004; Izurieta, 2000].

Annual influenza vaccination is currently the most effective means of controlling influenza and preventing its complications and mortality [WHO, 2016].

Please refer to the current Investigator's Brochure (IB) for information regarding the pre-clinical and epidemiological information for the investigational supra-seasonal universal influenza vaccines (SUIVs) and the adjuvants.

1.2. Rationale for the study and study design

1.2.1. Rationale for the study

Current seasonal influenza vaccines show good efficacy when they are well-matched with the circulating virus strains. However, influenza viruses constantly change their surface glycoproteins that are the targets of most immune responses, allowing them to escape pre-existing immunity, a process called antigenic drift. Therefore, seasonal influenza vaccines have to be reformulated and re-administered on an annual basis. In addition, novel viruses can appear at irregular intervals and cause influenza virus pandemics that can claim millions of lives.

Influenza A HAs are phylogenetically divided into influenza A group 1 (H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18) and influenza A group 2 (H3, H4, H7, H10, H14, and H15) based on sequence similarities. Protection from influenza viruses is usually correlated with antibodies that bind to the membrane distal head domain of the HA molecule, thereby blocking the virus from attaching to host cell receptors. However, the head domain has high plasticity and is the main site of antigenic drift.

The membrane proximal stalk domain of the HA is more conserved than the head domain and antibodies that target this domain have been shown to broadly neutralize influenza viruses across several subtypes. The stalk domain is immuno-subdominant compared to the head domain and is therefore usually not targeted by the immune system following exposure to influenza virus vaccines [Krammer, 2016].

The proposed approach of the GlaxoSmithKline (GSK) Biological's investigational SUIV is to circumvent the immunodominance of the head domain by sequential exposure of the immune system to chimeric HAs (cHAs) that pair the globular head region of an exotic (for example, avian) HA with the HA stalk of a currently circulating seasonal influenza virus. The purpose of this approach is to boost pre-existing cross-reactive memory responses to the HA stalk without further boosting strain-specific responses against the head region of HA. This approach has previously provided good protection in mice [Goff, 2013a; Krammer, 2014a; Nachbagauer, 2016] and ferrets [Krammer, 2014b]. The use of adjuvants enhanced the induction of stalk-reactive antibodies in mice [Krammer, 2014a; Goff, 2013b]. Although the final formulation of SUIV is planned to be a trivalent vaccine containing three inactivated split virion cHA components (i.e. one from influenza A group 1 (H1), one from influenza A group 2 (H3) and one from influenza B), the Company will first assess monovalent SUIVs, each containing one inactivated split virion cHA antigen from influenza A group 1. It is expected that achieving the optimal immune response will require improvement of the immunogenic properties of the chimeric HAs. This will be assessed in this clinical study by comparing two adjuvanted formulations (i.e. a formulation adjuvanted with AS03 and a formulation adjuvanted with AS01) with the immunogenicity of a non-adjuvanted formulation. These adjuvants were taken into consideration for inclusion in the investigational SUIVs as they are the most commonly used adjuvant systems in other GSK Biologicals' vaccines (such as in the pandemic influenza vaccine, *Pandemrix*, which is adjuvanted with $AS03_A$; and the malaria vaccine, *Mosquirix,* which is adjuvanted with $AS01_E$).

An informative study performed by Nachbagauer *et al.,* [Nachbagauer, 2014] demonstrated that human sera from subjects who had received an inactivated H5N1 vaccine were able to protect mice against challenge with an influenza virus expressing an unmatched HA head, but a similar HA stalk. This study supports the concept of a chimeric HA universal influenza vaccine based on re-focusing the immune response to the conserved stalk domain by sequential exposure to HAs with divergent globular head domains, but conserved stalk domains. It has also been demonstrated in mice that a chimeric HA-based vaccination regimen induced higher stalk antibody titers than the seasonal influenza vaccine. The stalk antibody responses were long lasting, cross-reactive to distantly related HAs and provided protection *in vivo* in a serum passive transfer / challenge model, which further support the development of a universal influenza virus vaccine candidate built on the chimeric HA technology platform [Nachbagauer, 2016].

1.2.2. Rationale for the study design

Approximately 470 subjects 18-39 years of age will be equally randomized in 10 different treatment groups to receive 2 or 3 doses of a monovalent influenza A group 1 investigational SUIV or to receive an annual quadrivalent inactivated seasonal influenza vaccine. Each SUIV contains a split inactivated influenza virus expressing a chimeric HA with the same stalk domain (H1 stalk) at each dose, but a different exotic influenza A group 1 head. The antigen dose of 15 μg in each vaccine in this study is based on the standard antigen dose for inactivated seasonal influenza vaccines.

Although it is assumed that a minimum of two priming doses followed by at least one booster dose may be required to induce long-term protection, the use of the adjuvant could significantly improve the anti-stalk immune response and induce adequate response with only one dose, especially in adults that have been previously exposed to the conserved HA stalk domain by natural exposure or vaccination. In a recent study, it has been shown that, in an adult population, anti-stalk antibodies are present at high titers pre-vaccination and that they are boosted after a single dose of *Pandemrix* (AS03-adjuvanted pandemic H1N1) [Tete, 2016]. In this study, it was also demonstrated that these H1 HA stalk-specific antibodies had neutralizing activity and they were detected already at baseline (i.e. prior to vaccination). Vaccination resulted in a significant increase in HA stalk-specific neutralizing antibodies in the absence of hemagglutination inhibition (HI) activity. It was also shown that the avidity of the anti-H1 stalk response was 3-fold greater relative to the avidity of the anti-HA head response to *Pandemrix*. Knowing that the H1N1pdm virus contained a novel HA head domain that was different from the pre-pandemic seasonal H1 virus, this paper provides rationale for testing, in adults who are likely to be already "primed", a single SUIV dose, followed by a booster dose 14 months later with a SUIV containing a different HA construct.

Therefore, the vaccine regimen in the SUIV groups will consist of sequential primary intramuscular immunization with one dose (Day 1) or 2 doses (Day 1 and Day 57) followed by a booster dose at Month 14 of a vaccine containing split inactivated influenza virus expressing a chimeric HA with at each dose the same stalk domain (H1 stalk) but a different exotic head. The SUIV vaccine will be adjuvanted with $AS03_A$ or $AS01_E$ or non-adjuvanted. The interval of two months between the two priming doses was selected to ensure optimal priming in a setting where the vaccine can be administered the whole year round without time pressure for completion of vaccination in contrast with a seasonal or pandemic influenza vaccination setting. The booster dose at Month 14 is anticipated to be necessary to obtain an adequate and persisting anti-stalk antibody response. In the control group, subjects will be administered one dose of *Fluarix Quadrivalent* inactivated influenza vaccine (IIV4) on Day 1 and then re-vaccinated at Month 14 with the next year's formulation.

The main purpose of this study will be to assess the safety and the reactogenicity of each SUIV compared to IIV4. This study will also evaluate the adjuvant effect of $AS03_A$ and $AS01_E$ on the immune response when compared to the non-adjuvanted formulation. In addition, the immune response after 1 priming dose and after 2 priming doses will be evaluated, as well as the immune response after a booster dose given 14 or 12 months after a one dose priming schedule or a 2-dose priming schedule, respectively. Since the vaccine sequence of the priming dose and the booster dose in the one dose-priming schedule groups varies, the effect of the chimeric HA vaccine sequence on the humoral immune response will also be assessed. Finally, the cell-mediated immune response and the protective effect *in vivo* of the anti-stalk antibodies will be explored. Passive surveillance will be put in place in order to capture the occurrence of ***influenza-like illnesses*** during the entire study period. **(Amended: 11 July 2019)**

1.3. Benefit : Risk Assessment

Please refer to the current IB for the summary of potential risks and benefits of the investigational SUIVs and the Adjuvant Systems AS03 and AS01.

Please refer to the Prescribing Information for information regarding the summary potential risks and benefits of *Fluarix Quadrivalent*.

The following section outlines the risk assessment and mitigation strategy for this study protocol:

1.3.1. Risk Assessment

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Investigational vaccine: adjuvanted SUIV		
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 [Perricone, 2013].	Close monitoring of potential immune-mediated diseases (pIMDs) in clinical development programs using adjuvants systems. The potential risk of events of possible autoimmune etiology to occur is mentioned in the Informed Consent Form (ICF). Subjects with a history or with a current diagnosis of an auto-immune disease will be excluded from this study.

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Influenza vaccines: *Fluarix Quadrivalent* and SUIVs		
Anaphylaxis	Anaphylaxis reactions have been reported in people who had influenza vaccination. People allergic to any ingredients in *Fluarix Quadrivalent* or SUIV vaccines could have an allergic reaction to the vaccine. The viruses for the vaccine are grown in eggs; therefore, people allergic to eggs could have an allergic reaction to *Fluarix Quadrivalent* or SUIVs.	The investigators are advised of possible anaphylaxis following *Fluarix Quadrivalent* or SUIVs administration by information included in product label or IB. As with all injectable vaccines, appropriate medical treatment and supervision should always be readily available in case of an anaphylactic event following the administration of the vaccine. Subjects will be closely monitored for at least 60 minutes (Phase I subjects) or 30 minutes (Phase II subjects) after vaccination. Subjects with history of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccine, or anaphylaxis following the administration of influenza vaccine(s) will be excluded from the study.
Narcolepsy	Epidemiological data developed by organizations not associated with GSK suggest an increased risk of narcolepsy following vaccination with *Pandemrix* H1N1 in children and adolescents. Due to the methodological limitations of the studies, which are retrospective observational studies, further research is needed to determine whether the observed risk is related to the vaccine, environmental effects, genetic factors, other factors or a combination of factors. *Arepanrix*, another AS03-adjuvanted vaccine produced in Quebec, Canada, with a slightly different H1N1 viral antigen manufacturing process, has not been associated with an increased risk of narcolepsy comparable to *Pandemrix* [Montplaisir, 2014].	Close monitoring of pIMDs in clinical development programs using adjuvants systems. The potential risk of events of possible autoimmune etiology to occur (like narcolepsy) is mentioned in the ICF. Subjects with a history or with a current auto-immune disease will be excluded from this study.

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
	GSK considers narcolepsy to be a clear signal that requires further investigation, and continues to support both epidemiological and mechanistic studies. No such risk has been identified in clinical trials of AS03-adjuvanted vaccines against other pandemic influenza antigens.	
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 advised of possible injection site hemorrhage in individuals with thrombocytopenia or any coagulation disorder following study vaccine 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 intramuscularly, all study vaccines should be given with caution to individuals with thrombocytopenia or any coagulation disorder since bleeding may occur following an intramuscular administration to these subjects.”
Syncope (fainting)	Syncope (fainting) can occur following or even before any vaccination as a psychogenic response to needle injection.	Vaccination will be done by a trained professional and procedures will be put in place to avoid injury from fainting.
Risk from blood sampling	Blood sampling-associated risk of discomfort, syncope, dizziness, infection at the site after or during venipuncture.	Blood samples will be obtained by a trained professional and medical assistance will be available. The potential risk of feeling faint, or experiencing mild local pain, bruising, irritation or redness at the site where blood was taken, will be mentioned in the ICF. The amount of blood to be taken for sampling will not be harmful to the subject’s health.

Vaccine-associated enhanced respiratory disease (VAERD) has been described in influenza-naïve pigs vaccinated with inactivated H1 influenza vaccine and subsequently challenged with an antigenically mismatched H1 virus [Vincent, 2008; Gauger, 2011]. Although VAERD is a reproducible observation under well-controlled laboratory conditions with pigs, it has never been observed in natural conditions or in other laboratory animal model used to study influenza viruses (i.e. mice and ferrets). VAERD has not been described with GSK Biologicals' AS03-adjuvanted H5N1-pandemic vaccination. The latter is a relevant surrogate for the SUIV candidate since it elicits a robust immune response to the H1 HA stalk domain without inducing a strong serum neutralizing antibody response to A/H1N1pdm09 virus. Although VAERD is not considered as a potential safety risk in the current study, surveillance will be performed to capture all influenza like illnesses for the duration of the study.

In addition, all cases of respiratory illness, as any safety data, will be closely monitored by the Independent Data Monitoring Committee (IDMC). The theoretical risk of VAERD will be stated in the ICF.

1.3.2. Benefit Assessment

Benefits considerations for the subjects include:

- Contribution to the process of developing a universal influenza vaccine that would potentially provide protection against all influenza A and B strains.
- Gaining information about their general health status through the medical evaluations/assessments associated with this study (i.e. physical examination, blood testing [hematology and biochemistry data]).
- Subjects may have the benefit of being protected against seasonal influenza A and B if they are enrolled in the IIV4 group.
- Subjects receiving one of the investigational monovalent influenza A group 1 SUIVs may potentially have the benefit of being protected against influenza A group 1 viruses (e.g. H1, H5, H9 …). However, the efficacy of the investigational SUIVs has not yet been assessed.

1.3.3. Overall Benefit:Risk Conclusion

It has been demonstrated in mice that the chimeric HA-based vaccination regimen with investigational SUIVs similar as the ones used in this study induced higher stalk antibody titers than the seasonal influenza vaccine. The stalk antibody responses were long lasting, cross-reactive to distantly related HAs and provided protection *in vivo* in a serum passive transfer challenge mouse model [Nachbagauer, 2016]. The investigational SUIVs are 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 SUIVs are justified by the potential benefits linked to the development of the SUIV.

2. OBJECTIVES

2.1. Primary objectives

- To assess the reactogenicity and safety of each vaccine dose throughout the entire study period, in all study groups.
- To describe the anti-H1 stalk humoral immune response 28 days after each priming dose (1 or 2 dose(s)) in all study groups.

Refer to Section 10.1 for the definition of the primary endpoints.

2.2. Secondary objectives

- To evaluate the adjuvant effect of AS03 and AS01 on the humoral immune response after 1 and 2 priming dose(s) of investigational SUIVs when compared to the non-adjuvanted formulations.
- To describe the persistence of the anti-H1 stalk humoral immune response after each priming dose (1 or 2 dose(s)) in all study groups up to Month 14.
- To describe the humoral immune response after a booster dose at Month 14.
- To describe the breadth of the humoral immune response after each vaccination in all study groups.
- To describe the effect of the chimeric HA vaccination-sequence on the humoral immune response.

Refer to Section 10.2 for the definition of the secondary endpoints.

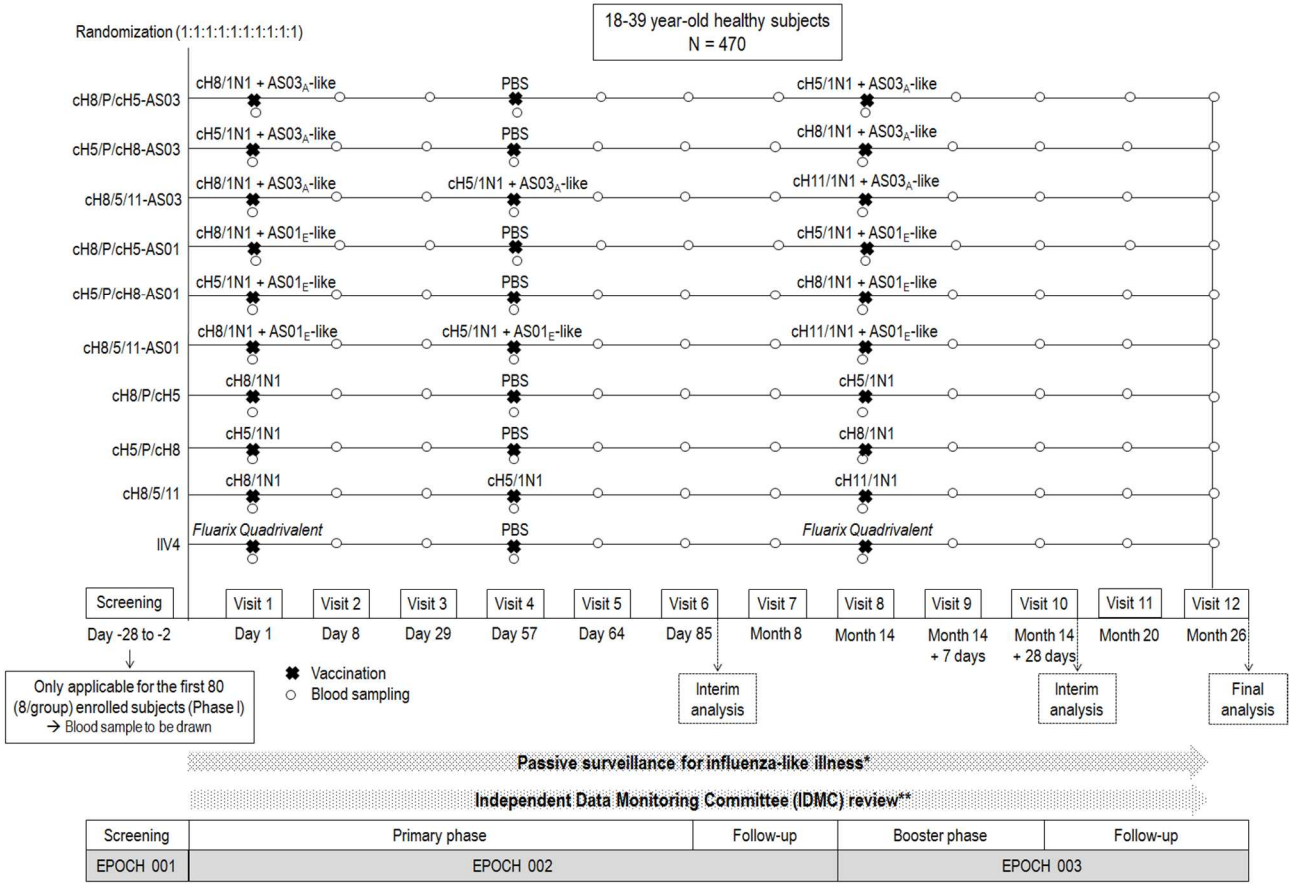
2.3. Tertiary objectives (Amended: 11 July 2019)

- To explore the cell-mediated immune responses (B-cells and T-cells).
- To explore the immune response against the HA head of cH5/1N1 ***and*** cH8/1N1 strain by hemagglutination inhibition (HI) assay.
- To explore the protective effect of the stalk-reactive antibodies induced by vaccination in a passive transfer challenge experiment in mice.
- To develop assays for evaluation/characterization of the humoral and cellular immune responses to the investigational vaccines.
- To explore anti-stalk antibody functionality, e.g., antibody-dependent cell-mediated cytotoxicity (ADCC).

Refer to Section 10.3 for the definition of the tertiary endpoints.

3. STUDY DESIGN OVERVIEW

Figure 1 Study design overview



*If a subject presents signs and symptoms of influenza-like illness (ILI) (see Section 5.4.2), nasal and throat swabs will be collected as soon as possible (preferably within 24 hours, but not later than 7 days) after the onset of an ILI to test for influenza and/or other respiratory pathogens by RT-PCR if deemed necessary or for storage. (Amended: 11 July 2019)
**IDMC reviews will be performed throughout the study (refer to Section 8.8).

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 of study procedures (Section 5.5), are essential and required for study conduct.

- **Experimental design:** Phase I/II, observer-blind, randomized, controlled, multi-centric study with 10 parallel groups.
- **Duration of the study:**
  - Epoch 001: Screening (Day -28 to -2) – only for Phase I subjects (refer to the Enrolment section).
  - Epoch 002: Primary starting at Visit 1 (Day 1) and ending at Visit 7 (Month 8).
  - Epoch 003: Booster starting at Visit 8 (Month 14) and ending at Visit 12 (Month 26).
- **Primary Completion Date (PCD):** Visit 12 (Month 26).

  Refer to glossary of terms for the definition of PCD.
- **End of Study (EoS):** Last testing results released of samples collected at Visit 12 (Month 26).

  Refer to glossary of terms for the definition of EoS.
- **Study groups:**
  - ***cH8/P/cH5-AS03 group***: subjects receiving one dose of cH8/1N1+AS03 at Day 1, one dose of PBS at Day 57 and one booster dose of cH5/1N1+AS03 at Month 14.
  - ***cH5/P/cH8-AS03 group***: subjects receiving one dose of cH5/1N1+AS03 at Day 1, one dose of PBS at Day 57 and one booster dose of cH8/1N1+AS03 at Month 14.
  - ***cH8/5/11-AS03 group***: subjects receiving one dose of cH8/1N1+AS03 at Day 1, one dose cH5/1N1+AS03 at Day 57 and one booster dose of cH11/1N1+AS03 at Month 14.
  - ***cH8/P/cH5-AS01 group***: subjects receiving one dose of cH8/1N1+AS01 at Day 1, one dose of PBS at Day 57 and one booster dose of cH5/1N1+AS01 at Month 14.
  - ***cH5/P/cH8-AS01 group***: subjects receiving one dose of cH5/1N1+AS01 at Day 1, one dose of PBS at Day 57 and one booster dose of cH8/1N1+AS01 at Month 14.
  - ***cH8/5/11-AS01 group***: subjects receiving one dose of cH8/1N1+AS01 at Day 1, one dose cH5/1N1+AS01 at Day 57 and one booster dose of cH11/1N1+AS01 at Month 14.
  - ***cH8/P/cH5 group***: subjects receiving one dose of cH8/1N1 at Day 1, one dose of PBS at Day 57 and one booster dose of cH5/1N1 at Month 14.

- ***cH5/P/cH8 group***: subjects receiving one dose of cH5/1N1 at Day 1, one dose of PBS at Day 57 and one booster dose of cH8/1N1 at Month 14.
- ***cH8/5/11 group***: subjects receiving one dose of cH8/1N1 at Day 1, one dose cH5/1N1 at Day 57 and one booster dose of cH11/1N1 at Month 14.
- ***IIV4 group***: subjects receiving one dose of *Fluarix Quadrivalent* at Day 1, one dose of PBS at Day 57 and one dose of *Fluarix Quadrivalent* at Month 14.

Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min - Max)	Epochs	
			Epoch 002	Epoch 003
cH8/P/cH5-AS03	47	18 years – 39 years	x	x
cH5/P/cH8-AS03	47	18 years – 39 years	x	x
cH8/5/11-AS03	47	18 years – 39 years	x	x
cH8/P/cH5-AS01	47	18 years – 39 years	x	x
cH5/P/cH8-AS01	47	18 years – 39 years	x	x
cH8/5/11-AS01	47	18 years – 39 years	x	x
cH8/P/cH5	47	18 years – 39 years	x	x
cH5/P/cH8	47	18 years – 39 years	x	x
cH8/5/11	47	18 years – 39 years	x	x
IIV4	47	18 years – 39 years	x	x

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/Product name	Study groups									
		cH8/P/cH5-AS03	cH5/P/cH8-AS03	cH8/5/11-AS03	cH8/P/cH5-AS01	cH5/P/cH8-AS01	cH8/5/11-AS01	cH8/P/cH5	cH5/P/cH8	cH8/5/11	IIV4
cH8/1N1+AS03$_A$-like*	cH8/1N1	x	x	x							
	AS03	x	x	x							
	PBS	x	x	x							
cH5/1N1+AS03$_A$-like*	cH5/1N1	x	x	x							
	AS03	x	x	x							
	PBS	x	x	x							
cH11/1N1+AS03$_A$-like*	cH11/1N1			x							
	AS03			x							
	PBS			x							
cH8/1N1+AS01$_E$-like#	cH8/1N1				x	x	x				
	AS01$_B$				x	x	x				
	PBS				x	x	x				
cH5/1N1+AS01$_E$-like#	cH5/1N1				x	x	x				
	AS01$_B$				x	x	x				
	PBS				x	x	x				
cH11/1N1+AS01$_E$-like#	cH11/1N1						x				
	AS01$_B$						x				
	PBS						x				
cH8/1N1	cH8/1N1							x	x	x	
	PBS							x	x	x	
cH5/1N1	cH5/1N1							x	x	x	
	PBS							x	x	x	
cH11/1N1	cH11/1N1									x	
	PBS									x	
Fluarix Quadrivalent	FLU D-QIV										x
PBS	PBS	x	x		x	x		x	x		x

*AS03$_A$-like is obtained by dilution of AS03 with PBS
#AS01$_E$-like is obtained by dilution of AS01$_B$ with PBS

- **Control:** active control (*Fluarix Quadrivalent*).
- **Vaccination schedule:**
  - Two primary doses at Visit 1 (Day 1) and Visit 4 (Day 57).
  - A booster dose at Visit 8 (Month 14).

  Phase I subjects will be vaccinated one at the time, at least 60 minutes apart, with a maximum of 10 subjects a day. This is applicable for Dose 1 (Day 1), Dose 2 (Day 57) and booster dose (Month 14). Dose 2 can only be provided to Phase I subjects upon favorable outcome of the 7-day post-Dose 1 safety data review by the IDMC of at least 60 Phase I subjects.
- **Treatment allocation**: randomized (1:1:1:1:1:1:1:1:1:1:1 ratio) using GSK Biologicals' Randomization System on Internet (SBIR).
- **Blinding:**

Table 3 Blinding of study epochs

Study Epochs	Blinding
Epoch 001	Not applicable
Epoch 002	Observer-blind
Epoch 003	Observer-blind

- **Sampling schedule: (Amended: 11 July 2019)**
  - Blood samples for safety assessment will be drawn from all subjects at all visits: Screening*, Days 1, 8, 29, 57, 64, 85, Month 8, Month 14, Month 14 + 7 days, Month 14 + 28 days, Month 20 and Month 26.

    *Only for subjects enrolled in Phase I (refer to the Enrolment section).
  - Blood samples for serology testing will be drawn from all subjects at Days 1 (Visit 1), 29 (Visit 3), 85 (Visit 6), Month 8 (Visit 7), Month 14 (Visit 8), Month 14 + 28 days (Visit 10), Month 20 (Visit 11) and Month 26 (Visit 12).
  - Blood samples for passive transfer experiment in animals will be drawn from all subjects at Days 1 (Visit 1), 85 (Visit 6), Month 14 (Visit 8)*.
  - Blood samples for cell-mediated immunity (CMI) assessment will be drawn from a sub-cohort of ~225 subjects at Days 1 (Visit 1), 8 (Visit 2), 29 (Visit 3), 64 (Visit 5), 85 (Visit 6), Month 14 (Visit 8)*, Month 14 + 7 days (Visit 9)* ***and*** Month 14 + 28 days (Visit 10)*. The sub-cohort will consist of the first Phase II subjects enrolled in pre-specified centers.
  - During the entire study period, nasal and throat swabs will be collected as soon as possible (preferably within 24 hours, but not later than 7 days) after the onset of an influenza-like illness (ILI) to test for influenza and/or other respiratory pathogens by RT-PCR ***if deemed necessary, or stored for future research*** (see Section 5.4.2).

    Note that samples already collected for these timepoints by the time of Protocol Amendment 4 implementation at site will not be tested and will be stored, unless deemed necessary based on medical review of the cases.

- **ILI surveillance:** ILI is defined as at least one of these systemic symptoms:
  - Temperature (oral) ≥ 37.8°C/98.6°F and/or,
  - Myalgia (widespread muscle ache);

  AND at least one of these respiratory symptoms:
  - Cough and/or,
  - Sore throat.

  Passive surveillance will be carried out from Visit 1 (after Dose 1) until the end of the study (Visit 12). Subjects will be instructed to contact the investigator/study staff as soon as they experience ILI symptoms.
- **Type of study:** self-contained.
- **Data collection:** electronic Case Report Form (eCRF).
- **Safety monitoring:** an IDMC consisting of clinical experts and a biostatistician, independent from the Sponsor, will review unblinded safety data (including laboratory assessment) at a regular frequency to monitor the safety of the subjects throughout the study. Refer to Section 8.8 for detailed description of holding rules and safety monitoring.
- **Enrolment**: the study will follow a staggered enrolment with 2 steps; the first being Phase I (N = ~80) and the second being Phase II (N = ~390):
  - Phase I: During the Phase I enrolment, subjects will be vaccinated one at a time, at least 60 minutes apart, with a maximum of 10 subjects/day until ~80 subjects are enrolled (i.e. to obtain treatment groups of at least 8 subjects/group). If no safety issue is identified by the IDMC upon review of the 7-day post-dose 1 safety data (Days 1-7) of all Phase I subjects (N = ~80), Phase II enrolment will be allowed to start.
  - Phase II: Subjects will be enrolled and vaccinated without limitation on the number of vaccinees per day or time between consecutive subjects.

4. STUDY COHORT

4.1. Number of subjects/centers

This study will be conducted in multiple centers. A total of 470 subjects (47/group) are planned to be enrolled in this study, in order to have 430 evaluable subjects for the primary immunogenicity endpoints. Refer to Section 10.4 for a detailed description of the criteria used in the determination of sample size.

Table 4 Sub-cohort (Amended: 11 July 2019)

Sub-cohort name	Description	Estimated number of subjects
Cell-mediated immunity (CMI) testing	Additional blood sample (~40 mL) to be taken at Visits 1, 2, 3, 5, 6, 8, 9 ***and*** 10 for CMI testing. This will be done at pre-specified sites with adequate material for such sampling procedure.	Approximately 225 The sub-cohort will consist of the first Phase II subjects enrolled in pre-specified sites.

4.2. Inclusion criteria for enrolment

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:

- Subjects who, in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g. completion of the diary cards, return for follow-up visits, contact the site in case of ILI).

  Please refer to the glossary of terms for the definition of ILI.
- Written informed consent obtained from the subject prior to performing any study specific procedure.
- A male or female between, and including, 18 and 39 years of age at the time of the first vaccination.
- Healthy subjects without acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality, as established by medical history and clinical examination before first vaccination and laboratory screening tests (the latter being only applicable for subjects enrolled in Phase I).
- Subjects with no history of influenza vaccination within 6 months prior to first study vaccination and who are willing to forego any influenza vaccination during the entire study period.
- Female subjects of non-childbearing potential may be enrolled in the study.
  - Non-childbearing potential is defined as pre-menarche, current bilateral tubal ligation or occlusion, hysterectomy, bilateral ovariectomy or post-menopause.

Please refer to the glossary of terms for the definition of menarche and menopause.

- Female subjects of childbearing potential may be enrolled in the study, if the subject:
  - Has practiced adequate contraception for 30 days prior to first vaccination, and
  - Has a negative 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 (last vaccination at Month 14).

Please refer to the glossary of terms for the definition of adequate contraception.

4.3. Exclusion criteria for enrolment

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:

- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccines during the period starting 30 days before the first dose of study vaccines (Day -29 to Day 1), or planned use during the study period.
- Any medical condition that in the judgment of the investigator would make intramuscular injection unsafe.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs during the period starting 6 months prior to the first vaccine dose. For corticosteroids, this will mean prednisone ≥ 20 mg/day, or equivalent. Topical steroids are allowed.
- Administration of long-acting immune-modifying drugs (e.g. infliximab, rituximab) within 6 months before first vaccination (Visit 1), or planned administration any time during the study period.
- Planned administration/administration of a vaccine not foreseen by the study protocol in the period starting 30 days before the first dose (Visit 1) up to the blood sampling at Day 85 (Visit 6) and in the period starting 30 days before booster vaccination at Month 14 (Visit 8) up to the blood sample at Month 14 + 28 days (Visit 10).
- 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).
- Previous vaccination against influenza within the 6 months preceding the first vaccination at Visit 1 or planned use of such vaccines during the study period.
- History of vaccination with a (pre)pandemic influenza vaccine other than an H1N1pdm09 vaccine or history of laboratory-confirmed influenza infection other than seasonal or other than H1N1pdm09 influenza.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination (no laboratory testing required).
- History of or current autoimmune disease.
- Subjects diagnosed with excessive daytime sleepiness (unintended sleep episodes during the day present almost daily for at least one month) or narcolepsy; or history of narcolepsy in a subject's parent or sibling.
- History of Guillain-Barré syndrome.

- History of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccines (including egg proteins); a history of anaphylactic-type reaction to consumption of eggs; or a history of severe adverse reaction to a previous influenza vaccine.
- Hypersensitivity to latex.
- Acute disease and/or fever at the time of enrolment.
  - 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.
  - For subjects with acute disease and/or fever at the time of enrolment, Visit 1 may be re-scheduled within the allowed time-window.
- Administration of immunoglobulins and/or any blood products during the period starting 3 months before the first dose of study vaccines or planned administration during the study period.
- Blood donation within 30 days before the first study blood sampling or planned blood donation within 30 days before and up to 30 days after any study blood sampling.
- Pregnant or lactating female.
- 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.
- Female planning to become pregnant or planning to discontinue contraceptive precautions.
- Any condition that puts the subject at risk for serious influenza-related complications, as identified by the Advisory Committee on Immunization Practices [Grohskopf, 2017] (note that only criteria applicable for the study population are listed below):
  - Chronic pulmonary (including asthma), cardiovascular (except isolated hypertension), renal, hepatic, neurologic, hematologic or metabolic disorders (including diabetes mellitus);
  - Persons who are immunocompromised due to any cause (including immunosuppression caused by medications or by HIV infection);
  - Adolescents (through 18 years) who are receiving aspirin- or salicylate-containing medications and who might be at risk for experiencing Reye syndrome after influenza virus infection;
  - Residents of nursing homes and other long-term care facilities;
  - American Indians/Alaska Natives; and
  - Persons who are extremely obese (Body Mass Index ≥ 40).

Additional criterion applicable for Phase I subjects:

- Hematological and/or biochemical parameters (complete blood cell count [red blood cells, white blood cells], white blood cells differential count [lymphocytes, neutrophils and eosinophils], platelets count or hemoglobin level, creatinine or blood urea nitrogen) outside the laboratory normal ranges, unless the laboratory abnormalities are considered not clinically significant by the investigator.
- Liver enzymes (alanine aminotransferase [ALT] or aspartate aminotransferase [AST]) outside of the normal laboratory ranges.

5. CONDUCT OF THE STUDY

5.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the International Conference on Harmonization (ICH) Guideline for GCP, all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

GSK will obtain favorable opinion/approval to conduct the study from the appropriate regulatory agency, in accordance with applicable regulatory requirements, prior to a site initiating the study in that country.

Conduct of the study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favorable opinion/approval of study protocol and any subsequent amendments.
- Subject informed consent.
- Investigator reporting requirements as stated in the protocol.

GSK will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written or witnessed informed consent must be obtained from each subject, as appropriate, prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the ICH GCP and GSK Biologicals required elements. While it is strongly recommended that this model ICF is to be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgement, local regulations and requirements should guide the final structure and content of the local version of the ICF.

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

5.2. Subject identification and randomization

5.2.1. 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 each study center.

5.2.2. Randomization of treatment

5.2.2.1. Randomization of supplies

The randomization of supplies within blocks will be performed at GSK Biologicals, using MATerial EXcellence (MATEX), a program developed for use in Statistical Analysis System (SAS®) (Cary, NC, USA) by GSK Biologicals. Entire blocks will be shipped to the study centers/warehouse(s).

To allow GSK Biologicals to take advantage of greater rates of recruitment than anticipated at individual centers in this multi-center study and to thus reduce the overall study recruitment period, an over-randomization of supplies will be prepared.

5.2.2.2. Treatment allocation to the subject

The treatment numbers will be allocated by dose.

5.2.2.2.1. Study group and treatment number allocation

The target will be to enroll ~470 eligible subjects who will be randomly assigned to 10 study groups in a (1:1:1:1:1:1:1:1:1:1) ratio (~47 subjects in each group).

Allocation of the subject to a study group at the investigator site will be performed using SBIR. The randomization algorithm will use a minimization procedure accounting for center, sex, age (18-30 years vs. 31-39 years) and history of influenza vaccination since the 2014/2015 season (yes vs. no). Minimization factors will have equal weight in the minimization algorithm.

After obtaining the signed and dated ICF from the subject and having checked the eligibility of the subject, the site staff in charge of the vaccine/product administration will access SBIR. Upon providing 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.

5.2.2.2.2. Treatment number allocation for subsequent doses

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

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

5.3. Method of blinding

Data will be collected in an observer-blind manner. By observer-blind, it is meant that during the course of the study, the vaccines/products recipient and those responsible for the evaluation of any study endpoint (e.g. safety, reactogenicity) will all be unaware of which vaccine/product was administered. To do so, vaccine/product preparation and administration will be done by authorized medical personnel who will not participate in any of the study clinical evaluation assays.

The site staff will work in an observer-blind manner during the entire study. As the vaccines appearance and preparation are different, two teams of study personnel will be set up:

- A team of unblinded personnel (responsible for the reception, preparation and administration of the vaccines).
- A team of blinded personnel (responsible for the clinical safety evaluation of the subjects).

Please refer to the SPM for more information on vaccine preparation and administration while maintaining the blind.

An analysis of data collected up to Day 85 and Month 14 + 28 days will be done on as cleaned as possible data up to Visit 6 and Visit 10, respectively (refer to Section 10.11.1 for more information). At Day 85 analysis, the GSK statistician will be unblinded (i.e. will have access to the individual subject treatment assignment). The remaining GSK study personnel will stay blinded (i.e. 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) (e.g. an AE/SAE occurring only in a single group). Therefore anyone having access to the analysis of Day 85 could become unblinded regarding those specific cases.

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.

5.4. General study aspects

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 site personnel with administrative and detailed technical information that does not impact the safety of the subjects.

5.4.1. Independent data monitoring committee

An IDMC will be established by GSK Biologicals for the purpose of monitoring the study and to provide independent, non-binding advice on safety and ethics. The IDMC will provide recommendations about stopping, continuing or modifying the trial. Refer to Section 8.8 for a description of the holding rules and safety monitoring put in place for this study.

5.4.2. Influenza-like illness surveillance

ILI is defined as at least one of these systemic symptoms:

- Temperature (oral) ≥ 37.8°C/98.6°F and/or,
- Myalgia (widespread muscle ache);

AND at least one of these respiratory symptoms:

- Cough and/or,
- Sore throat.

All ILIs will be reported as unsolicited AEs or SAEs, as applicable.

Passive surveillance will be carried out from Visit 1 (after Dose 1) until the end of the study (Visit 12). All study participants will be instructed to contact the investigator/study staff in case they experience signs symptoms of ILI as defined above.

The investigator/study staff will schedule a visit for the collection of nasal and throat swab specimens preferably within 24 hours (but not later than 7 days) after the onset of the ILI. Refer to the SPM for a detailed description of the procedures.

5.5. Outline of study procedures

The list of study procedures is presented in Table 5 for Phase I subjects and in Table 6 for Phase II subjects.

Table 5 List of study procedures for Phase I subjects (Amended: 11 July 2019)

Epoch	Screening	Primary							Booster					In case of ILI**
Type of contact	Screening Visit*	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	
Timepoints	Pre-Day 1	Day 1	Day 8	Day 29	Day 57	Day 64	Day 85	M8	M14	M14+7D	M14+28D	M20	M26	
Sampling timepoints	Screening	PRE	PId7	PId28	PId56	PIId7	PIId28	M8	M14	PIIId7	PIIId28	M20	M26	
Informed consent	●													
Check inclusion/exclusion criteria	●	O												
Collect demographic data	●													
Medical history	O	●												
History of influenza vaccination within previous 3 seasons (2014/2015, 2015/2016, 2016/2017)	●													
Physical examination	●	●	O	O	O	O	O	O	O	O	O	O	O	
Pregnancy test §	●	●			●				●					
Check contraindications and warnings and precautions to vaccination		O			O				O					
Pre-vaccination body temperature		●			●				●					
Measure/record height and weight	●													
Vaccine														
Study group and treatment number allocation		O												
Treatment number allocation for subsequent doses					O				O					
Recording of administered treatment number		●			●				●					
Vaccine administration		●†			●†				●†					
60 minutes post-vaccination observation		O			O				O					
Laboratory assays														
Blood sampling for antibody determination (~12 mL)		●		●			●	●	●		●	●	●	
Blood sampling for hematology/biochemical analysis (~5.5 mL)	●	●‡	●	●	●	●	●	●	●	●	●	●	●	

Epoch	Screening	Primary							Booster					In case of ILI**
Type of contact	Screening Visit*	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	
Timepoints	Pre-Day 1	Day 1	Day 8	Day 29	Day 57	Day 64	Day 85	M8	M14	M14+7D	M14+28D	M20	M26	
Sampling timepoints	Screening	PRE	PId7	PId28	PId56	PIId7	PIId28	M8	M14	PIIId7	PIIId28	M20	M26	
Blood sampling for passive transfer (~2.5 mL)		●					●		●					
Nasal and throat swab sampling														●
ILI surveillance														
Surveillance for ILI		O	O	O	O	O	O	O	O	O	O	O	O	
Documentation of symptoms and signs of ILI		●	●	●	●	●	●	●	●	●	●	●	●	●
Safety assessments														
Record any concomitant medications/vaccinations	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Record any intercurrent medical conditions		●	●	●	●	●	●	●	●	●	●	●	●	
Distribution of diary cards [1]		O		O	O		O	O	O		O	O		
Recording of solicited AEs within 7 days post-vaccination		●	●		●	●			●	●				
Recording of unsolicited AEs within 28 days post-vaccination		●	●	●	●	●	●		●	●	●			
Recording of ILIs as unsolicited AEs/SAE, as applicable		●	●	●	●	●	●	●	●	●	●	●	●	●
Return of diary cards			O	O	O	O	O	O	O	O	O	O	O	O
Diary card transcription by investigator			●	●	●	●	●	●	●	●	●	●	●	●
Recording of AEs and SAEs leading to withdrawal from the study		●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of SAEs, MAEs, pIMDs and pregnancies		●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Screening conclusion	●													
Study conclusion													●	

Note: the double-line border following Day 85 and Month 14 + 28 days indicates the analyses which will be performed on all data (i.e. data that are as clean as possible) obtained up to Day 85 and Month 14 + 28 days, respectively.

D = day; **M** = month (= 28 days); **PRE** = pre-vaccination; **PI** = post-dose 1; **PII** = post-dose 2; **PIII** = post-dose 3 (booster)

AE = adverse events, **ILI** = influenza-like illness; **SAE** = serious adverse event; **MAE** = medically attended event; **pIMD** = potential immune-mediated disease

● is used to indicate a study procedure that requires documentation in the individual eCRF

○ is used to indicate a study procedure that does not require documentation in the individual eCRF
* Screening evaluations may be completed 2 to 28 days before Day 1. Site staff should allow sufficient time between the Screening and Visit 1 to receive and review screening safety laboratory test results. If a delay occurs such that the interval between Screening and the Visit 1 vaccination exceeds 28 days, a re-screening visit should be scheduled before Visit 1 during which blood sample collection for safety laboratory assessment must be repeated; an interim medical history and physical examination must be obtained and inclusion / exclusion criteria must be re-reviewed. Only data from the re-screening visit, if it occurs, will be recorded in the eCRF and taken into consideration.
** This visit is only applicable following passive surveillance contacts for subjects with ILI (see Section 5.4.2).
§ Only for females subjects of childbearing potential. An urine pregnancy test will be performed. A serum pregnancy test instead of a urine pregnancy test should only be considered if required by local or ethics committee regulations. If a serum pregnancy test instead of a urine pregnancy test is required by country, local or ethics committee regulations, a blood sample will be collected from women of childbearing potential at these visits and will be used for the test as per local guidance. The results must be obtained and be confirmed negative before vaccination.
† Blood samples must be taken before vaccine administration
‡ Blood sample for hematology/biochemical assessment will not be drawn at Visit 1 if this visit occurs less than one week after the screening visit
1 Diary cards will allow the collection of solicited and unsolicited symptoms, concomitant medications/vaccinations, MAEs and episode(s) of ILI. Refer to the SPM for more details on the usage of the diary cards.

Table 6 List of study procedures for Phase II subjects (Amended: 11 July 2019)

Epoch	Primary						Booster						In case of ILI *
Type of contact	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	
Timepoints	Day 1	Day 8	Day 29	Day 57	Day 64	Day 85	M8	M14	M14+7D	M14+28D	M20	M26	
Sampling timepoints	PRE	PId7	PId28	PId56	PIId7	PIId28	M8	M14	PIIId7	PIIId28	M20	M26	
Informed consent	●												
Check inclusion/exclusion criteria	●												
Collect demographic data	●												
Medical history	●												
History of influenza vaccination within previous 3 seasons (2014/2015, 2015/2016, 2016/2017)	●												
Physical examination	●	O	O	O	O	O	O	O	O	O	O	O	
Pregnancy test §	●			●				●					
Check contraindications and warnings and precautions to vaccination	O			O				O					
Pre-vaccination body temperature	●			●				●					
Measure/record height and weight	●												
Vaccine													
Study group and treatment number allocation	O												
Treatment number allocation for subsequent doses				O				O					
Recording of administered treatment number	●			●				●					
Vaccine administration	●†			●†				●†					
30 minutes post-vaccination observation	O			O				O					
Laboratory assays													
Blood sampling for antibody determination (~12 mL)	●		●			●	●	●		●	●	●	
Blood sampling for CMI response (~40 mL)#	●	●	●		●	●		●	●	●			
Blood sampling for hematology/biochemical analysis (~5.5 mL)	●	●	●	●	●	●	●	●	●	●	●	●	
Blood sampling for passive transfer (~2.5 mL)	●					●		●					
Nasal and throat swab sampling													●

Epoch	Primary						Booster						In case of ILI *
Type of contact	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**	**Visit 10**	**Visit 11**	**Visit 12**	
Timepoints	**Day 1**	**Day 8**	**Day 29**	**Day 57**	**Day 64**	**Day 85**	**M8**	**M14**	**M14+7D**	**M14+28D**	**M20**	**M26**	
Sampling timepoints	**PRE**	**PId7**	**PId28**	**PId56**	**PIId7**	**PIId28**	**M8**	**M14**	**PIIId7**	**PIIId28**	**M20**	**M26**	
ILI surveillance													
Surveillance for ILI	O	O	O	O	O	O	O	O	O	O	O	O	
Documentation of symptoms and signs of ILI	●	●	●	●	●	●	●	●	●	●	●	●	●
Safety assessments													
Record any concomitant medications/vaccinations	●	●	●	●	●	●	●	●	●	●	●	●	●
Record any intercurrent medical conditions	●	●	●	●	●	●	●	●	●	●	●	●	
Distribution of diary cards [1]	O		O	O		O	O	O		O	O		
Recording of solicited AEs within 7 days post-vaccination	●	●		●	●			●	●				
Recording of unsolicited AEs within 28 days post-vaccination	●	●	●	●	●	●		●	●	●			
Recording of ILIs as unsolicited AEs/SAE, as applicable	●	●	●	●	●	●	●	●	●	●	●	●	●
Return of diary cards		O	O	O	O	O	O	O	O	O	O	O	O
Diary card transcription by investigator		●	●	●	●	●	●	●	●	●	●	●	●
Recording of AEs and SAEs leading to withdrawal from the study	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of SAEs, MAEs, pIMDs and pregnancies	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●	●	●	●	●	●	●	●	●
Study conclusion												●	

Note: the double-line border following Day 85 and Month 14 + 28 days indicates the analyses which will be performed on all data (i.e. data that are as clean as possible) obtained up to Day 85 and Month 14 + 28 days, respectively.

D = day; **M** = month (= 28 days); **PRE** = pre-vaccination; **PI** = post-dose 1; **PII** = post-dose 2; **PIII** = post-dose 3 (booster)

CMI = cell-mediated immunity; **AE** = adverse events, **ILI** = influenza-like illness; **SAE** = serious adverse event; **MAE** = medically attended event; **pIMD** = potential immune-mediated disease

● is used to indicate a study procedure that requires documentation in the individual eCRF

○ is used to indicate a study procedure that does not require documentation in the individual eCRF

* This visit is only applicable following passive surveillance contacts for subjects with ILI (see Section 5.4.2).

§ Only for females subjects of childbearing potential. An urine pregnancy test will be performed. A serum pregnancy test instead of a urine pregnancy test should only be considered if required by local or ethics committee regulations. If a serum pregnancy test instead of a urine pregnancy test is required by country, local or ethics committee regulations, a blood sample will be collected from women of childbearing potential at these visits and will be used for the test as per local guidance. The results must be obtained and be confirmed negative before vaccination.

† Blood samples must be taken before vaccine administration

Only for subjects included in the CMI sub-cohort

1 Diary cards will allow the collection of solicited and unsolicited symptoms, concomitant medications/vaccinations, MAEs and episode(s) of ILI. Refer to the SPM for more details on the usage of the diary cards.

Whenever possible, the investigator should arrange study visits within the interval described in Table 7.

Table 7 Intervals between study visits

Interval	Optimal length of interval	Allowed interval**
Screening to Visit 1*	2-28 days	
Visit 1 → Visit 2	7 days	7-9 days
Visit 1 → Visit 3	28 days	28-38 days
Visit 1 → Visit 4	56 days	56-66 days
Visit 4 → Visit 5	7 days	7-9 days
Visit 4 → Visit 6	28 days	28-38 days
Visit 4 → Visit 7	168 days	168-196 days
Visit 4 → Visit 8	336 days	336-364 days
Visit 8 → Visit 9	7 days	7-9 days
Visit 8 → Visit 10	28 days	28-38 days
Visit 8 → Visit 11	168 days	168-196 days
Visit 8 → Visit 12	336 days	336-364 days

*Only applicable for Phase I subjects. Screening evaluations may be completed 2 to 28 days before Day 1. Site staff should allow sufficient time between the screening and the Day 1 visits to receive and review screening safety laboratory test results. If a delay occurs such that the interval between screening and the Day 1 vaccination exceeds 28 days, a re-screening visit should be scheduled before Visit 1.
**Visits out of the allowed interval can lead to elimination from the Per-Protocol set for immunogenicity analysis.

5.6. Detailed description of study procedures

5.6.1. Informed consent

The signed/witnessed informed consent of the subject must be obtained before study participation. Refer to Section 5.1 for the requirements on how to obtain informed consent.

5.6.2. Check inclusion and exclusion criteria

Check all inclusion and exclusion criteria as described in Sections 4.2 and 4.3 before enrolment.

5.6.3. Collect demographic data

Record demographic data such as date of birth (only month and year), sex, race (ethnicity and geographic ancestry) in the subject's eCRF.

5.6.4. Medical history

Obtain the subject's medical history by interview and/or review of the subject's medical records and record any pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination in the eCRF.

5.6.5. History of influenza vaccination

Any history of influenza vaccination in the past three seasons (2014/2015; 2015/2016 and 2016/2017) will be recorded. Self-reporting by the subject is acceptable (medical records are not required).

5.6.6. Physical examination

Perform a physical examination of the subject, including assessment of oral body temperature and resting vital signs: systolic/diastolic blood pressure, heart rate and respiratory rate after at least 10 minutes of rest, pulmonary auscultation. Collected information needs to be recorded in the eCRF.

Any findings from the(se) physical examination(s) need to be recorded in the subject's medical record, in the medical history screen of the eCRF or reported as an AE or SAE, as applicable, if they meet the protocol definition of AE or SAE (see Sections 8.1.1 and 8.1.2).

If the investigator determines that the subject's health on the day of vaccination temporarily precludes vaccination, the visit will be rescheduled within the allowed interval for that visit (see Table 7).

Treatment of any abnormality observed during physical examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

5.6.7. Pregnancy test

Female subjects of childbearing potential are to have a urine pregnancy test prior to any study vaccine administration. The test result is to be recorded in the eCRF. The study vaccines/products may only be administered if the pregnancy test is negative.

A serum pregnancy test instead of a urine pregnancy test should only be considered if required by country, local or ethics committee regulations. If a serum pregnancy test instead of a urine pregnancy test is required by country, local or ethics committee regulations, a blood sample will be collected from women of childbearing potential at the vaccination visits and will be used for the test as per local guidance.

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

5.6.8. Check contraindications, warnings and precautions to vaccination

Contraindications, warnings and precautions to vaccination must be checked at the beginning of each vaccination visit. Refer to Sections 6.5 and 6.6 for more details.

5.6.9. Assess pre-vaccination body temperature

The oral body temperature of each subjects needs to be measured prior to any study vaccines/products administration. If the subject has fever (fever is defined as temperature ≥ 38.0°C/100.4°F) on the day of vaccination, the vaccination visit will be rescheduled within the allowed interval for that visit (see Table 7).

5.6.10. Measure/record height and weight

Measure and record the height and weight of the subjects in the eCRF.

5.6.11. Study group and treatment number allocation

Study group and treatment number allocation will be performed as described in Section 5.2.2. The number of each administered treatment must be recorded in the eCRF.

5.6.12. Sampling

Refer to the Module on Biospecimen Management in the SPM for detailed instructions for the collection, handling and processing of the samples.

5.6.12.1. Blood sampling for safety and immune response assessments

Blood samples will be taken during certain study visits as specified in Section 5.5 List of Study Procedures:

- A volume of approximately 12 mL of whole blood should be drawn from all subjects for each analysis of humoral immune response at each pre-defined timepoint. After centrifugation, serum samples should be kept at –20°C/ –4°F or below until shipment. Refer to the SPM for more details on sample storage conditions.
- A volume of approximately 40 mL of whole blood should be drawn from all subjects included in the CMI sub-cohort for analysis of CMI response at each pre-defined timepoint. The blood should be stored at the investigator's site at room temperature and it must not be centrifuged. Samples will be shipped at room temperature (20 to 25°C/68 to 77°F) to the designated laboratory for cell separation to be performed within 24 hours. Refer to the SPM for more details on sample storage conditions.
- A volume of approximately 5.5 mL of whole blood should be drawn from all subjects for each hematology and biochemistry analysis at each pre-defined timepoint. Blood will be collected in 2 tubes (2 mL for hematology and 3.5 mL for biochemistry) or in one tube, but then will be separated in 2 aliquots. The aliquot for biochemistry analysis will be centrifuged. Serum samples should be kept at –20°C/–4°F or below until shipment. Refer to the SPM for more details on sample storage conditions.
- A volume of approximately 2.5 mL of whole blood should be drawn from all subjects for the passive transfer testing at each pre-defined timepoint. After

centrifugation, serum samples should be kept at –20°C/ –4°F or below until shipment. Refer to the SPM for more details on sample storage conditions.

5.6.12.2. Nasal and throat sampling

Cells and secretions from the nasopharynx will be collected using sterile nasal and throat swabs any time during the study if a subject presents with an ILI. Samples should be kept at –70°C/–94°F or below until shipment. Refer to the SPM for more details on sample storage conditions.

5.6.13. Study vaccines/products administration

- After completing all prerequisite procedures prior to vaccination, one dose of study vaccines/products will be administered intramuscularly (IM) in the deltoid of the non-dominant arm (refer to Section 6.3 for detailed description of the vaccines/products administration procedure). If the investigator or delegate determines that the subject's health on the day of administration temporarily precludes vaccines/products administration, the visit will be rescheduled within the allowed interval for this visit (refer to Table 7).
- The subjects will be observed closely for at least 60 minutes (Phase I subjects) or 30 minutes (Phase II subjects) following the administration of the vaccines/products, with appropriate medical treatment readily available in case of anaphylaxis.

5.6.14. Surveillance for influenza-like illness and documentation of signs and symptoms

Subjects will be instructed to contact the investigator/study staff as soon as they experience ILI signs or symptoms from Visit 1 (after Dose 1) until the end of the study (Visit 12) (see Section 5.4.2). The subjects will be requested to come to the study site to collect nasal and throat swabs as soon as possible after the onset of the ILI symptoms (preferably within 24 hours and not later than 7 days after the onset of an ILI). In case the subject has no possibility to come, a study staff member will visit the subject to collect nasal and throat swabs. All samples will be obtained, if possible, before antimicrobial/influenza antiviral therapy is started. However, if microbial/antiviral therapy has started, the swab will be collected and the therapy will be recorded on the ILI diary cards. The most suitable time for collecting the swabs is in the morning before a meal. If the sample cannot be collected in the morning, the subject should be instructed not to take any meal at least one hour before sampling.

The ILI date of onset, the ILI signs and symptoms and the date of swab specimen collection will be collected in the eCRF. In addition, all cases of ILI have to be recorded as unsolicited AE or SAE in the eCRF.

An ILI not reported to the study team within 7 days following the onset of ILI will be considered and recorded in the CRF as a miscase. For these miscases, no swabs will be collected.

5.6.15. Check and record concomitant medication/vaccination and intercurrent medical conditions

Concomitant medication/vaccination must be checked and recorded in the eCRF as described in Section 6.7.

Intercurrent medical conditions must be checked and recorded in the eCRF as described in Section 6.8.

5.6.16. Recording of AEs, MAEs, SAEs, pregnancies and pIMDs

- Refer to Section 8.3 for procedures for the investigator to record AEs, medically attended events (MAEs), SAEs, pregnancies and potential immune-mediated diseases (pIMDs). Refer to Section 8.4 for guidelines and how to report SAE, pregnancy and pIMD reports to GSK Biologicals.
- The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.
- At each visit (except Visits 2, 5 and 9), diary cards will be provided to the subject to record AEs, MAE(s) and episode(s) of ILI. The subject will be instructed to measure and record the body temperature (preferably measured orally), and any solicited local/general AEs (i.e. on the day of vaccination and during the next 6 days) or any unsolicited AEs (i.e. on the day of vaccination and during the next 27 days) occurring after vaccination. In addition, MAE(s) and episode(s) of ILI occurring during the entire study period will be collected. The subject will be instructed to return the completed diary card to the investigator at the next study visit.
- Collect and verify completed diary cards during discussion with the subject at each visit.
- Any unreturned diary cards will be sought from the subject through telephone call(s) or any other convenient procedure.
- The investigator and/or delegate will transcribe the collected information into the eCRF in English.

5.6.17. Study conclusion

The investigator will:

- review data collected to ensure accuracy and completeness.
- complete the Study Conclusion screen in the eCRF.

5.7. Biological sample handling and analysis

Please refer to the SPM for details on biospecimen management (handling, storage and shipment).

Samples will not be labelled with information that directly identifies the subject but will be coded with the identification number for the subject (subject number).

- Collected samples will be used for protocol mandated research and purposes related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test methods, as well as making sure that new tests are comparable to previous methods and work reliably.
- It is also possible that future findings may make it desirable to use the samples acquired in this study for future research, not described in this protocol. Therefore, all subjects in countries where this is allowed will be asked to give a specific consent to allow GSK or a contracted partner to use the samples for future research. Future research will be subject to the laws and regulations in the respective countries and will only be performed once an independent Ethics Committee or Review Board has approved this research.

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

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

Refer also to the Investigator Agreement, where it is noted that the investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

If additional testing is performed, the marker priority ranking given in Section 5.7.4 may be changed.

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

5.7.1. Use of specified study materials

When materials are provided by GSK Biologicals, it is MANDATORY that all clinical samples (including serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the per-protocol analysis (see Section 10.5 for the definition of analysis sets). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals does not provide material for collecting and storing clinical samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM.

5.7.2. Biological samples

Table 8 Biological samples (Amended: 11 July 2019)

Sample type	Quantity	Unit	Timepoint	Sub-cohort name**
Blood	Approximately 20	mL	Visits 1, 6, 8	All subjects
	Approximately 5.5*	mL	Visits 2, 4, 5, 9	
	Approximately 17.5	mL	Visits 3, 7, 10, 11***, 12***	
Blood	Approximately 40	mL	Visits 1, 2, 3, 5, 6, 8, 9, 10	In addition for the CMI sub-cohort
Nasal and throat swabs	Not defined	-	Within 24 hours (but not later than 7 days) after the onset of the ILI	All subjects

*Note that an additional blood sample (5.5 mL) will be drawn at Screening visit for Phase I subjects.
**Refer to Table 4 for sub-cohort description.

Note that if a serum pregnancy test instead of a urine pregnancy test is required, a blood sample (~2 mL) will be collected from women of child-bearing potential at screening (Phase I subjects), Visits 1, 4 and 8, and will be used for the test as per local guidance.

5.7.3. Laboratory assays

Please refer to APPENDIX A for a detailed description of the assays performed in the study. Please refer to APPENDIX B for the address of the clinical laboratories used for sample analysis.

Humoral immune responses

Serological assays (quantification of antibodies by ELISA, microneutralization [MN] and hemagglutination inhibition [HI] assays) will be performed at GSK Biologicals' laboratory or in a laboratory designated by GSK Biologicals using standardized procedures (Table 9).

Cell-mediated immunity

T-cell, B-memory cell and plasmablast responses will be evaluated at GSK Biologicals' laboratory or in a laboratory designated by GSK Biologicals using standardized procedures (Table 10).

Hematology and biochemistry

Hematology and biochemistry assays for safety assessment will be performed in a central laboratory (Table 11).

Molecular biology (PCR tests) (Amended: 11 July 2019)

At the onset of an ILI episode (refer to Section 5.4.2 for the ILI definition), nasal and throat swab specimens will be taken according the procedure described in the SPM. ***N***asal and throat swab specimen***s*** will be tested by RT-PCR for influenza and/or other respiratory virus infections at GSK Biologicals' central laboratory or in a laboratory designated by GSK Biologicals***, if deemed necessary, or will be stored for future research*** (Table 12).

Passive transfer experiment

The passive transfer experiment in mice will be conducted at GSK Biologicals' laboratory or in a laboratory designated and validated by GSK Biologicals using standardized procedures. Refer to APPENDIX D for more details.

Table 9 Humoral immunity (Amended: 11 July 2019)

System	Component (Strain or Antigen Description)	Method	Kit/Manufacturer	Unit	Cut-off	Laboratory*
ELISAs						
Serum	**cH6/1 HA** = Recombinant antigen based on A/mallard/Sweden/81/2002 (H6N1) head domain with stalk domain from HA of H1N1 virus A/California/04/09	Anti-H1 HA stalk ELISA	In-house assay	ELISA units per mL (EU/mL)	***66***	GSK Biologicals' laboratory** or laboratory designated by GSK Biologicals
	H2 HA full length = Recombinant antigen based on A/Japan/305/1957 (H2N2) HA	Anti-H2 HA full length ELISA			***22***	
	H18 HA full length = Recombinant antigen based on A/flat-faced bat/Peru/033/2010 (H18N11) HA	Anti-H18 HA full length ELISA			***43***	
Microneutralization (MN) assays						
Serum	**cH6/1N5 virus:** HA head: A/mallard/Sweden/81/2002 (H6N1) HA stalk: A/California/04/2009 (H1N1) N5: A/mallard/Sweden/86/2003 (H12N5)	Anti-H1 HA stalk MN Assay	In-house assay	1/DIL (IC_{50})	***20***	GSK Biologicals' laboratory** or laboratory designated by GSK Biologicals
	H1N1 swine influenza virus: A/Swine/Jiangsu/40/2011 (H1N1)	Anti-heterosubtypic HA Group 1 virus MN Assay			***20***	
	IIV4 H1N1 strains$	Anti-heterosubtypic HA Group 1 virus MN Assay			***20***	
Hemagglutination Inhibition (HI) assay						
Serum	**Chimeric vaccine strains**: cH5/1N1 ***and*** cH8/1N1	HI assay	In-house assay	1/DIL	***10***	GSK Biologicals' laboratory** or laboratory designated by GSK Biologicals

TBD = to be determined; **DIL** = dilution

*Refer to APPENDIX B for the laboratory addresses.

**GSK Biologicals laboratory refers to Clinical Laboratory Sciences (CLS) in Rixensart, Belgium; Wavre, Belgium; Dresden, Germany

$ IIV4 H1N1 strains will depend on the World Health Organization recommendation for the 2017/2018 season (Dose 1 at Day 1) and the 2018/2019 season (booster dose at Month 14).

Table 10 Cell-mediated immunity

System	Component	Challenge	Method	Unit	Laboratory*
PBMCs	T-cells stained with probes for various activation markers (such as IL-2, TNF-α, IFN-γ, CD40-L)	None, H1 (A/California/04/2009) stalk peptide pool	T-cell response by ICS assay	Frequencies of CD4+/CD8+ T-cells expressing activation markers/million CD4+/CD8+ T-cells	GSK Biologicals**
PBMCs	B-cells reactive to "challenge" antigens	None, H1 stalk domain (cHA 6/1)	B-memory cells by ELISPOT	Frequencies of antigen-specific memory B-cells/million memory B-cells	GSK Biologicals**
PBMCs	Plasmablasts detected using cH6/1 biotinylated probe	H1 stalk domain (cHA 6/1)	Plasmablast detection to HA by flow cytometry	Frequencies of antigen-specific plasmablasts/million PBMCs	GSK Biologicals**

PBMC = Peripheral blood mononuclear cells; **IL-2** = interleukin-2; **TNF-α** = Tumor Necrosis Factor-alpha; **IFN-γ** = interferon-gamma; **CD40-L** = Cluster of Differentiation 40-Ligand; **ICS** = intracellular cytokine staining
*Refer to APPENDIX B for the laboratory addresses.
**GSK Biologicals laboratory refers to CLS in Rixensart, Belgium; Wavre, Belgium

Table 11 Hematology/biochemistry

System	Discipline	Component	Method	Scale**	Laboratory
Whole blood	Hematology	Leukocytes (white blood cells)	As per central laboratory procedure	Quantitative	Central laboratory***
		Neutrophils*			
		Lymphocytes*			
		Basophils*			
		Monocytes*			
		Eosinophils*			
		Hemoglobin			
		Platelets			
		Erythrocytes (red blood cells)			
Serum	Biochemistry	Alanine aminotransferase (ALT)	As per central laboratory procedure	Quantitative	
		Aspartate aminotransferase (AST)			
		Creatinine[1]			
		Urea nitrogen[1]			

*For white blood cell differential count.
**Grading of laboratory parameters will be based on the Food and Drug Administration (FDA) Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (refer to APPENDIX C).
***Refer to APPENDIX B for the laboratory addresses
1 The Blood Urea Nitrogen (BUN)-to-creatinine ratio is to be calculated.

Note that hematology and biochemistry data will be transferred from the central laboratory performing the testing into the clinical database as soon as possible to ensure that all necessary information are available for IDMC reviews. In addition results will be communicated as soon as possible to the investigators via lab reports, hard copies, faxes or emails.

Additional exploratory 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.

Further characterization of the vaccine-induced immune responses may include assessment of HLA-DRB10401 and HLA-A201 alleles expression to evaluate the presence of H1 stalk specific CD4+ and CD8+ T-cells using tetramer technology.

Table 12 Molecular Biology (PCR tests)

Component	Kit/ Manufacturer	Method	Unit	Laboratory
Nasal swab samples				
Influenza A virus (Flu A) Influenza B virus (Flu B)	In-house	RT-PCR	Qualitative assay (positive/negative)	GSK Biologicals* or designated laboratory
Human Influenza A virus subtype H1 (Flu A-H1) Human Influenza A virus subtype H3 (Flu A-H3)	In-house	RT-PCR	Qualitative assay (positive/negative)	
RSV A virus (RSV A) RSV B virus (RSV B)	In-house	Qualitative RT-PCR	Copies/mL or pos/neg	
Human adenovirus (AdV) Human metapneumovirus (MPV) Human enterovirus (HEV) Human parainfluenza virus 1 (PIV1) Human parainfluenza virus 2 (PIV2) Human parainfluenza virus 3 (PIV3) Human parainfluenza virus 4 (PIV4) Human bocavirus (HBoV) Human rhinovirus (HRV) Human coronavirus 229E (CoV 229E) Human coronavirus NL63 (CoV NL63) Human coronavirus OC43 (CoV OC43)	*Allplex* Respiratory Panel or equivalent'	Multiplex real-time PCR	Qualitative assay (positive/negative)	

Pos/neg = positive/negative
*GSK Biologicals laboratory refers to the CLS in Rixensart, Belgium; Wavre, Belgium.

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

5.7.4. Biological samples evaluation

5.7.4.1. Immunological read-outs

Table 13 Immunological read-outs for humoral immunity and cell-mediated immunity (Amended: 11 July 2019)

Blood sampling timepoint		Sub-cohort Name	No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Humoral immunity					
Visit 1 (Day 1) Visit 3 (Day 29) Visit 6 (Day 85) Visit 7 (Month 8) Visit 8 (Month 14) Visit 10 (Month 14 + 28 days) Visit 11 (Month 20) Visit 12 (Month 26)	PRE PId28 PIId28 M8 M14 PIIId28 M20 M26	All subjects	~470	Anti-H1 HA stalk ELISA	P P D
				Anti-H2 HA full length ELISA	P P D
				Anti-H18 HA full length ELISA	P P D
Visit 1 (Day 1) Visit 3 (Day 29) Visit 6 (Day 85)	PRE PId28 PIId28	All subjects	~470	Anti-H1 HA stalk MN assay	P P D
				Anti-heterosubtypic HA Group 1 virus MN assay (H1N1 swine)	P P D
				Anti-heterosubtypic HA Group 1 virus MN assay (IIV4 H1N1 strains)	P P D
				HI with cH5/1N1 and cH8/1N1 virus	P P D
Cell-mediated immunity					
Visit 1 (Day 1) Visit 3 (Day 29) Visit 6 (Day 85)	PRE PId28 PIId28	CMI sub-cohort*	~225	T-cell response by ICS assay	P P D
Visit 1 (Day 1) Visit 2 (Day 8) Visit 3 (Day 29) Visit 5 (Day 64) Visit 6 (Day 85)	PRE PId7 PId28 PIId7 PIId28	CMI sub-cohort*	~225	B memory cells by ELISPOT	P P D
Visit 1 (Day 1) Visit 2 (Day 8) Visit 5 (Day 64))	PRE PId7 PIId7	CMI sub-cohort*	~225	Plasmablast detection to HA by flow cytometry	P P D

PRE = pre-vaccination; **PI** = post-dose 1; **PII** = post-dose 2; **PIII** = post-dose 3 (booster); **D** = day; **M** = month; **ELISA** = enzyme-linked immunosorbent assay; **MN** = microneutralization; **ICS** = intracellular cytokine staining
*CMI sub-cohort comprising ~50% of the enrolled Phase II subjects.

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 13.

5.7.4.2. Hematology/Blood Chemistry

Table 14 Read-outs for hematology/blood chemistry

Blood sampling timepoint		Number of subjects	Component
Type of contact and timepoint	Sampling timepoint		
Screening	Screening	All screened subjects*	Hematology: leukocytes, neutrophils, lymphocytes, basophils, monocytes, eosinophils, hemoglobin, platelets, erythrocytes
			Biochemistry: ALT, AST, creatinine**, urea nitrogen**
Visit 1 (Day 1) Visit 2 (Day 8) Visit 3 (Day 29) Visit 4 (Day 57) Visit 5 (Day 64) Visit 6 (Day 85) Visit 7 (Month 8) Visit 8 (Month 14) Visit 9 (Month 14 + 7 days) Visit 10 (Month 14 + 28 days) Visit 11 (Month 20) Visit 12 (Month 26)	PRE PId7 PId28 PId56 PIId7 PIId28 M8 M14 PIIId7 PIIId28 M20 M26	All	Hematology: leukocytes, neutrophils, lymphocytes, basophils, monocytes, eosinophils, hemoglobin, platelets, erythrocytes
			Biochemistry: ALT, AST, creatinine**, urea nitrogen**

ALT = alanine aminotransferase
AST = aspartate aminotransferase
*Only for Phase I subjects
**The BUN-to-creatinine ratio is to be calculated.

5.7.4.3. Molecular biology

Table 15 Read-outs for molecular biology tests (Amended: 11 July 2019)

Sampling timepoint		Number of subjects	Component
Type of contact and timepoint	Sampling timepoint		
Assessment visit	Unscheduled	***E***vent-driven	Influenza A virus (Flu A) Influenza B virus (Flu B) Human Influenza A virus subtype H1 (Flu A-H1) Human Influenza A virus subtype H3 (Flu A-H3) RSV A virus (RSV A) RSV B virus (RSV B) Human adenovirus (AdV) Human metapneumovirus (MPV) Human enterovirus (HEV) Human parainfluenza virus 1 (PIV1) Human parainfluenza virus 2 (PIV2) Human parainfluenza virus 3 (PIV3) Human parainfluenza virus 4 (PIV4) Human bocavirus (HBoV) Human rhinovirus (HRV) Human coronavirus 229E (CoV 229E) Human coronavirus NL63 (CoV NL63) Human coronavirus OC43 (CoV OC43)

Additional viral/bacterial diagnosis testing on the nasal and throat swabs, such as (but not limited to) multiplex PCR and/or high-throughput sequencing, may be done, if deemed necessary, provided specific assays become available at GSK Biologicals' laboratory or a laboratory designated by GSK Biologicals.

5.7.5. Immunological correlates of protection

For the investigational SUIVs, no generally accepted immunological correlate of protection has been demonstrated so far for the antigen(s) used in the vaccines.

6. STUDY VACCINES/PRODUCTS AND ADMINISTRATION

6.1. Description of study vaccines/products

All candidate vaccines/products to be used have been developed and manufactured by GSK Biologicals.

The investigational SUIVs have been developed by the Icahn School of Medicine at Mount Sinai (New York, United States) in cooperation with GSK Biologicals.

The Quality Control Standards and Requirements for each candidate vaccines/products are described in separate Quality Assurance documents (e.g. release protocols, certificate of analysis) and the required approvals have been obtained.

The vaccines/products are labelled and packed according to applicable regulatory requirements.

Table 16 Study vaccines/products

Treatment name	Vaccine/product name	Formulation	Presentation	Volume to be administered	Number of doses
cH5/1N1 + $AS03_A$-like$^{\alpha}$	cH5/1N1	HA head,A/Vietnam/1203/2004 (H5N1); HA stalk,A/California/04/2009 (H1N1); NA,A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL**	1
	AS03	Emulsion containing tocopherol,tocopherol=47.44mg/ml	Whitish to yellowish homogenous milky liquid emulsion in multi-dose vial		
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		
cH8/1N1 + $AS03_A$-like$^{\alpha}$	cH8/1N1	HA head,A/mallard/Sweden/24/2002 (H8N4); HA stalk,A/California/04/2009 (H1N1); NA,A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL**	1
	AS03	Emulsion containing tocopherol,tocopherol=47.44mg/ml	Whitish to yellowish homogenous milky liquid emulsion in multi-dose vial		
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		
cH11/1N1 + $AS03_A$-like$^{\alpha}$	cH11/1N1	HA head,A/Northern Shoveler/ Netherlands/18/1999 (H11N9); HA stalk,A/California/04/2009 (H1N1); NA,A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL**	1
	AS03	Emulsion containing tocopherol,tocopherol=47.44mg/ml	Whitish to yellowish homogenous milky liquid emulsion in multi-dose vial		
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		
cH5/1N1 + $AS01_E$-like^	cH5/1N1	HA head,A/Vietnam/1203/2004 (H5N1); HA stalk,A/California/04/2009 (H1N1); NA,A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL**	1
	AS01B	MPL=50µg; QS21=50µg; Liposomes*	Liquid in vial		
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		
cH8/1N1 + $AS01_E$-like^	cH8/1N1	HA head,A/mallard/Sweden/24/2002 (H8N4); HA stalk,A/California/04/2009 (H1N1); NA,A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL**	1
	AS01B	MPL=50µg; QS21=50µg; Liposomes*	Liquid in vial		
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		
cH11/1N1 + $AS01_E$-like^	cH11/1N1	HA head,A/Northern Shoveler/ Netherlands/18/1999 (H11N9); HA stalk,A/California/04/2009 (H1N1); NA,A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL**	1
	AS01B	MPL=50µg; QS21=50µg; Liposomes*	Liquid in vial		
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		

Treatment name	Vaccine/product name	Formulation	Presentation	Volume to be administered	Number of doses
cH5/1N1	cH5/1N1	HA head,A/Vietnam/1203/2004 (H5N1); HA stalk,A/California/04/2009 (H1N1); NA,A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL***	1
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		
cH8/1N1	cH8/1N1	HA head,A/mallard/Sweden/24/2002 (H8N4); HA stalk,A/California/04/2009 (H1N1); NA,A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL***	1
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		
cH11/1N1	cH11/1N1	HA head,A/Northern Shoveler/ Netherlands/18/1999 (H11N9); HA stalk,A/California/04/2009 (H1N1); NA,A/California/04/2009 (H1N1); HA=15µg	Freeze-dried pellet	0.5 mL***	1
	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial		
PBS	PBS	Na_2HPO_4=1.3mg; KH_2PO_4=186µg; NaCl=3.85mg; KCl=100µg; $MgCl_2$=50µg	Liquid in vial	0.5 mL	1
Fluarix Quadrivalent	FLU-D-QIV	A/H1N1=15µg; A/H3N2=15µg; B/Yamagata=15µg; B/Victoria=15µg #	Pre-filled syringe	0.5 mL	2

MPL = Monophosphoryl Lipid A

$\alpha AS03_A$-like will be obtained by dilution of the AS03 with PBS

$^{\wedge}AS01_E$-like will be obtained by dilution of the $AS01_B$ with PBS

*QS-21 = *Quillaja saponaria Molina*, fraction 21 (Licensed by GSK from Antigenics Inc, a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation)

**After dilution and reconstitution

***After reconstitution

#The strains will depend on the World Health Organization recommendation for the Northern Hemisphere 2017/2018 season (Dose 1 at Day 1) and the Northern Hemisphere 2018/2019 season (booster dose at Month 14)

6.2. Storage and handling of study vaccines/products

The study vaccines/products 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 pre-study 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 SPM for more details on storage of the study vaccines/products.

Temperature excursions must be reported in degree Celsius.

Any temperature excursion outside the range of 2.0 to +8.0°C (for +2 to +8°C/+36 to +46°F label storage condition) impacting investigational medicinal products (IMPs) must be reported in the appropriate (electronic) temperature excursion decision form ([e]TDF). The impacted IMPs must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from the sponsor.

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 vaccines/products.

6.3. Dosage and administration of study vaccines/products

Dose preparation is to be performed by an unblinded member of the site staff. Detailed instructions for vaccine preparation will be provided separately in the SPM.

For study vaccines adjuvanted with AS03, preparation of the $AS03_A$-like adjuvant through dilution of AS03 (presented in multi-dose vials) with the accompanying PBS diluent is required prior to mixing with the investigational flu freeze-dried cake for reconstitution. After reconstitution, the individual dose of the appropriate study vaccine will be withdrawn into a syringe using aseptic technique. The needles used for vial withdrawal are to be disposed of and replaced by new needles for intramuscular (IM) injection.

For study vaccines adjuvanted with AS01, preparation of the $AS01_E$-like adjuvant through dilution of $AS01_B$ (presented in single-dose vials) with the accompanying PBS diluent is required prior to mixing with the investigational flu freeze-dried cake for reconstitution. After reconstitution, the individual dose of the appropriate study vaccine will be withdrawn into a syringe using aseptic technique. The needles used for vial withdrawal are to be disposed of and replaced by new needles for IM injection.

For non-adjuvanted study vaccines, thorough mixing of two components (accompanying PBS diluent and investigational flu freeze-dried cake) is required for reconstitution. The individual dose of the appropriate study vaccine will be withdrawn into a syringe using aseptic technique. The needles used for vial withdrawal are to be disposed of and replaced by new needles for IM injection.

PBS, when given as placebo, is presented in a vial and does not require mixing.

The IIV4 will be delivered in a monodose syringe, ready to use.

All vials of vaccines/products provided in this study are intended for single use only.

All vials of vaccines/products should be kept at room temperature for a few minutes prior to reconstitution/preparation. All vaccines/products must be administered within one hour after being taken out of the fridge.

All used vials will be retained for monitoring and reconciliation purposes.

All doses must have a volume of 0.5 mL for injection and are to be administered IM into the deltoid region of the non-dominant arm. All study vaccines will be administered by an unblinded member of the site staff who may also participate in dose preparation, but who is barred from participation in any other study functions and may not contribute any observations.

The needle for any IM injection should be long enough to reach the muscle mass and prevent vaccine from seeping into subcutaneous tissue, but not so long as to involve underlying nerves and blood vessels or bone. Vaccinators should be familiar with the anatomy of the area into which they are injecting vaccine. Refer to Table 17.

Table 17 Dosage and administration

Type of contact and timepoint	Study group	Treatment name	Volume to be administered	Route	Site	
					Location	Laterality
Visit 1 (Day 1)	cH5/P/cH8-AS03	cH5/1N1 + $AS03_A$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/P/cH5-AS03	cH8/1N1 + $AS03_A$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/5/11-AS03					
	cH5/P/cH8-AS01	cH5/1N1 + $AS01_E$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/P/cH5-AS01	cH8/1N1 + $AS01_E$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/5/11-AS01					
	cH5/P/cH8	cH5/1N1	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/5/11	cH8/1N1	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/P/cH5					
	IIV4	FLU D-QIV	0.5 mL	IM	Deltoid	Non-Dominant**
Visit 4 (Day 57)	cH8/5/11-AS03	cH5/1N1 + $AS03_A$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/5/11-AS01	cH5/1N1 + $AS01_E$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/5/11	cH5/1N1	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/P/cH5-AS03	PBS	0.5 mL	IM	Deltoid	Non-Dominant**
	cH5/P/cH8-AS03					
	cH8/P/cH5-AS01					
	cH5/P/cH8-AS01					
	cH8/P/cH5					
	cH5/P/cH8					
	IIV4					
Visit 8 (Month 14)	cH8/P/cH5-AS03	cH5/1N1 + $AS03_A$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH5/P/cH8-AS03	cH8/1N1 + $AS03_A$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/5/11-AS03	cH11/1N1 + $AS03_A$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/P/cH5-AS01	cH5/1N1 + $AS01_E$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH5/P/cH8-AS01	cH8/1N1 + $AS01_E$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/5/11-AS01	cH11/1N1 + $AS01_E$-like	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/P/cH5	cH5/1N1	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH5/P/cH8	cH8/1N1	0.5 mL*	IM	Deltoid	Non-Dominant**
	cH8/5/11	cH11/1N1	0.5 mL*	IM	Deltoid	Non-Dominant**
	IIV4	FLU D-QIV	0.5 mL	IM	Deltoid	Non-Dominant**

IM = Intramuscular
*After reconstitution
**The non-dominant arm is the preferred arm of injection. In case it is not possible to administer the vaccine in the non-dominant arm, an injection in the dominant arm may be performed.

6.4. Replacement of unusable vaccine/product doses

In addition to the vaccine/product doses provided for the planned number of subjects (including over-randomization when applicable), at least 5% additional vaccine/product doses will be supplied to replace those that are unusable.

6.5. Contraindications to subsequent vaccination

The following events constitute absolute contraindications to further administration of any study vaccines/products. If any of these events occur during the study, the subject must not receive additional doses of vaccines/products but may continue other study procedures at the discretion of the investigator (see Section 8.5):

- Anaphylaxis following the administration of vaccine(s)/product(s).
- Pregnancy (see Section 8.2.1).
- Any condition that in the judgment of the investigator would make intramuscular injection unsafe.
- Occurrence of an SAE judged to be vaccine-related by the investigator.
- Occurrence of a new pIMD or the exacerbation of an existing pIMD. Refer to Section 8.1.6.1 for the definition of pIMDs.
- Discovery of any health condition which, in the investigator's opinion, places the subject at increased risk from receiving further study vaccines dose(s); or discovery of a change in the subject's health status which make him/her unable to comply with protocol-mandated safety follow-up.
- Hypersensitivity to the active substances or to any of the excipients or to any component that may be present as traces.

The following events constitute contraindications to administration of any study vaccines/products at that point in time; if any of these events occur at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol (see Section 5.5), or the subject may be withdrawn at the discretion of the investigator (see Section 8.5):

- 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/products.

6.6. Warnings and precautions

Refer to the approved product label/package insert of *Fluarix Quadrivalent*.

6.7. 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.

6.7.1. Recording of concomitant medications/products and concomitant vaccinations

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 during the period starting on the day of administration of the first dose of study vaccines up to study end (Day 1 to Month 26).
- Any concomitant vaccination administered during the period starting 30 days before the first dose of study vaccines/products and ending at the last study visit (Day -30 to Month 26).
- Prophylactic medication (i.e. medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination).

  E.g. an anti-pyretic 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 listed in Section 6.7.2.
- Any concomitant medications/products/vaccines relevant to a SAE/pIMD to be reported as per protocol or administered at any time during the study period for the treatment of a SAE/pIMD. In addition, concomitant medications relevant to SAEs and pIMD need to be recorded on the expedited Adverse Event report.

6.7.2. 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 10.5 for the analysis sets.

- Any investigational or non-registered product (drug or vaccine) other than the study vaccines/products used during the study period.
- Immunosuppressants or other immune-modifying drugs administered chronically (i.e. more than 14 days in total) during the study period. For corticosteroids, this will mean prednisone ≥ 20 mg/day, or equivalent. Inhaled and topical steroids are allowed.

- Long-acting immune-modifying drugs administered at any time during the study period (e.g. infliximab, rituximab).
- A vaccine not foreseen by the study protocol administered during the period starting 30 days before the first dose of study vaccines/products (Visit 1) up to the blood sampling at Day 85 (Visit 6) and in the period starting 30 days before the booster dose at Month 14 (Visit 8) up to the blood sampling at Month 14 + 28 days (Visit 10)*.

  *In case an emergency mass vaccination for an unforeseen public health threat (e.g. a pandemic) is organized by the public health authorities, outside the routine immunization program, the time period described above can be reduced if necessary for that vaccine provided it is licensed and used according to its Summary of Product Characteristics (SmPC) or Prescribing Information and according to the local governmental recommendations and provided a written approval of the Sponsor is obtained.
- Administration of any influenza vaccine during the study period.
- Immunoglobulins and/or any blood products administered during the study period.
- Drug and/or alcohol abuse.

6.8. Intercurrent medical conditions that may lead to elimination of a subject from Per-Protocol analyses

At each study visit subsequent to the first vaccination visit, it must be verified if the subject has experienced or is experiencing any intercurrent medical condition. If it is the case, the condition(s) must be recorded in the eCRF.

Subjects' data may be eliminated from the Per-Protocol set for 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 (i.e. if any confirmed or suspected immunosuppressive or immunodeficient condition appears during the study period).

7. HEALTH ECONOMICS

Not applicable.

8. SAFETY

The investigator or site staff is/are responsible for the detection, documentation and reporting of events meeting the criteria and definition of an AE or SAE as provided in this protocol.

Each subject will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

8.1. Safety definitions

8.1.1. Definition of an adverse event

An AE is any untoward medical occurrence in a 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. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Examples of an AE include:

- 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 vaccines/products administration even though they may have been present prior to 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 vaccines/products or a concurrent medication (overdose *per se* should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with study vaccines/products administration.
- Significant failure of expected pharmacological or biological action.
- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of subject's previous therapeutic regimen).

AEs to be recorded as endpoints (solicited AEs) are described in Section 8.1.3. All other AEs will be recorded as UNSOLICITED AEs.

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE/SAE.

- Situations where an untoward medical occurrence did not occur (e.g. 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.

8.1.2. Definition of a serious adverse event

A SAE is any untoward medical occurrence that:

a. Results in death,

b. Is life-threatening,

   Note: 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, had it been 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 out-patient setting. Complications that occur during hospitalization are also considered AEs. If a complication prolongs hospitalization or fulfills 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, OR

   Note: 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 like illness, and accidental trauma (e.g. sprained ankle) which may interfere 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 judgement 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 one 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.

8.1.3. Solicited adverse events

Solicited AEs (Table 18 and Table 19) occurring during the 7-day follow-up period after each vaccination (day of vaccination and 6 subsequent days) will be recorded in the appropriate section of the eCRF. The investigator should record in the eCRF any pain relief and/or antipyretics taken by the subject to correct the AEs (local and/or general) during the 7-day follow-up period after vaccination.

8.1.3.1. Solicited local (injection-site) adverse events

The following local (injection-site) AEs will be solicited:

Table 18 Solicited local adverse events

Pain at injection site
Redness at injection site
Swelling at injection site

8.1.3.2. Solicited general adverse events

The following general AEs will be solicited:

Table 19 Solicited general adverse events

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 (preferred route) 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.

8.1.4. Unsolicited adverse events

Unsolicited AEs occurring during the 28-day follow-up period (day of vaccination and 27 subsequent days) after each vaccination will be recorded in the appropriate section of the eCRF.

8.1.5. Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

In absence of diagnosis, abnormal laboratory findings (e.g. clinical chemistry, hematology, urinalysis) or other abnormal assessments (e.g. physical examination findings) 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 (refer to Sections 8.1.1 and 8.1.2). The grading of laboratory parameters will be based on the Food and Drug Administration (FDA) Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (refer to APPENDIX C). 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.

In case of clinically significant grade 3 and above abnormal laboratory findings that cannot be reasonably explained (e.g. 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) for a repeat test to confirm the result.

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

8.1.6. Adverse events of specific interest

8.1.6.1. Potential immune-mediated diseases

AEs of specific interest for safety monitoring include pIMDs, a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology. AEs that need to be recorded and reported as pIMDs include those listed in Table 20.

However, the investigator will exercise his/her medical and scientific judgement in deciding whether other diseases have an autoimmune origin (i.e. pathophysiology involving systemic or organ-specific pathogenic autoantibodies) and should also be recorded as a pIMD.

Once a pIMD is diagnosed (serious or non-serious) in a subject, the investigator (or designate) must complete, date and sign the electronic Expedited Adverse Events Report.

Table 20 List of potential immune-mediated diseases

Neuro-inflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve disorders, including paralyses/paresis (e.g. 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 (e.g. non-infectious 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 • Anti-synthetase 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 (Morphoea)

Vasculitides	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 angiitis or eosinophilic granulomatosis with polyangiitis) – Buerger's disease (thromboangiitis obliterans) – Necrotizing vasculitis (cutaneous or systemic) – Anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified) – Henoch-Schonlein purpura (IgA vasculitis) – Behcet's syndrome – Leukocytoclastic vasculitis.	• 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 – Membrano-proliferative glomerulonephritis – Mesangio-proliferative glomerulonephritis – Tubulo-intestinal nephritis and uveitis syndrome • Ocular autoimmune diseases, including: – Autoimmune uveitis – Autoimmune retinitis • Autoimmune myocarditis • Sarcoidosis • Stevens-Johnson syndrome • Sjögren's syndrome • Alopecia areata • Idiopathic pulmonary fibrosis • Good pasture 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 I • Addison's disease • Polyglandular autoimmune syndrome • Autoimmune hypophysitis

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

In order to facilitate the documentation of pIMDs in the eCRF, a pIMD standard questionnaire and a list of preferred terms (PTs) and PT codes corresponding to the above diagnoses will be available to investigators at study start.

8.2. Events or outcomes not qualifying as adverse events or serious adverse events

8.2.1. Pregnancy

Female subjects who are pregnant or lactating at the time of vaccination must not receive additional doses of study vaccines/products but may continue other study procedures at the discretion of the investigator.

While pregnancy itself 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 itself should always be recorded on the electronic pregnancy report.

The following should always be considered as SAE and will be reported as described in Sections 8.4.1 and 8.4.3:

- 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 cut-off in gestational age is based on WHO-ICD 10 noted in the EMA Guideline on pregnancy exposure [EMA, 2006]. It is recognized that national regulations might be different.

- Any early neonatal death (i.e. death of a live born infant occurring within the first 7 days of life).
- Any congenital anomaly or birth defect (as per [CDC MACDP] guidelines) 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 post-study pregnancy AND considered by the investigator to be reasonably related to the study vaccines/products will be reported to GSK Biologicals as described in Section 8.4.3. While the investigator is not obligated to actively seek this information from former study participants, he/she may learn of a pregnancy through spontaneous reporting.

8.3. Detecting and recording adverse events, serious adverse events and pregnancies

8.3.1. Time period for detecting and recording adverse events, serious adverse events and pregnancies

All AEs starting within 28 days following administration of each dose of study vaccines/products (vaccination day and 27 subsequent days) must be recorded into the appropriate section of the eCRF, irrespective of intensity or whether or not they are considered vaccination-related.

All cases of ILI (refer to Section 5.4.2 for the ILI definition) starting after Dose 1 administration (Day 1) up to the study conclusion (Month 26) must be recorded into the appropriate sections of the eCRF (specific screen for recording of ILI and AE/SAE screen, as applicable), irrespective of intensity, or whether or not they are considered vaccination-related, or whether or not they are associated with a medically attended visit.

The time period for collecting and recording MAEs/SAEs will begin at the first receipt of study vaccines/products and will end 12 months following administration of the last dose of study vaccines/products for each subject (Day 1 to Month 26). See Section 8.4 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 vaccines/product.

In addition to the above-mentioned reporting requirements and in order to fulfill international reporting obligations, SAEs that are related to study participation (i.e. 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 vaccines/products and will end 12 months following administration of the last dose of study vaccines/products. See section 8.4 for instructions on reporting of pregnancies.

The time period for collecting and recording of pIMDs will begin at the first receipt of study vaccines/products and will end 12 months following administration of the last dose of study vaccines/products. See section 8.4 for instructions on reporting of pIMDs.

An overview of the protocol-required reporting periods for AEs, SAEs, MAEs, pIMDs and pregnancies is given in Table 21.

Table 21 Reporting periods for collecting safety information

Event	Screen. Visit*	Visit 1			Visit 4			Visit 8			Study Conclusion
		Day 1	7 days post-vacc	28 days post-vacc	Day 57	7 days post-vacc	28 days post-vacc	Month 14	7 days post-vacc	28 days post-vacc	Month 26
Solicited local and general AEs											
Unsolicited AEs											
ILIs											
AEs/SAEs leading to withdrawal from the study											
SAEs MAEs											
SAEs related to study participation or concurrent GSK medication/vaccine**											
Pregnancies											
pIMDs											

Vacc = vaccination
*Only applicable for Phase I subjects
**To be collected as of consent obtained (e.g. at Screening visit for Phase I subjects and at Visit 1 for Phase II subjects)
Screen. = screening; AEs = adverse events; ILIs = influenza-like illnesses; SAEs = serious adverse events; MAEs = medically attended events; pIMDs = potential immune-mediated diseases

8.3.2. Post-study adverse events and serious adverse events

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE reporting period defined in Table 21. Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study vaccines/products, the investigator will promptly notify the Study Contact for Reporting SAEs.

8.3.3. Evaluation of adverse events and serious adverse events

8.3.3.1. Active questioning to detect adverse events and serious adverse events

As a consistent method of collecting AEs, the subject should be asked a non-leading question such as:

'*Have you felt different in any way since receiving the vaccines/products or since the previous visit?*'

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. 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 Biologicals instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

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.

8.3.3.2. Assessment of adverse events

8.3.3.2.1. Assessment of intensity

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

Table 22 Intensity scales for solicited symptoms

Adverse Event	Intensity grade	Parameter
Pain at injection site	0	None
	1	Mild: Any pain neither interfering with nor preventing normal every day activities.
	2	Moderate: Painful when limb is moved and interferes with every day activities.
	3	Severe: Significant pain at rest. Prevents normal every day activities.
Redness at injection site		Record greatest surface diameter in mm
Swelling at injection site		Record greatest surface diameter in mm
Fever*		Record temperature in °C/°F
Headache	0	Normal
	1	Mild: Headache that is easily tolerated
	2	Moderate: Headache that interferes with normal activity
	3	Severe: Headache that prevents normal activity
Fatigue	0	Normal
	1	Mild: Fatigue that is easily tolerated
	2	Moderate: Fatigue that interferes with normal activity
	3	Severe: Fatigue that prevents normal activity
Gastrointestinal symptoms (nausea, vomiting, diarrhea and/or abdominal pain)	0	Normal
	1	Mild: Gastrointestinal symptoms that are easily tolerated
	2	Moderate: Gastrointestinal symptoms that interfere with normal activity
	3	Severe: Gastrointestinal symptoms that prevent normal activity
Arthralgia	0	Normal
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity
Myalgia	0	Normal
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity
Shivering	0	Normal
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity

*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 maximum intensity of local injection site redness/swelling/fever will be scored at GSK Biologicals as follows:

	Redness/swelling
0:	≤ 20 mm
1:	> 20 - ≤ 50 mm
2:	> 50 - ≤ 100 mm
3:	> 100 mm

Temperature > 39°C/102.2°F will be considered as grade 3 fever.

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 judgement.

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

1 (mild)	=	An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
2 (moderate)	=	An AE which is sufficiently discomforting to interfere with normal everyday activities.
3 (severe)	=	An AE which prevents normal, everyday activities. In adults, such an AE would, for example, prevent attendance at work/school and would necessitate the administration of corrective therapy.

An AE that is assessed as Grade 3 (severe) should not be confused with a SAE. Grade 3 is a category used for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 8.1.2.

Note that the grading of laboratory parameters will be based on the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (refer to Section 8.1.5 and APPENDIX C).

8.3.3.2.2. Assessment of causality

The investigator is obligated to assess the relationship between study vaccines/products and the occurrence of each AE/SAE using clinical judgement. In case of concomitant administration of multiple vaccines/products, if possible, the investigator should specify if the AE could be causally related to a specific vaccine/product administered (i.e. investigational, control/placebo or co-administered vaccine). When causal relationship to a specific vaccine(s)/product(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 vaccines/products will be considered and investigated. The investigator will also consult the IB and/or SmPC and/or Prescribing Information for marketed products to determine his/her assessment.

There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. 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 Biologicals. 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 one 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/product?*

YES : There is a reasonable possibility that the study vaccines/products contributed to the AE.

NO : There is no reasonable possibility that the AE is causally related to the administration of the study vaccines/products. There are other, more likely causes and administration of the study vaccines/products is not suspected to have contributed to the AE.

If an event meets the criteria to be determined as 'serious' (see Section 8.1.2), 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 vaccines/products, if applicable.
- Erroneous administration.
- Other cause (specify).

8.3.3.3. Assessment of outcomes

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).

8.3.3.4. Medically attended events

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.

8.4. Reporting of serious adverse events, pregnancies, and other events

8.4.1. Prompt reporting of serious adverse events, pregnancies, and other events to GSK Biologicals

SAEs that occur in the time period defined in Section 8.3 will be reported promptly to GSK within the timeframes described in Table 23, once the investigator determines that the event meets the protocol definition of a SAE.

Pregnancies that occur in the time period defined in Section 8.3 will be reported promptly to GSK within the timeframes described in Table 23, once the investigator becomes aware of the pregnancy.

pIMDs that occur in the time period defined in Section 8.3 will be reported promptly to GSK within the timeframes described in Table 23, once the investigator determines that the event meets the protocol definition of a pIMD.

Table 23 Timeframes for submitting serious adverse event, pregnancy and other events reports to GSK Biologicals

Type of Event	Initial reports		Follow-up of relevant information on a previous report	
	Timeframe	Documents	Timeframe	Documents
SAEs	24 hours*‡	electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report
Pregnancies	2 weeks*	electronic pregnancy report	2 weeks*	electronic pregnancy report
pIMDs	24 hours**‡	electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report

*Timeframe allowed after receipt or awareness of the information.
**Timeframe allowed once the investigator determines that the event meets the protocol definition of a pIMD.
‡ The investigator will be required to confirm review of the SAE/pIMD causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE/pIMD.

8.4.2. Contact information for reporting serious adverse events, pregnancies and pIMDs

Study Contact for Reporting SAEs, pIMDs and pregnancies
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs, pIMDs and pregnancies
24/24 hour and 7/7 day availability GSK Biologicals Clinical Safety & Pharmacovigilance US sites only: Fax: PPD Canadian sites only: Fax: PPD Outside US & Canada sites: Fax: PPD or PPD Email address: PPD

8.4.3. Completion and transmission of SAE reports to GSK Biologicals

Once an investigator becomes aware that a 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 a 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.

8.4.3.1. 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 Biologicals 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.

8.4.4. Completion and transmission of pregnancy reports to GSK Biologicals

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 2 WEEKS.

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

8.4.5. Reporting of pIMDs to GSK Biologicals

Once a pIMD is diagnosed (serious or non-serious) 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 to specify that the event is a pIMD and whether it is serious or non-serious. The report will always be completed as thoroughly as possible with all available details of the event, in accordance with the pIMD standard questionnaire provided. Even if the investigator does not have all information regarding a pIMD, 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 pIMD causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the pIMD.

Refer to Section 8.4.3.1 for back-up system in case the electronic reporting system does not work.

8.4.6. Updating of SAE, pregnancy, and pIMD information after removal of write access to the subject's eCRF

When additional SAE, pregnancy or pIMD 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 Biologicals Clinical Safety and Pharmacovigilance department within the designated reporting time frames specified in Table 23.

8.4.7. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section 8.4.1. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

Investigator safety reports are prepared according to the current GSK policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a SAE(s) that is both attributable to the study vaccines/products and unexpected. The purpose of the report is to fulfill specific regulatory and GCP requirements, regarding the product under investigation.

8.5. Follow-up of adverse events, serious adverse events, and pregnancies

8.5.1. Follow-up of adverse events and serious adverse events

8.5.1.1. Follow-up 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 Biologicals (within 24 hours for SAEs; refer to Table 23).

All MAEs, AEs and pIMDs (serious or non-serious) documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the last visit of the subject.

All AEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until 30 days after the last vaccination.

8.5.1.2. Follow-up after the subject is discharged from the study

The investigator will follow subjects:

- With MAEs, SAEs, pIMDs (serious or non-serious), 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.
- With other non-serious AEs, until the event is otherwise explained or they are lost to follow-up.

If the investigator receives additional relevant information on a previously reported SAE, he/she will provide this information to GSK Biologicals using a paper/electronic Expedited Adverse Events Report and/or pregnancy report as applicable.

GSK Biologicals 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 Biologicals will be provided with any available post-mortem findings, including histopathology.

8.5.2. 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 Biologicals 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 six to eight weeks after the estimated date of delivery.

Regardless of the reporting period for SAEs for this study, if the pregnancy outcome is a SAE, it should always be reported as SAE.

8.6. 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 a SAE/pIMD should be recorded in Expedited Adverse Event Report of the subject's eCRF (refer to Section 6.7).

8.7. 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.

8.8. Holding rules and safety monitoring

Study holding rules (see Section 8.8.1) and safety monitoring (see Section 8.8.2) have been defined.

The investigator is not permitted to administer Dose 2 of the study vaccine to the subjects enrolled in the Phase I until receipt of the favorable outcome of the IDMC safety evaluation based on safety data collected up to 7 days post-Dose 1 in at least 60 Phase I subjects.

Vaccination in Phase II subjects can only start after receipt of the favorable outcome of the IDMC safety evaluation based on safety data collected up to 7 days post-Dose 1 in all Phase I subjects (N = ~80) (refer to Enrolment design).

During the entire study period, there will be regular IDMC reviews (monthly during the vaccination phases and every 6 months during the safety follow-up phases).

Dose 3 (booster) can only be administered to Phase II subjects upon favorable outcome of the 7-day post-Dose 3 safety data review of Phase I subjects by the IDMC.

In addition, if any safety concern is identified by the investigator (i.e. meeting of holding rules 1a-1d [see Table 24] or any other safety concern), he/she should inform GSK Biologicals immediately, and vaccination may be put on hold as a consequence.

If any safety concern is identified in other study(ies) in which the investigational SUIV is used, GSK Biologicals might also request to put the vaccination in this study on hold during the period needed to perform the assessment of safety data of the other study(ies).

8.8.1. Holding rules

Study holding rules which will be applied during the safety evaluation are defined below.

If a holding rule 1a through 1d is observed by the investigator, it will be reported promptly to GSK within 24 hours. Meeting the other holding rules will be determined by IDMC review of safety data at regular intervals.

These holding rules have been written under the assumption that safety data of all subjects will be available. Therefore, the investigator will be requested to record safety data meeting the criteria of holding rules in the eCRF within 24 hours after reception of the data to ensure that all necessary information are available for IDMC reviews. If the data from all subjects are not available, the holding rules will be assessed on a pro-rata basis.

Upon meeting of any holding rules, further vaccination will be immediately put on hold pending safety review and consultation with the GSK Biologicals' Vaccine Safety Monitoring Board (VSMB), as appropriate.

GSK Biologicals' decision on whether to suspend, modify, or continue the conduct of the study on all groups or on selected groups will be communicated to the investigators, to CBER, and to the IRBs/IECs.

In case of a safety concern, the final responsibility to recommend whether or not the trial should be stopped permanently rests with the sponsor, after having considered all the safety information available. If the trial is stopped, a letter indicating the reasons for stopping the study will be sent to the IRBs/IECs via the investigator(s), and to CBER via GSK Biologicals.

Table 24 Study holding rules

Holding rules	Event	Number/ percentage of subjects
1a	Death or any life-threatening **SAE.**	≥ 1
1b	Any **SAE** that cannot reasonably be attributed to a cause other than vaccination	≥ 1
1c	Any **withdrawal** from the study or **withdrawal** from study vaccine(s)/product(s) (by investigator or subject request) following a grade 3 AE that cannot reasonably be attributed to a cause other than vaccination.	≥ 1
1d	Any local or general solicited AE leading to **hospitalization**, or **fever > 40°C** (104°F) (oral route) that cannot reasonably be attributed to a cause other than vaccination, or **necrosis** at the injection site, within the 7-day (Days 1-7) post-vaccination period.	≥ 1
2a	Any **grade 3 solicited local** AE lasting 48h or more in an investigational group, within the 7-day (Days 1-7) post-vaccination period.	≥ 30% (and ≥ 2 subjects in one group)
2b	Any **grade 3 solicited general** AE lasting 48h or more in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 7-day (Days 1-7) post-vaccination period.	≥ 20% (and ≥ 2 subjects in one group)
2c	Any **grade 3 unsolicited** AE in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 28-day (Days 1-28) post-vaccination period OR Any grade 3 abnormality in pre-specified hematological or biochemical **laboratory parameters*** in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 7 days post-vaccination.	≥ 20% (and ≥ 2 subjects in one group)

*Grading of laboratory parameters will be based on the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (refer to APPENDIX C).

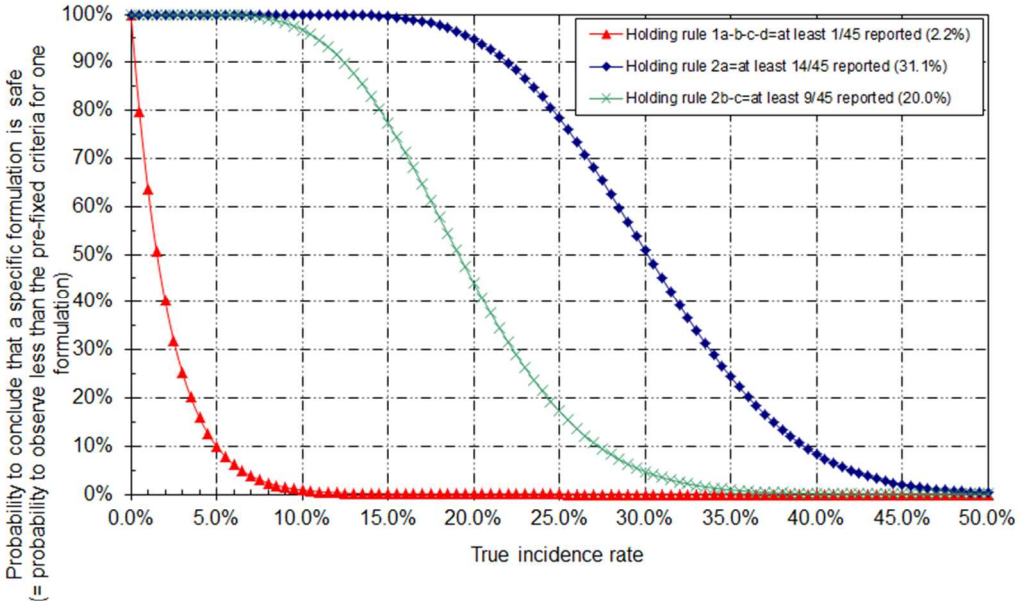
Figure 2 gives the probability of not meeting each holding rule 1 and 2 for 45 subjects in an investigational group. This evaluation was performed for the sample size of the initial protocol (i.e. 450 subjects, 45/group) and it was not repeated at the time of the protocol amendment 1 because of the small difference in the number of subjects per group (now 470 subjects in total, 47/group).

Each holding rule 1a-d has 80% chance of not being met for an event with a true incidence rate below 1.0% and has less than 5% of chance of not being met for an event with a true incidence rate above 7%.

Holding rule 2a has more than 90% chance of not being met for an event with a true incidence rate below 20% and has less than 52% of chance of not being met for an event with a true incidence rate above 30%.

Holding rules 2b and 2c have more than 90% chance of not being met for an event with a true incidence rate below 10% and has less than 45% of chance of not being met for an event with a true incidence rate above 20%.

Figure 2 Probability of successfully completing the study in function of adverse events incidence rates



8.8.2. Safety monitoring

An IDMC will be established by GSK Biologicals for the purpose of monitoring the study and to provide independent, non-binding advice on safety and ethics. The IDMC will provide recommendations about stopping, holding, continuing or modifying the trial. During the whole study period, there will be regular IDMC reviews (monthly during the vaccination phases and every 6 months during the safety follow-up phases. The frequency of these reviews may be adapted upon IDMC recommendation if deemed necessary).

During the Phase I enrolment, subjects will be vaccinated one at a time, at least 60 minutes apart, with a maximum of 10 subjects per day until ~80 subjects are enrolled (i.e. to obtain treatment groups of at least 8 subjects/group):

- If no safety issue is identified upon review of the 7 days post-Dose 1 safety data (Days 1-7) of at least 60 Phase I subjects, Dose 2 will be administered to Phase I subjects.
- If no safety issue is identified upon review of the 7-day post-Dose 1 safety data (Days 1-7) of all Phase I subjects (N = ~80), Phase II enrolment will start.

Phase I subjects will be vaccinated one at the time, at least 60 minutes apart, with a maximum of 10 subjects a day. This is applicable for Dose 1 (Day 1), Dose 2 (Day 57) and Dose 3 (booster) (Month 14) of the Phase I subjects.

Phase II subjects will be enrolled and vaccinated without limitation on the number of vaccinees per day or time between consecutive subjects.

The frequency of IDMC sessions and other operational details are described in the IDMC charter. In case of a serious safety issue during the study, GSK Biologicals will inform the IDMC as well as fulfil its regulatory obligation expeditiously.

9. SUBJECT COMPLETION AND WITHDRAWAL

9.1. Subject completion

A subject who returns for the concluding visit foreseen in the protocol (Visit 12) is considered to have completed the study.

9.2. Subject withdrawal

Withdrawals will not be replaced.

9.2.1. Subject withdrawal from the study

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 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.

Information relative to the 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:

- Serious adverse event.
- Unsolicited non-serious adverse event.
- Solicited adverse event
- Protocol violation (specify).
- Consent withdrawal, not due to an adverse event*.
- Moved from the study area.
- Lost to follow-up.
- 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 a SAE/AE until resolution of the event (see Section 8.5.1.2).

9.2.2. Subject withdrawal from study vaccines/products

A 'withdrawal' from the study vaccines/products refers to any subject who does not receive the complete treatment, i.e. when no further planned dose is administered from the date of withdrawal. A subject withdrawn from the study vaccines/products may not necessarily be withdrawn from the study as further study procedures or follow-up may be performed (safety or immunogenicity) if planned in the study protocol.

Information relative to premature discontinuation of the study vaccines/products will be documented on the Vaccine Administration screen of the eCRF. The investigator will document whether the decision to discontinue further vaccination/treatment was made by the subject himself/herself, or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- Serious adverse event.
- Unsolicited non-serious adverse event.
- Solicited adverse event
- Not willing to be vaccinated
- Other (specify).

9.3. Extension study

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

9.4. Screening failures

Only applicable for Phase I subjects: Screening and baseline failures are defined as subjects who withdraw or are withdrawn from the study after giving informed consent, but before receiving the study vaccine at Day 1. Data reported to the eCRF for these subjects will be limited to:

- ICF information,
- Demographic data,
- Inclusion/exclusion criteria,

- Blood sampling for hematology/biochemistry (if performed).
- Screening conclusion and study conclusion data.

Medical history, physical examination, height, weight and vital signs data, and history of previous influenza vaccination do not need to be reported to the eCRF.

10. STATISTICAL METHODS

10.1. Primary endpoints

Reactogenicity and safety

- Occurrence of solicited local and general AEs after each vaccination:
  - Occurrence of solicited local AEs during a 7-day follow-up period (i.e. on the day of vaccination and 6 subsequent days) after each vaccine dose, in all vaccine groups.
  - Occurrence of solicited general AEs during a 7-day follow-up period (i.e. on the day of vaccination and 6 subsequent days) after each vaccine dose, in all vaccine groups.
- Occurrence of unsolicited AEs after each vaccination:
  - Occurrence of unsolicited AEs during a 28-day follow-up period (i.e. on the day of vaccination and 27 subsequent days) after each vaccine dose, in all vaccine groups.
- Occurrence of hematological and biochemical laboratory abnormalities after each vaccination:
  - Any hematological (red blood cells, white blood cells and differential count, platelets count and hemoglobin level) or biochemical (alanine aminotransferase, aspartate aminotransferase, creatinine, blood urea nitrogen [BUN] and BUN-to-creatinine ratio) laboratory abnormality at each visit subsequent to Day 1, in all vaccine groups.
- Occurrence of MAEs, pIMDs and SAEs:
  - Occurrence of MAEs, pIMDs and SAEs throughout the entire study period, in all vaccine groups.

Immunogenicity

Anti-H1 stalk immune response measured by ELISA and by MN assay 28 days after each priming dose:

- Levels of anti-H1 stalk antibody titers by ELISA and by MN assay.

  The following aggregate variables will be calculated for the above parameters with 95% confidence interval (CI):

  - Seropositivity rates and geometric mean titers (GMTs) at Days 1, 29 and 85.

- Percentage of subjects with a ≥ 4-fold increase from Day 1 to Days 29 and 85.
- Percentage of subjects with a ≥ 10-fold increase from Day 1 to Days 29 and 85.
- Mean geometric increase (MGI) from Day 1 to Days 29 and 85.

10.2. Secondary endpoints (Amended: 11 July 2019)

Immunogenicity

Adjuvant effect on the anti-stalk immune response in terms of:

- GMT ratio for anti-stalk ELISA titer SUIV+AS03 or AS01/SUIV non-adjuvanted, 28 days post vaccination (i.e. at Day 29 to evaluate the adjuvant effect post-dose 1 and at Day 85 to evaluate the adjuvant effect post-dose 2).

Anti-H1 stalk immune response measured by ELISA and by MN assay:

- Levels of anti-H1 stalk antibody titers by ELISA post-each vaccination.

  The following aggregate variables will be calculated for the above parameters with 95% CI:

  - Seropositivity rates and GMTs at Days 1, 29, 85, Month 8, Month 14, Month 14 + 28 days, Month 20 and Month 26.
  - Percentage of subjects with a ≥ 4-fold increase in antibody titers by ELISA from Day 1 to each subsequent timepoint listed above.
  - Percentage of subjects with a ≥ 10-fold increase in antibody titers by ELISA from Day 1 to each subsequent timepoint listed above.
  - MGI in antibody titers by ELISA from Day 1 to each subsequent timepoint listed above.
- Levels of anti-H1 stalk antibody titers by MN assay.

  The following aggregate variables will be calculated for the above parameters with 95% CI:

  - Seropositivity rates and GMTs at Days 1, 29 ***and*** 85.
  - Percentage of subjects with a ≥ 4-fold increase in antibody titers by MN assay from Day 1 to each subsequent timepoint listed above.
  - Percentage of subjects with a ≥ 10-fold increase in antibody titers by MN assay from Day 1 to each subsequent timepoint listed above.
  - MGI in antibody titers by MN assay from Day 1 to each subsequent timepoint listed above.

Breadth of the immune response:

- Levels of anti-H2 and anti-H18 antibody titers by ELISA.

  The following aggregate variables will be calculated for the above parameters with 95% CI:

  - Anti-H2 and anti-H18 seropositivity rates and GMTs at Days 1, 29, 85, Month 8, Month 14, Month 14 + 28 days, Month 20 and Month 26.
  - Percentage of subjects with a ≥ 4-fold increase in anti-H2 and anti-H18 antibody titers from Day 1 to each subsequent timepoint listed above.
  - Percentage of subjects with a ≥ 10-fold increase in anti-H2 and anti-H18 antibody titers from Day 1 to each subsequent timepoint listed above.
  - MGI in anti-H2 and anti-H18 antibody titers from Day 1 to each subsequent timepoint listed above.

- Levels of antibody titers by MN assay for H1N1 swine influenza and IIV4 H1N1 vaccine strains.

  The following aggregate variables will be calculated for the above parameters with 95% CI:

  - Seropositivity rates and GMTs at Days 1, 29 ***and*** 85.
  - Percentage of subjects with a ≥ 4-fold increase in antibody titers from Day 1 to each subsequent timepoint listed above.
  - Percentage of subjects with a ≥ 10-fold increase in antibody titers from Day 1 to each subsequent timepoint listed above.
  - MGI in antibody titers from Day 1 to each subsequent timepoint listed above.

10.3. Tertiary endpoints (Amended: 11 July 2019)

- Evaluation of CMI parameters in terms of frequencies of:
  - Antigen-specific CD4+/CD8+ T-cells identified as producing at least two markers among CD40L, IL-2, TNF-α and IFN-γ upon *in vitro* stimulation at Days 1, 29 ***and*** 85.
  - B-memory cells reactive with the challenge antigen(s) at Days 1, 8, 29, 64 ***and*** 85.
  - Plasmablasts reactive with the challenge antigens at Days 1, 8 ***and*** 64.
- Levels of HI antibody to chimeric vaccine strains ***cH5/1N1 and cH8/1N1***:

  The following aggregate variables will be calculated with 95% CI:

  - Seropositivity rates and GMTs at Days 1, 29 ***and*** 85.
  - Seroprotection rate (SPR) at each timepoint listed above.
  - Seroconversion rate (SCR) at Days 29 ***and*** 85.
  - MGI from Day 1 to each subsequent timepoint listed above.
- Assessment of the in vivo protective effect of the anti-stalk antibodies when transferring Day 1 ***and*** Day 85 pooled serum from all evaluable subjects of each vaccine groups to mice that will be subsequently challenged with cH6/1N5* or with H1N1 contained in the IIV4, using the following endpoints [refer to APPENDIX D]:

- Survival over 14 days post-challenge (day of death/euthanasia for weight loss > 25% baseline body weight) in groups of 35 mice**/serum pool/vaccine group/timepoint.
- Weight loss (change from baseline over 14 days post-challenge) in groups of 35 mice**/serum pool/vaccine group/timepoint.
- Lung virus titer in $TCID_{50}$/mg (log_{10} fold change [Day 1 minus Day 85]), within challenge group.
- Pre- and post-transfer titer of human IgG to cH6/1N5* by ELISA or HI.
- Pre- and post-transfer titer of human IgG to H1N1 by ELISA or HI.
- Pre- and post-transfer titer of human IgG to recombinant HA protein by ELISA.

*Or an alternative challenge virus with similar attributes but more fit for purpose.

**If sufficient serum volumes are not available, and depending on the challenge virus pathogenicity, the number of mice can be reduced to as low as 10 mice per timepoint and virus challenge.

10.4. Determination of sample size

10.4.1. Descriptive objectives

The primary objectives of the study are to assess the reactogenicity and safety of each vaccine dose throughout the study and to describe the anti-H1 stalk immune response 28 days after each priming dose.

At the time of the protocol amendment 1, it was decided to increase the sample size from 450 to 470 subjects to take into account a larger rate of non-evaluable subjects than anticipated. The power computations from the initial protocol relative to safety endpoints were not modified because of the small sample size increase (about 2 subjects per group, from 45 to 47). The immunogenicity endpoints evaluation will be done primarily on the Per-Protocol set, which is not deemed to increase (about 43 subjects per group).

Table 25 shows the true proportions associated with a 90% probability to observe an event in 45 subjects (e.g. SAE, pIMD).

Table 25 True proportions associated with a 90% probability to observe a certain number of adverse events within a group (45 subjects)

True proportion	Number of adverse events observed with > 90% probability
0.049	> 0
0.083	> 1
0.114	> 2
0.142	> 3

Table 26 illustrates the 95% CIs for different possible numbers of AEs within each group.

Table 26 Two-sided exact 95% confidence intervals for the true adverse event rate at different possible observed adverse event rates (45 subjects)

Observed number of adverse events	Observed adverse event proportion	95% exact confidence interval	
		Lower limit	Upper limit
0	0.000	0.000	0.079
1	0.022	0.001	0.118
2	0.044	0.005	0.152
3	0.067	0.014	0.183
4	0.089	0.025	0.212
5	0.111	0.037	0.241
10	0.222	0.112	0.371
20	0.444	0.296	0.600
30	0.667	0.511	0.800

Table 27 presents the 95% CIs for different possible rates of immunological response within each group. A rate of unevaluable subjects of 2/45 (4%) has initially been considered for the immune response post-primary vaccination. As from protocol amendment 1, a rate of non-evaluable subjects of 4/47 (9%) has been considered for the immune response post-primary vaccination.

Table 27 Two-sided exact 95% confidence intervals for the true immunological response rate at different possible observed response rates (43 evaluable subjects)

Observed number of responses	Observed response proportion	95% exact confidence interval	
		Lower limit	Upper limit
25	0.581	0.421	0.730
30	0.698	0.539	0.828
35	0.814	0.667	0.916
40	0.930	0.809	0.985

*Response rate can be either seropositivity rate, percentages of subjects with a 4-fold increase or percentages of subjects with a 10-fold increase

The advantage of adding an adjuvant and the preference for a specific priming sequence/number of priming doses will be assessed by making use of the factorial nature of the trial design, which is summarized in Table 28. The anti-H1 stalk antibody titers by ELISA will primarily be modelled. The standard deviation (SD) used for the calculation cannot be obtained at the present stage due to unavailability of the final assay under development at Néomed. As a consequence, the assumed SD of $\log_{10}$ titers was derived from study FLU-CC-SUIV-001 (e-track number 201598). In that study, anti-H1 HA stalk ELISA antibody titers were obtained using the existing anti-stalk ELISA [Pica, 2012], and the observed SD of $\log_{10}$ titers was 0.43 (Day 42). SDs of 0.45 and 0.50 were thus considered in the present power calculations.

Table 28 Factorial study design: repartition of the subjects according to the nature of the priming sequence and to the type of adjuvant system

N evaluable subjects	1 priming dose (cH8/1N1)	1 priming dose (cH5/1N1)	2 priming doses (cH8/1N1 and cH5/1N1)	Total
AS01	43 subjects	43 subjects	43 subjects	129 subjects
AS03	43 subjects	43 subjects	43 subjects	129 subjects
No adjuvant	43 subjects	43 subjects	43 subjects	129 subjects
Total	129 subjects	129 subjects	129 subjects	387 subjects

Using a 1-way Analysis of Variance (ANOVA) power analysis in PASS 12.0.10, considering 3 pooled groups of size n = 129 subjects, 2-sided alpha = 0.10, SD of $\log_{10}$ (titers) = 0.45 to 0.50, and a scenario with a maximum difference in GMTs of 2-fold (repartition type 1-1-2 or 1-2-2), we obtain the SD of group means associated with 80% power to detect differences among the means versus the alternative of equal means. This value is then converted in fold increase according to the scenario. The values are presented in Table 29.

Table 29 Detectable fold increase in GMTs with 80% power (N/group = 129, 2-sided alpha = 0.10, 1-way ANOVA power analysis)

SD of $\log_{10}$ (titers)	SD of group Means	Detectable Fold increase
0.45	0.064	1.36
0.50	0.071	1.41

10.4.2. Confirmatory objective

The most important secondary objective in the study is to establish whether an adjuvant system is needed. This evaluation will primarily be based on levels of anti-H1 stalk titers by ELISA at Day 29 and Day 85 (i.e. 28 days post-last vaccination). Power calculations were conducted for the detection of differences in GMTs between each of the 3 pooled groups of 129 subjects (AS01, AS03, no adjuvant) considering a margin of superiority of 1.5 fold. The 2-sided significance level (alpha) of each test is 0.0554; it corresponds to a critical value from a 2-sided Dunnett test (accounting for the comparison of AS01 to non adjuvanted and AS03 to non-adjuvanted) of 1.922 (2-sided alpha = 0.10, DF = 384 (129 subjects/pooled group), 3 pooled groups).

Table 30 provides the power associated with different true GMT ratio values using PASS 12.0.2 Two-Sample T-Test (Superiority by a Margin) Power Analysis. Again, SDs of $\log_{10}$ (titers) of 0.45 and 0.50 were considered. With a standard deviation of 0.45, the power reaches 80% if the actual difference is 2.14 fold or greater. Similarly, with a SD of 0.50, the power reaches 80% if the actual difference is 2.23 fold or greater.

Table 30 Power to show a difference in means using a 1.5 superiority margin (N/pooled group = 129, 2-sided alpha = 0.055375)

Actual difference in means (fold-difference)	SD of log10 (titers)	Power
2.00	0.45	0.620
2.00	0.50	0.533
2.14	0.45	0.800
2.14	0.50	0.714
2.23	0.45	0.875
2.23	0.50	0.800
2.50	0.45	0.979
2.50	0.50	0.949
3.00	0.45	0.999
3.00	0.50	0.998

The success criterion for this objective can be defined as follows: *the use of the adjuvant (AS01 or AS03) will be considered justified if the lower limit of the 94.46% CI of the GMT ratio (adjuvanted vs non-adjuvanted) is > 1.50.*

10.5. Analysis sets

10.5.1. Exposed set

The Exposed Set (ES) will include all subjects with at least one vaccine administration documented:

- A safety analysis based on the ES will include all vaccinated subjects.
- An immunogenicity analysis based on the ES will include all vaccinated subjects for whom immunogenicity results are available.

The ES analyses will be performed per treatment actually administered.

10.5.2. Per-Protocol set for analysis of immunogenicity

The Per-Protocol set will be adapted by timepoint to include all eligible subjects' data up to the time of important protocol deviation, namely:

- Dose of study vaccine not according to protocol procedures and to their random assignment.
- Randomisation code broken.
- Non-compliance with the procedures and intervals defined in the protocol (refer to Table 7).
- Intake of concomitant medication/product/vaccination leading to elimination from the Per-Protocol analysis.
- Occurrence of medical condition leading to elimination from the Per-Protocol analysis (refer to Section 6.7.2).

10.6. Derived and transformed data

- The cut-off value is defined by the laboratory before the analysis and is described in Section 5.7.3. A seronegative subject is a subject whose titre is below the cut-off value. A seropositive subject is a subject whose titre is greater than or equal to the cut-off value.
- The GMTs calculations are performed by taking the anti-log of the mean of the log titre transformations. Antibody titres below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMT calculation.
- The MGI is defined as the geometric mean of the fold increase in serum HI titres post-vaccination compared to Day 1.
- All CIs are 95% CIs. The 95% CIs for GMT are obtained within each group separately. The 95% CI for the mean of log-transformed titre is first obtained assuming that log-transformed titres are normally distributed with unknown variance. The 95% CI for the GMT is then obtained by exponential-transformation of the 95% CI for the mean of log-transformed titre.
- Handling of missing data: for a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, analysis will exclude subjects with missing or non-evaluable measurements.
- Reactogenicity and safety
  - For the analysis of solicited symptom, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited symptoms based on the ES will include only subjects/doses with documented safety data (i.e. symptom screen completed).
  - For the analysis of unsolicited AEs/MAEs/ILIs/SAEs/concomitant medication, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.

10.7. Analysis of demographics

Demographic characteristics (center, age at study vaccination in years, gender, ethnicity, geographic ancestry, history of influenza vaccination since the 2014/2015 season) and withdrawal status will be summarized by group in the ES, using descriptive statistics:

- Frequency tables will be generated for categorical variable such as center.
- Mean, median, standard deviation will be provided for continuous data such as age.

10.8. Analysis of safety

The analysis will be performed on the ES.

All analyses will be descriptive. Data will be presented by dose, overall/dose and overall/subject. Outputs will be presented by study group. Analyses will be repeated pooling groups according to the adjuvant (AS01, AS03, no adjuvant).

- The percentage of subjects with at least one local AE (solicited and unsolicited), with at least one general AE (solicited and unsolicited) and with any AE during the solicited follow-up period will be tabulated with exact 95% CI. The same calculations will be performed for AEs rated as grade 3.
- The percentage of subjects reporting each individual solicited local and general AE during the solicited follow-up period will be tabulated with exact 95% CI. The same tabulation will be performed for grade 3 AEs and for AEs with relationship to vaccination.
- The verbatim reports of unsolicited AEs will be reviewed by a physician and the signs and AEs will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). The percentage of subjects with at least one report of unsolicited AE classified by the MedDRA and reported up to 28 days after vaccination will be tabulated with exact 95% CI. The same tabulation will be performed for grade 3 unsolicited AEs and for unsolicited AEs with a relationship to vaccination.
- The percentage of subjects with MAE(s) will be summarized by group with exact 95% CI.
- The percentage of subjects with episode(s) of ILI will be summarized by group with exact 95% CI. **(Amended: 11 July 2019)**
- At each hematology/biochemistry sampling timepoint, by study group, individual hematological and biochemical values will be presented as number of subjects out of range (above and below normal range) and tabulated by toxicity grading (refer to APPENDIX C). In addition, changes from baseline (median/interquartile range) will be presented.
- SAEs and pIMDs will be described in detail. Withdrawals due to (S)AEs will also be summarized.

10.9. Analysis of immunogenicity

The analysis of immunogenicity will be performed primarily on the Per-Protocol set. If 5% or more of the vaccinated subjects are eliminated from the Per-Protocol set at one timepoint, a second analysis will be performed on the ES.

10.9.1. Within group assessment

10.9.1.1. Humoral immunogenicity assessment

For each study group, at each timepoint at which the tests are done and results are available, for each humoral immunity parameter, the following analyses will be performed:

- Seropositivity rates and GMTs, with exact 95% CI.
- MGI from Day 1, with 95% CI.
- Percentage of subjects with at least 4-fold increase from Day 1, with exact 95% CI.
- Percentage of subjects with at least 10-fold increase from Day 1, with exact 95% CI.
- Distribution of antibody titers using reverse cumulative distribution curves.

The correlation between anti-H1 HA stalk ELISA and anti-H1 HA stalk MN assay results will be explored.

10.9.1.2. CMI assessment

For each study group, ***at Days 1, 8, 29, 64 and 85***, the frequency of specific CD4+/CD8+ T-cells, B-memory cells and plasmablasts will be summarised using descriptive statistics. **(Amended: 11 July 2019)**

10.9.2. Between group assessment

10.9.2.1. ANCOVA modelling

The anti H1 HA stalk ELISA titers will be modelled using an ANCOVA model. Twenty-eight days post priming/post booster $\log_{10}$ (titers) will be modelled as a function of the adjuvant (AS01, AS03, no adjuvant) and of the priming sequence (cH8/1N1, cH5/1N1, cH8/1N1 and cH5/1N1), including the pre-vaccination titer as covariate. The primary analysis will not include any interaction term.

For the parameter related to the priming sequence, in absence of a reference group, the overall test of difference (to reject the null hypothesis of no difference) will be done at significance level 0.10. If the test is statistically significant at level 0.10, the different pairwise comparisons will be performed at the same alpha level.

For the parameter related to the adjuvant, the pairwise comparisons to the non-adjuvant reference group (AS01 vs no adjuvant and AS03 vs no adjuvant) are planned to be performed without preamble. Therefore, a Dunnett test will be used for the pairwise comparisons.

10.9.2.2. Descriptive assessment

GMT ratios and their 2-sided 95% CI will be computed after fitting an ANCOVA model on the $\log_{10}$ transformation of ELISA/MN titers, including vaccine group as fixed effect and the pre-vaccination titer as covariate.

Differences in percentage of subjects with a fold increase from baseline and their 95% CIs will be calculated.

The assessment timepoints will be described in the Statistical Analysis Plan, but generally speaking, the 4 weeks post-dose results will be compared.

The following group ratios/differences will be provided:

- Evaluation of the proof of principle:
  - cH8/5/11-AS03 vs IIV4.
  - cH8/5/11-AS01 vs IIV4.
  - cH8/5/11 vs IIV4.
- Evaluation of the number of priming doses:
  - cH8/5/11-AS03 vs cH8/P/cH5-AS03.
  - cH8/5/11-AS01 vs cH8/P/cH5-AS01.
  - cH8/5/11 vs cH8/P/cH5
- Assessment of the adjuvant systems:
  - cH8/5/11-AS03 vs cH8/5/11-AS01.
  - cH8/P/cH5-AS03 vs cH8/P/cH5-AS01.
  - cH5/P/cH8-AS03 vs cH5/P/cH8-AS01.
- Description of the priming sequence:
  - cH8/P/cH5-AS03 vs cH5/P/cH8-AS03.
  - cH8/P/cH5-AS01 vs cH5/P/cH8-AS01.

Additional ratios/differences might be considered if deemed necessary at the time analysis.

10.10. Interpretation of analyses

Comparative analyses will be descriptive with the aim to characterise the difference in reactogenicity/immunogenicity between groups.

With respect to the secondary objective and decision rule linked to the use of an adjuvant, the interpretation will be done according to the CI for the GMT ratios (pooled AS01 vs pooled non-adjuvanted and pooled AS03 vs pooled non-adjuvanted). The use of the adjuvant (AS01 or AS03) will be considered justified if the lower limit of the 94.46% CI of the GMT ratio (adjuvanted vs non-adjuvanted) is > 1.50.

10.11. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final Study Report.

10.11.1. Sequence of analyses

All interim analyses will be conducted on data as clean as possible. The final analysis will be performed on fully clean data.

Excluding the IDMC monitoring analyses, the analyses will be performed in a stepwise manner:

- Interim analyses will be performed when safety, reactogenicity and immunogenicity (including at least H1 anti-stalk ELISA) data from all subjects are available up to Day 85 and up to Month 14 + 28 days*. The GSK statistician/statistical analyst will be unblinded for these analyses (i.e. will have access to the individual subject treatment assignment). The remaining GSK study personnel will remain blinded (see Section 5.3).

  *Note that for this timepoint, an additional interim analysis will be performed on all available immunogenicity data when Phase I subjects eligible for booster vaccination have completed their Visit 10 according to the allowed interval.

- A final analysis of all data will be performed when all data up to study conclusion are available. This analysis will be reported in an integrated Study Report and made available to the investigators.

If the data for tertiary endpoints become available at a later stage, (an) additional analysis/analyses will be performed. These data will be documented in annex(es) to the Study Report and will be made available to the investigators at that time.

10.11.2. Statistical considerations for interim analyses

No statistical adjustment will be made for the interim analyses, which are intended to provide final outputs related to the different endpoints and timepoints in a phased manner.

11. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality, public disclosure requirements and publications must be fulfilled.

11.1. electronic Case Report Form instructions

A validated GSK defined electronic data collection tool will be used as the method for data collection.

In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with a CD-ROM of the final version of the data generated at the investigational site once the database is archived and the study report is complete and approved by all parties.

11.2. Study Monitoring by GSK Biologicals

GSK will monitor the study to verify that, amongst other items, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform a eCRF review and a Source Document Verification (SDV). By SDV we understand verifying eCRF entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the eCRF. This document should be completed and signed by the site monitor and investigator and should be filed in the investigator's study file. Any data item for which the eCRF will serve as the source must be identified, agreed and documented in the source documentation agreement form.

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and GSK procedures.

11.3. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g. audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures, otherwise, the minimum retention period will default to 25 years after completion of the study report.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

11.4. Quality assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

11.5. Posting of information on publicly available clinical trial registers and publication policy

GSK assures that the key design elements of this protocol will be posted on the GSK website and in publicly accessible database(s) such as clinicaltrials.gov, in compliance with the current regulations.

GSK also assures that results of this study will be posted on the GSK website and in publicly accessible regulatory registry(ies) within the required time-frame, in compliance with the current regulations. The minimal requirement is to have primary endpoint summary results disclosed at latest 12 months post PCD and to have secondary endpoint disclosed at latest 12 months after the Last Subject Last Visit as described in the protocol.

GSK also aims to publish the results of these studies in searchable, peer reviewed scientific literature and follows the guidance from the International Committee of Medical Journal Editors.

11.6. Provision of study results to investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK Biologicals 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.

11.7. Data Sharing

Under the framework of the SHARE initiative, results of GSK studies may be combined with non-GSK studies, to investigate further about the study product(s) and other product(s), and/or the disease/condition under investigation and related diseases and conditions.

12. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

13. REFERENCES

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APPENDIX A LABORATORY ASSAYS

Anti-stalk ELISA protocol:

The prevalence of anti-stalk antibodies will be measured by ELISA using reagents based on HA-group specific chimeric HAs. The chimeric HA consists of an exotic head domain on top of a vaccine-antigen stalk domain. For example, the cH6/1 antigen has been constructed to measure anti-stalk antibodies against H1. Since humans are naïve to the H6 head domain, reactivity measured with this substrate indicates reactivity to the H1 stalk. Chimeric antigens will be developed for the two HA groups (group A1 and group A2) and will be used in a classical ELISA. Briefly, the antigen is coated on 96 well plates. After blocking, the serum is added and sequentially diluted. After incubation and washing steps, a detection antibody is used to distinguish serum antibodies attached to the antigen and optical density measurement provides quantitative information about the amount of antibodies in the serum. Positive and negative controls are developed in addition of an antigen-specific standard.

The breadth of the immune response within the group 1 HA subtypes will be measured using a full length HA ELISA.

Microneutralization (MN) assay protocol:

The functionality of the stalk-reactive antibodies is evaluated by MN assays developed using chimeric viruses. As done for the ELISA, the objective is to avoid interference caused by antibodies directed against HA head but also caused by antibodies directed against NA; for that purpose we use viruses expressing an HA with an HA group-specific stalk and an HA head domain and NA to which humans are naïve. Briefly, measurements are conducted on thawed frozen serum samples. Samples are heat inactivated for 30 min at 56°C. A standardized amount of virus is mixed with serial dilutions of serum and incubated to allow binding of the antibodies to the virus. A cell suspension, containing a defined amount of Madin-Darby Canine Kidney (MDCK) cells is then added to the mixture of virus and antiserum and incubated at 35°C (± 2°C). After the incubation period, virus replication is visualized (by hemagglutination of red blood cells or OD reading) and a neutralization titer is calculated between the highest serum dilution able to totally neutralize the virus and one of the next serum dilution where viruses remain detectable. Each serum sample is tested once.

Hemagglutination inhibition (HI) assay protocol:

HI antibody titers are determined using the method derived from the WHO Manual on Animal Influenza Diagnosis and Surveillance, WHO/CDS/CSR/NCS/2002.5.

Measurements are conducted on thawed frozen serum samples with a standardized and comprehensively validated micro-method using 2 hemagglutinating units (2 HAU) of the appropriate antigens and a 0.45% fowl erythrocyte suspension. Non-specific serum inhibitors are removed by heat treatment and receptor-destroying enzymes.

Starting with an initial dilution of 1:10, a dilution series (by a factor of 2) is prepared up to an end dilution of 1:10240. The titration end-point is taken as the highest dilution step that shows complete inhibition of hemagglutination. All assays are performed in duplicate. The usual cut-off value is 10 1/DIL.

ELISPOT (memory B cell detection assay):

The B-cell ELISPOT allows the quantification of antigen-specific memory B-cells. These cells are responsible of long term (humoral) memory and will be the cells recalled during an infection subsequent to vaccination. This assay is designed to evaluate the frequency (per million memory B-cells) of HA stalk specific memory B-cells from peripheral blood samples.

The protocol is adapted from the assay developed by [Crotty, 2004], and involves the incubation of PBMC that have been differentiated into antibody secreting cells in nitro-plates coated with either the antigen of interest (for the detection of antigen-specific memory B-cells) or anti-human Ig (for the detection of total memory B-cells). A conventional immuno-enzymatic procedure [Crotty, 2004] is applied to detect antibody/antigen spots enumerating memory B-cells and the results are expressed as the frequencies of antigen-specific memory B-cells within the total memory B-cell population.

Flow Cytometry B-cells (FCB) for plasmablasts detection

The FCB assay has been developed to allow detection of HA stalk specific plasmablasts. Upon vaccination or natural challenge, these differentiated B-cells are transiently present in the periphery (peak at Day 7 post-antigen encounter) and are the cells producing the antibodies. The FCB assay has been set up to detect the cells producing the anti-HA stalk antibodies that the SUIV is designed to induce.

The PBMC are thawed and immuno-stained for surface markers allowing phenotypic identification of the plasmablasts (CD14- / IgD- / CD20- / CD19+ / CD38+). Cells are then permeabilized and immune-stained with a specific biotinylated probe (here the chimeric HA protein cH6/1) which will bind to the cognate antibodies (here the anti-HA stalk antibodies) within the plasmablasts. The probe is then detected with a fluorescently labelled streptavidin, and analysis performed by multi-parametric flow cytometry. Results are expressed as frequencies of antigen specific plasmablasts per million PBMC.

T-cell detection by Intracellular Cytokine Staining (ICS) assay:

This assay is applied to measure the frequency of antigen-specific T-lymphocytes in peripheral blood. CD4+ T-lymphocytes are critical helper cells supporting the differentiation of antibody secreting cells (B-cells). CD8+ T-lymphocytes are direct effector cells able to kill virus-infected cells.

Blood samples are collected by venipuncture and PBMCs are prepared by centrifugation onto a *Lymphoprep* cushion within 24 hours following collection. PBMC suspensions are stored in liquid nitrogen until analysis. To measure T-cell responses elicited by the vaccine candidate, samples are thawed and stimulated with relevant antigen (synthetic peptides covering the HA stalk domain). Re-stimulated cells are then immunostained for surface markers such as CD3, CD4 and CD8 followed by cell permeabilization and immunostaining for effector molecules (cytokines/activation marker) such as IL-2, IFN-γ, TNF-α and CD40L (additional effector molecules could also be explored). Analysis is performed by multiparametric flow cytometry, and the results are expressed as frequencies of CD4+ (or CD8+) T-cells producing various combinations of the cytokine/activation markers assessed per million CD4+ (or CD8+) T-cells.

RT-qPCR assay for influenza detection:

Viral RNAs extracted from the clinical sample are amplified and detected using Flu-A and Flu-B specific primers and probes (designed in the Matrix gene). Viral Load values are quantified and the sample is considered positive when the measured Viral Load is equal to or above the assay cut-off. Several controls are included throughout the process, both at the extraction and RT-PCR steps to monitor the extraction and RT-PCR efficiencies and any potential contamination that may occur during each run.

RT-PCR assay for influenza A/H1N1 and A/H3N2 typing:

A RT-PCR allowing the identification of Flu-A/H1N1 and Flu-A/H3N2 is performed on the nucleic acids generated for Flu-A and Flu-B detection. Specific primers designed in the HA gene of Flu-A/H1N1 and Flu-A/H3N2 are used to perform the discrimination. In addition, Flu-A/H1N1 and Flu-A/H3N2 RT-PCR positive controls corresponding to the RNA from reference strains are evaluated in parallel with the samples. No template controls are also used to monitor any potential contamination during each RT-PCR run.

RT-qPCR assay for RSV detection:

Viral RNAs extracted from the clinical sample are amplified and detected using RSV-A and RSV-B specific primers and probes designed in the N gene encoding the nucleocapsid protein. Viral load values are quantified and the sample is considered positive when the measured viral load is equal to or above the assay cut-off. Several controls are included throughout the process, both at the extraction and RT-PCR steps to monitor the extraction and RT-PCR efficiencies and any potential contamination that may occur during each run.

Multiplex RT-PCR assay for viral pathogen detection:

A qualitative PCR multiplex assay is used for the detection and identification of multiple respiratory virus nucleic acids in nasal and throat swabs from individuals suspected of respiratory tract infections. The following virus types and subtypes can be identified in the assay:

- Parainfluenza 1 virus
- Parainfluenza 2 virus
- Parainfluenza 3 virus
- Parainfluenza 4 virus
- Human Metapneumovirus
- Rhinovirus
- Enterovirus
- Adenovirus
- Coronavirus 229E
- Coronavirus OC43
- Coronavirus NL63
- Human Bocavirus

Following total nucleic acids extraction, viruses are detected by multiplex real-time RT-PCR assays targeting the above mentioned viruses. A comparative analysis of the fluorescence intensities of each target is performed to detect the viruses present in the sample. Several controls are included throughout the process, both at the extraction and RT-PCR steps to monitor the extraction and RT-PCR efficiencies and any potential contamination that may occur during each run.

APPENDIX B CLINICAL LABORATORIES

Table 31 GSK Biologicals' 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
GSK Dresden GlaxoSmithKline Biologicals Branch of SmithKline Beecham Pharma GmbH & Co. KG	Zirkusstrasse 40, D-01069 Dresden Germany

Table 32 Outsourced laboratories

Laboratory	Address
Néomed-Labs Inc.	525, Cartier Ouest Laval Quebec Canada H7V 3S8 NÉOMED-LABS Inc.
Q^2 Solutions Clinical Trials (US)	27027 Tourney Road, Suite 2E Valencia, CA 91355, USA
Q^2 Solutions Clinical Trials (UK)	Unit B1, Parkway West Industrial Estate Cranford Lane – Heston, Middlesex TW5 9QA UK

APPENDIX C FDA GUIDANCE FOR INDUSTRY: TOXICITY GRADING SCALE FOR HEALTHY ADULT AND ADOLESCENT VOLUNTEERS ENROLLED IN PREVENTIVE VACCINE CLINICAL TRIALS (SEPTEMBER 2007)

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

i. INTRODUCTION

Preventive vaccines are usually developed to prevent disease in a healthy population. The Office of Vaccines Research and Review, Centre for Biologics Evaluation and Research, regulates preventive vaccines under authority of section 351 of the Public Health Service Act (42 U.S.C. 262), as well as specific sections of the Federal Food, Drug, and Cosmetic Act, and reviews investigational new drug applications (INDs) and biologics license applications (BLAs). (See, for example, Title 21 Code of Federal Regulations (CFR) Parts 312, 600, and 601). Most of the clinical trials of preventive vaccines conducted to support INDs and BLAs enroll healthy volunteers in all phases of vaccine testing. The enrolment of healthy volunteers warrants a very low tolerance for risk in those clinical trials.

This guidance provides you, sponsors, monitors, and investigators of vaccine trials, with recommendations on assessing the severity of clinical and laboratory abnormalities in healthy adult and adolescent volunteers enrolled in clinical trials. The grading system described in the table can also be useful in defining a particular study's stopping rules (e.g. a certain number of AEs, as defined in the table, may call for stopping the study). Less extreme observations (e.g. mild) may not require discontinuing the study vaccine but can still contribute to evaluating safety by identifying parameters to focus upon in subsequent product development. Uniform criteria for categorizing toxicities in healthy volunteers can improve comparisons of safety data among groups within the same study and also between different studies. We, FDA, recommend using toxicity grading scale tables, provided below, as a guideline for selecting the assessment criteria to be used in a clinical trial of a preventive vaccine. We recommend incorporation of such appropriate, uniform, criteria into the investigational plan, case report forms, and study reports and correspondence with FDA, sponsors, monitors, investigators, and IRBs.

This guidance finalizes the draft guidance of the same title dated April 2005 (70 FR 22664, May 2, 2005).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word should in FDA's guidances means that something is suggested or recommended, but not required.

ii. BACKGROUND

Standardized toxicity assessment scales have been widely used to evaluate products treating specific diseases. For example, the National Cancer Institute's Common Toxicity Criteria Scale and the Division of AIDS' Toxicity Grading Scale standardize the evaluation of adverse events (AEs) among patients with cancer and HIV/AIDS, respectively (Refs. 1, 2). The defined toxicity parameters in those scales are designed for patients who may already experience mild, moderate, or severe adverse clinical or laboratory events due to the disease process, and may not be appropriate for healthy volunteers.

In the development of the toxicity grading scales for healthy volunteers, we chose parameter limit values based on published information, when such values were available (Refs. 1-6). For example, the Brighton Collaboration has developed case definitions and guidelines to evaluate some AEs associated with administering vaccines (Ref. 3). In some cases, parameter limit values were based on clinical experience and experience reviewing vaccine clinical trials that enroll normal healthy subjects.

Toxicity grading scales for laboratory abnormalities should consider the local laboratory reference values when the parameter limit values are defined. The characterization of laboratory parameters among some populations of healthy adults and adolescents may require the exercise of clinical judgment, for example, consideration of the potential for ethnic differences in white blood cell (WBC) counts or gender differences in creatine phosphokinase (CPK) values.

iii. TOXICITY GRADING SCALE TABLES

AEs in a clinical trial of an investigational vaccine must be recorded and monitored and, when appropriate, reported to FDA and others involved in an investigation (sponsors, IRBs, and investigators). (See, for example, 21 CFR 312.32, 312.33, 312.50, 312.55, 312.56, 312.60, 312.62, 312.64, and 312.66). Although the use of a toxicity grading scale for AEs would not replace these regulatory requirements, using a scale to categories adverse events observed during a clinical trial may assist you in monitoring safety and making required reports. Nonetheless, we believe that categorization or grading of data as outlined in this document is supplementary to and should not replace full and complete data analysis.

These guidelines for toxicity grading scales are primarily intended for healthy adult and adolescent volunteers. The parameters in the tables below are not necessarily applicable to every clinical trial of healthy volunteers. The parameters monitored should be appropriate for the specific study vaccine. For some preventive vaccines under development, it may be appropriate to include additional parameters to be monitored during a clinical trial or to alter the choice of values in the toxicity table. For example, additional parameters might be added based on one or more of the following: safety signals observed in pre-clinical toxicology studies, the biological plausibility of the occurrence of certain AEs, or previous experience with a similar licensed product.

As discussed above, the tables do not represent a recommendation to monitor all the listed parameters in all clinical trials of healthy volunteers, nor do the tables represent all possible parameters to be monitored. In addition, these tables do not represent study inclusion or exclusion criteria. We recommend that the parameters monitored be appropriate for the study vaccine administered to healthy volunteers participating in the clinical trial.

a. Tables for Clinical Abnormalities

Note from the sponsor: The tables in this section of the guidance will not be used in this particular study. Instead, the parameters as provided in the study protocol are to be used.

b. Tables for Laboratory Abnormalities

The laboratory values provided in the tables below serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Only those parameters that will be assessed as part of the study have been maintained in the tables below.

Table 33 FDA toxicity grading scales for hematology/biochemistry parameters

Serum*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Blood Urea Nitrogen – BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN

ULN = upper limit of the normal range.
*The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
**The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a Grade 3 parameter (125-129 mE/L) should be recorded as a Grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

Hematology*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm³	10 800 – 15 000	15 001 – 20 000	20 001 – 25 000	> 25 000
WBC Decrease - cell/mm³	2 500 – 3 500	1 500 – 2 499	1 000 – 1 499	< 1 000
Lymphocytes Decrease - cell/mm³	750 – 1 000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm³	1 500 – 2 000	1 000 – 1 499	500 – 999	< 500
Eosinophils - cell/mm³	650 – 1 500	1 501 – 5 000	> 5 000	Hyper-eosinophilic
Platelets Decreased - cell/mm³	125 000 – 140 000	100 000 – 124 000	25 000 – 99 000	< 25 000

*The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

iv. REFERENCES for Appendix C

1. National Cancer Institute Common Toxicity Criteria, April 30, 1999. (http://ctep.cancer.gov/reporting/CTC-3.html)
2. Division of AIDS Table for Grading Severity of Adult Adverse Experiences; August 1992. (http://rcc.tech-res-intl.com/tox_tables.htm)
3. The Brighton Collaboration. Finalized Case Definitions and Guidelines. (http://brightoncollaboration.org/internet/en/index/definition___guidelines.html)
4. HIV Vaccine Trials Network Table for Grading Severity of Adverse Experiences; September 18, 2002. (http://rcc.tech-res-intl.com/tox_tables.htm)
5. Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, December 2004. (http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/Safety/DAIDSAEGradingTable.pdf)
6. Kratz A, Ferraro M, Sluss PM, Lewandrowski KB. Laboratory Reference Values. New England Journal of Medicine. 2004;351:1548-1563.

APPENDIX D SERUM PASSIVE TRANSFER/VIRUS CHALLENGE EXPERIMENT IN BALB/C MICE FROM ADULT SUBJECTS INVOLVED IN THE FLU D-SUIV-ADJ-001 STUDY COHORT

Objectives (Amended: 11 July 2019)

This study is considered as part of exploratory read-outs in the clinical protocol.

1. The *in vivo* protective effect of transferring pooled adult human serum from subjects from the FLU D-SUIV-ADJ-001 study cohort to mice and subsequently challenging them with chimeric head-stalk HA virus, for example cHx/1Ny virus (Hx = most likely H6/1, Ny = most likely N5; to be decided) or circulating H1N1 (most likely H1N1 strain contained in the IIV4 control annual vaccines in accordance with WHO recommendation) will be assessed in terms of the following endpoints:

   – 1A: Survival over 14 days post-challenge (day of death or euthanasia for weight loss > 25% baseline body weight) in groups of 10 to 35 mice/human-group/timepoint (number of mice = most likely 25, to be decided upon the pathogenicity of the challenge virus in mice; the exact sample size will be specified in an annex statistical report).

   – 1B: Weight loss (change from baseline over 14 days post-challenge) in groups of 10 to 35 mice/human-group/timepoint.

   – 1C: Lung virus titer in $TCID_{50}$/mg on Day 3 and/or Day 6 post-challenge in subset of 5 mice for each timepoint. This assessment will be performed at Day 3 or Day 6 post-challenge, or both, depending on anticipated additional supportive pre-clinical data.

     Comparisons between FLU D-SUIV-ADJ-001 study groups and/or timepoints (Day 1 ***and*** Day 85) will be investigated.

2. The association between pre- or post-transfer human IgG binding antibody titers to the challenge viruses and the lung virus titer at Day 3 and/or Day 6 and the post-challenge outcome will be explored.

   – 2A: Measure of the pre- and post-transfer geometric mean human IgG binding antibody specific to cH6/1Nx virus, H1N1 virus or recombinant HA proteins in blood collected from mice receiving either one of the serum pools (Day 1 ***and*** Day 85).

   – 2B: Descriptive analyses will be conducted to detect possible associations between the antibody titers as measured in 2A and post-challenge endpoints.

     a. Proportion of survival over 14 days post-challenge,

     b. Weight loss,

     c. Geometric mean lung virus titer.

Protocol endpoints **(Amended: 11 July 2019)**:

Objective	Endpoint
1A	Survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 10 to 35 mice*/serum pool/timepoint
1B	Weight loss (change from baseline over 14 days post-challenge) in groups of 10 to 35 mice*/serum pool/timepoint
1C	Lung virus titer in $TCID_{50}$/mg (log_{10} fold change (D1 - D85)), within challenge group
2A	Pre- or post-transfer of human IgG binding antibody titers to cH6/1Nx virus by ELISA or HI, Pre- or post-transfer of human IgG binding antibody titers to H1N1 virus by ELISA or HI Pre- or post-transfer of human IgG binding antibody titers to recombinant HA proteins by ELISA

*If sufficient serum volumes are not available and depending on the challenge virus pathogenicity, the number of mice can be reduced to as low as 10 mice per timepoint and virus challenge

Methods

Viruses:

Challenge virus	Surface glycoprotein attributes	Virus-specific protection mediated by
cH6/1Nx (or an alternative challenge virus with similar attributes but more fit for purpose)	• Exotic HA head domain **not matched** to vaccines strains • HA stalk domain from H1-representative of all group A1 influenza stalk, **matched** to vaccine strains • Exotic neuraminidase **not matched** to vaccine strain	HA stalk antibody responses
H1N1 virus (most likely strain contained in the IIV4 control)	• HA head domain, **matched** to circulating strain • HA stalk domain **matched** to vaccine strain • Neuraminidase **matched** to vaccine strain	HA (stalk and head) and NA antibody responses

Both viruses will be pre-assessed for mouse lethality in LD_{50} experiments (in the presence of control adult human serum pools). The volume and route of inoculation will be 0.05 mL intra-nasal/intra-tracheal administered to anesthetized mice.

Human serum pools:

Serum pools will be created using serum samples from all evaluable subjects to constitute an aliquot of an equal volume across the groups and the timepoints Day 1 ***and*** Day 85 from the FLU D-SUIV-ADJ-001 cohort. **(Amended: 11 July 2019)**

Passive transfer experimental method:

- Twenty to 35 mice per timepoint per virus challenge will be transferred with a standard amount of undiluted human pooled serum (volume to be determined, within the range 150-250 μL). The exact sample size by challenge study will be specified in an annex statistical report. Also, most likely all the human group serums will be tested but some groups might be excluded depending on the results obtained, as the clinical trial progresses and in accordance with the animal ethical 3R's principles. Pre-transfer pooled serum human IgG titers against HA proteins or influenza virus will be evaluated by ELISA as previously mentioned.
- Two hours post serum transfer, the mice will be sedated and:
  - Blood will be collected for determination of post-transfer human IgG titers against HA proteins or influenza viruses by ELISA.
  - Then challenged with 10 x LD_{50} delivered by the IN/IT route.
- Mice will be monitored daily for 14 days for weight-loss and will be euthanized if they lose > 25% of their initial body weight or if any human endpoints defined in the ethical protocol are met.
- On Day 3 and/or Day 6 post-infection, 5 mice per timepoint/virus will be euthanized to assess viral lung titers. This will occur at Day 3 or Day 6 post-challenge, or both, depending on anticipated additional supportive pre-clinical data. Upon results, lung histopathology may be performed at selected timepoints on relevant groups.
- Weighing of the mice and lungs processing will be performed in an open fashion. Viral plaques will be counted by a blinded technician.
- Lung suspensions will be made by homogenization in PBS and frozen at -80°C for later plaque assay using a standard method.

Statistical analytical plan:

Statistical analysis will be performed to evaluate the improved survival after challenge of any vaccine groups (mice receiving human pooled serum from chimeric HA- approach groups) compared to control group (mice receiving human pooled serum from IIV4 group), measured at each timepoint. Additional analysis might be added to the study to detect potential association between post-transfer titers and survival, post-transfer titers and weight loss or between lung-titers at Day 3 or Day 6 and survival.

The analyses and reporting of those data will be handled by the pre-clinical team according to their processes. No data will be incorporated in the clinical study report, but presented as an appendix.

Ethical statement:

The study protocol will be ethically reviewed and approved by the appropriate ethical committee and the study will be carried out in accordance with national and international directives and the GSK Biologicals' policy on the care, welfare and treatment of animals.

APPENDIX E AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals SA Vaccines R&D **Protocol Amendment 1**	
eTrack study number and Abbreviated Title	207543 (FLU D-SUIV-ADJ-001)
IND number	17602
EudraCT number	2017-001584-20
Amendment number:	Amendment 1
Amendment date:	24 October 2017
Co-ordinating author:	PPD, Scientific Writer (XPE Pharma & Science for GSK Biologicals)

Rationale/background for changes:

This protocol is amended for the following reasons:

- To ensure that only healthy subjects with no risk factors for severe influenza-related complications can be enrolled, an additional exclusion criterion has been added:
  - Any condition that puts the subject at risk for serious influenza-related complications, as identified by the Advisory Committee on Immunization Practices.
- To account for an higher rate of non-evaluable subjects, the sample size has been increased by 20 subjects.
- The possibility to use a qualitative assay for the RSV-PCR assay has been added.
- To add the possibility to assess the lung titers in mice in the passive transfer experiment at Day 3 or at Day 6 post-challenge, or both, depending on anticipated additional supportive pre-clinical data; and to clarify that the pool of serum will be obtained using serum samples from evaluable subjects.
- To update the list of potential Immune-Mediated Diseases (pIMDs) to be aligned with the new version of the Medical Dictionary for Regulatory Activities. In addition, gout was added as the monitoring plan for this event (now considered a pIMD) became a commitment for the Company.

- To clarify that the vaccines/products must be brought to room temperature before reconstitution/preparation instead of before use.
- To correct values in the Table 27 presenting 2-sided exact 95% confidence intervals for the true immunological response rate at different possible observed response rates.
- To include the IND number which was assigned after protocol finalization.

In addition, minor corrections/updates have been made and the list of contributing authors has been updated.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Cover page

The symbol ™ has been deleted and the trademark has been put in italics:

Other study vaccines/products	• GSK Biologicals' quadrivalent split virion influenza vaccine *Fluarix Quadrivalent*~~™~~

The following change has also been implemented on the Sponsor and Investigator Approval pages:

Investigational New Drug (IND) number	~~*To be provided*~~ ***17602***
Contributing authors (*continued*)	• […] • PPD, PPD, Global Patent representative***s*** • PPD, PPD, ***Pre-clinical representatives***

Glossary of terms

Protocol amendment:	***The International Conference on Harmonisation (ICH) defines a protocol amendment as: "A written description of (a) change(s) to or formal clarification of a protocol". GSK Biologicals 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.***

Trademarks

The symbols ™ have been deleted and the trademarks have been put in italics:

**Arepanrix™*
Fluarix™
Mosquirix™
***Pandemrix™*

Section 1.2.2 Rationale for the study design (+ in synopsis)

Approximately ~~450~~ ***470*** subjects 18-39 years of age will be equally randomized in 10 different treatment groups to receive […]

Section 3 Study design overview (+ in synopsis)

Figure 1: Study design overview

The number of subjects has been updated (at the top of the figure):

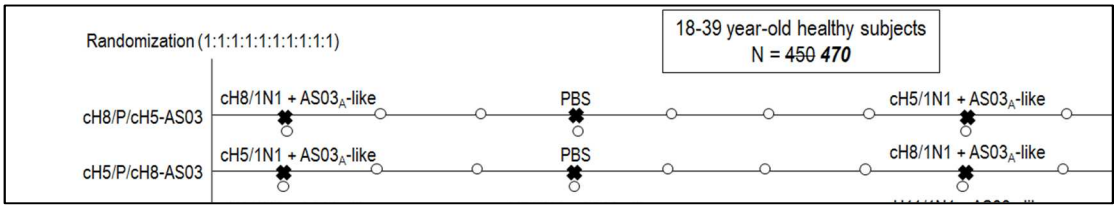

Table 1: Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min - Max)	Epochs	
			Epoch 002	Epoch 003
cH8/P/cH5-AS03	~~45~~ ***47***	18 years – 39 years	x	x
cH5/P/cH8-AS03	~~45~~ ***47***	18 years – 39 years	x	x
cH8/5/11-AS03	~~45~~ ***47***	18 years – 39 years	x	x
cH8/P/cH5-AS01	~~45~~ ***47***	18 years – 39 years	x	x
cH5/P/cH8-AS01	~~45~~ ***47***	18 years – 39 years	x	x
cH8/5/11-AS01	~~45~~ ***47***	18 years – 39 years	x	x
cH8/P/cH5	~~45~~ ***47***	18 years – 39 years	x	x
cH5/P/cH8	~~45~~ ***47***	18 years – 39 years	x	x
cH8/5/11	~~45~~ ***47***	18 years – 39 years	x	x
IIV4	~~45~~ ***47***	18 years – 39 years	x	x

- **Sampling schedule:**
  - […]
  - Blood samples for cell-mediated immunity (CMI) assessment will be drawn from a sub-cohort of ~~~50% of~~ ***~225*** subjects at Days 1 (Visit 1), […]
- **Enrolment**: the study will follow a staggered enrolment with 2 steps; the first being Phase I (N = ~80) and the second being Phase II (N = ~~~370~~ ***390***):

Section 4.1 Number of subjects/centers (+ in synopsis)

This study will be conducted in multiple centers. A total of ~~450~~ ***470*** subjects (~~45~~ ***47***/group) are planned to be enrolled in this study, in order to have 430 evaluable subjects for the primary immunogenicity endpoints. […]

Table 4 Sub-cohort

Sub-cohort name	Description	Estimated number of subjects
Cell-mediated immunity (CMI) testing	Additional blood sample (~40 mL) to be taken at Visits 1, 2, 3, 5, 6, 8, 9, 10 and 12 for CMI testing. This will be done at pre-specified sites with adequate material for such sampling procedure.	Approximately 225 ~~(~ 50% of the enrolled subjects)~~ The sub-cohort will consist of the first Phase II subjects enrolled in pre-specified sites.

Section 4.3 Exclusion criteria for enrolment

- Chronic administration (defined as more than 14 days in total) of immunosuppressants […], or equivalent. ~~Inhaled and~~ Topical steroids are allowed.
- ***[…]***
- ***Any condition that puts the subject at risk for serious influenza-related complications, as identified by the Advisory Committee on Immunization Practices [Grohskopf, 2017] (note that only criteria applicable for the study population are listed below):***
  - ***Chronic pulmonary (including asthma), cardiovascular (except isolated hypertension), renal, hepatic, neurologic, hematologic or metabolic disorders (including diabetes mellitus);***
  - ***Persons who are immunocompromised due to any cause (including immunosuppression caused by medications or by HIV infection);***
  - ***Adolescents (through 18 years) who are receiving aspirin- or salicylate-containing medications and who might be at risk for experiencing Reye syndrome after influenza virus infection;***
  - ***Residents of nursing homes and other long-term care facilities;***
  - ***American Indians/Alaska Natives; and***
  - ***Persons who are extremely obese (Body Mass Index ≥ 40).***

Section 5.2.2.2.1 Study group and treatment number allocation

The target will be to enroll ~~450~~ ***470*** eligible subjects who will be randomly assigned to 10 study groups in a (1:1:1:1:1:1:1:1:1:1) ratio (~~45~~ ***47*** subjects in each group).

Section 5.7.3 Laboratory assay

Table 12 Molecular Biology (PCR tests)

Component	Kit/ Manufacturer	Method	Unit	Laboratory
Nasal swab samples				
[…]	[…]	[…]	[…]	GSK Biologicals* or designated laboratory
RSV A virus (RSV A) RSV B virus (RSV B)	In-house	Quantitative ***or qualitative*** RT-PCR	Copies/mL ***or pos/neg***	

Pos/neg = positive/negative
*GSK Biologicals laboratory refers to the CLS in Rixensart, Belgium; Wavre, Belgium.

Section 5.7.4.1 Immunological read-outs

Table 13 Immunological read-outs for humoral immunity and cell-mediated immunity

Blood sampling timepoint		Sub-cohort Name	No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Humoral immunity					
Visit 1 (Day 1) […]	PRE […]	All subjects	~~450~~ ***470***	Anti-H1 HA stalk ELISA	P P
				[…]	[…]
Visit 1 (Day 1) […]	PRE […]	All subjects	~~450~~ ***470***	Anti-H1 HA stalk MN assay	P P
				[…]	[…]
Visit 1 (Day 1) […]	PRE […]	All subjects	~~450~~ ***470***	HI with IIV4 H1N1 strain from 2017/2018 season	P P
Visit 8 (Month 14) […]	M14 […]	All subjects	~~450~~ ***470***	HI with IIV4 H1N1 strain from 2018/2019 season	P P
				[…]	[…]

Section 6.3 Dosage and administration of study vaccines/products

[…]

All vials of vaccines/products should be kept at room temperature for ~~at least 15~~ ***a few*** minutes prior to ~~use~~ ***reconstitution/preparation***. All vaccines/products must be administered within one hour ~~of reconstitution/preparation~~ ***after being taken out of the fridge***.

Section 8.1.6.1 Potential immune-mediated diseases

Table 20 List of potential immune-mediated diseases

Neuro-inflammatory disorders	Musculoskeletal disorders	Skin disorders
• […] • Myasthenia gravis, including Lambert-Eaton myasthenic syndrome • ~~Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy).~~ • ***Demyelinating peripheral neuropathies including:*** – ***Chronic inflammatory demyelinating polyneuropathy*** – ***Multifocal motor neuropathy*** – ***Polyneuropathies associated with monoclonal gammopathy*** • Narcolepsy	• […] • Systemic scleroderma (systemic sclerosis), including: – Diffuse ~~systemic form~~ ***scleroderma*** – CREST syndrome • […] • Rheumatoid arthritis, and associated conditions including: – Juvenile ~~chronic~~ ***idiopathic*** arthritis – Still's disease • Polymyalgia rheumatica • ~~Spondyloarthritis~~ ***Spondyloarthropathies***, including: – […] – Psoriatic ~~arthropathy~~ ***arthritis*** – ***Enteropathic arthritis*** • […] • ***Gout***	• […] • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) • ~~Alopecia areata~~ • […]

Vasculitides	Blood disorders	Others
• Large vessels vasculitis including: – Giant cell arteritis (***temporal arteritis***) ~~such as~~ – Takayasu's arteritis~~and temporal arteritis.~~ • Medium sized and/or small vessels vasculitis including: – […] – Wegener's granulomatosis (***granulomatosis with polyangiitis***) – Churg–Strauss syndrome (allergic granulomatous angiitis ***or eosinophilic granulomatosis with polyangiitis***) – Buerger's disease (thromboangiitis obliterans), – Necrotizing vasculitis (***cutaneous or systemic***), • Anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), • Henoch-Schonlein purpura (***IgA vasculitis***), • […]	• Autoimmune hemolytic anemia • […]	• Autoimmune glomerulonephritis, including: – […] – ***Tubulo-intestinal nephritis and uveitis syndrome*** • Ocular autoimmune diseases, including: – Autoimmune uveitis – Autoimmune ~~retinopathy~~ ***retinitis*** • Autoimmune myocarditis~~/cardiomyopathy~~ • […] • ***Alopecia areata*** • […]

Liver disorders	Gastrointestinal disorders	Endocrine disorders
• Autoimmune hepatitis • […]	• Inflammatory Bowel disease, including: – Crohn's disease – […]	• Autoimmune thyroiditis (~~including~~ Hashimoto thyroiditis) • […]

Section 8.8.1 Holding rules

[…]

Figure 2 gives the probability of not meeting each holding rule 1 and 2 for ~~the~~ 45 subjects in an investigational group. ***This evaluation was performed for the sample size of the initial protocol (i.e. 450 subjects, 45/group) and it was not repeated at the time of the protocol amendment 1 because of the small difference in the number of subjects per group (now 470 subjects in total, 47/group).***

Section 10.3 Tertiary endpoints (+ in synopsis)

- Assessment of the *in vivo* protective effect of the anti-stalk antibodies when transferring Day 1, Day 85, Month 14 and Month 26 pooled serum from all ***evaluable*** subjects of each vaccine groups […]

Section 10.4.1 Descriptive objectives

[…]

At the time of the protocol amendment 1, it was decided to increase the sample size from 450 to 470 subjects to take into account a larger rate of non-evaluable subjects than anticipated. The power computations from the initial protocol relative to safety endpoints were not modified because of the small sample size increase (about 2 subjects per group, from 45 to 47). The immunogenicity endpoints evaluation will be done primarily on the Per-Protocol set, which is not deemed to increase (about 43 subjects per group).

[…]

Table 27 presents the 95% CIs for different possible rates of immunological response within each group. A rate of unevaluable subjects of 2/45 (4%) has ***initially*** been considered for the immune response post-primary vaccination. ***As from protocol amendment 1, a rate of non-evaluable subjects of 4/47 (9%) has been considered for the immune response post-primary vaccination***.

Table 27 Two-sided exact 95% confidence intervals for the true immunological response rate at different possible observed response rates (43 evaluable subjects)

Observed number of responses	Observed response proportion	95% exact confidence interval	
		Lower limit	Upper limit
25	0.581	0.421	0.730
30	0.698	~~0.547~~ ***0.539***	0.828
35	~~0.781~~ ***0.814***	0.667	0.916
40	0.930	0.809	0.985

*Response rate can be either seropositivity rate, percentages of subjects with a 4-fold increase or percentages of subjects with a 10-fold increase

Section 13 References

Grohskopf LA, Sokolow LZ, Broder KR, et al. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices - United States, 2017–18 Influenza Season. MMWR Recomm Rep 2017; 66 (No. RR-2): 1-20.

Appendix A Laboratory assays

Anti-stalk ELISA protocol:

The prevalence of anti-stalk antibodies will be measured by ELISA using reagents based on HA-group specific chimeric HAs […]. Chimeric antigens will be developed for the ~~3~~ ***two*** HA groups (group A1~~,~~ ***and*** group A2 ~~and group B~~) and will be used in a classical ELISA […].

Appendix D Serum passive transfer/virus challenge experiment in BALB/c mice from adult subjects involved in the FLU D-SUIV-ADJ-001 study cohort

Objectives

This study is considered as part of exploratory read-outs in the clinical protocol.

1. The *in vivo* protective effect of transferring pooled adult human serum […] will be assessed in terms of the following endpoints:
   - […]
   - 1C: Lung virus titer in pfu/µg [log_{10} fold-change (Day 1 – Day 85), (Day 1 – Month 14), (Day 1 – Month 26)] on Day 3 and***/or*** Day 6 post-challenge in subset of 5 mice for each timepoint. ***This assessment will be performed at Day 3 or Day 6 post-challenge, or both, depending on anticipated additional supportive pre-clinical data.***
2. The association between post-transfer ELISA titer of human IgG to the challenge viruses (anti-stalk IgG) at Day 3 and***/or*** Day 6 and the post-challenge outcome will be explored.
   - 2A: Measure of the post-transfer geometric mean ELISA titer […] upon challenge and at Day 3 and***/or*** Day 6 post-transfer.

Methods

Human serum pools:

Serum pools will be created using ~~all residual~~ serum samples ***from all evaluable subjects*** ~~with sufficient volume to furnish~~ ***to constitute*** an aliquot of an equal volume across the groups and the timepoints Day 1, Day 85, Month 14 and Month 26 from the FLU D-SUIV-ADJ-001 cohort.

Passive transfer experimental method:

- Twenty to 35 mice per timepoint per virus challenge will be transferred […], the number of mice can be reduced to as low as 10 mice per timepoint and virus for the survival monitoring and ~~10~~ ***5*** for the lung virus titer assays.
- […]
- On Day 3 and***/or*** Day 6 post-infection, 5 mice per timepoint/virus will be euthanized to assess viral lung titers (5 mice per timepoint/virus x 2 harvesting days x 5 timepoints x 2 virus; 100 mice in total). ***This will occur at Day 3 or Day 6 post-challenge, or both, depending on anticipated additional supportive pre-clinical data.***

GlaxoSmithKline Biologicals SA

Vaccines R&D

Protocol Amendment 2

eTrack study number and Abbreviated Title	207543 (FLU D-SUIV-ADJ-001)
IND number	17602
EudraCT number	2017-001584-20
Amendment number:	Amendment 2
Amendment date:	16 March 2018
Coordinating author:	PPD, Scientific Writer (XPE Pharma & Science for GSK Biologicals)

Rationale/background for changes:

This protocol is amended for the following reasons:

- Based on recommendations from the Independent Data Monitoring Committee, wording has been added to ensure that subjects with clinically significant grade 3 and above abnormal laboratory findings that cannot be reasonably explained may be followed up with a repeat test.
- To add an hemagglutination inhibition (HI) assay against cH6/1N5, H5N8 and H1N1 swine virus (as a tertiary objective/endpoint). These strains are the same as those to be tested by micro-neutralization (MN) assay .This will permit the measurement of the induction of antibodies reactive against the head domain of the antigens.
- To change the description of the influenza virus strain for the H2 full length ELISA testing.
- The results of the MN assay were removed from the data to be included the interim analysis planned at Day 85 due a delay in the availability of this testing.
- The protocol for the passive transfer/virus challenge experiment has been updated.

In addition, minor corrections/updates have been made and the list of contributing authors has been updated.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Cover page

Contributing authors

- PPD, PPD Clinical Research & Development Lead*s*
- PPD, Lead Statistician*s*
- […]
- PPD, Clinical and Epidemiology Project Lead*s*

Section 2.3 Tertiary objectives (+ in synopsis)

- To explore the immune response against the IIV4 H1N1, ~~and~~ the HA head of cH5/1N1, cH8/1N1, cH11/1N1, ***the chimeric cH6/1N5 strain, H5N8 virus strain and H1N1 swine virus strain*** by hemagglutination inhibition (HI) assay.

Section 5.7.3 Laboratory assays

Table 9 Humoral immunity

System	Component (Strain or Antigen Description)	Method	[…]	Cut-off*
Serum	**cH6/1 HA** = Recombinant antigen based on A/mallard/Sweden/81/2002 (H6N1) head domain with stalk domain from HA of H1N1 virus A/California/04/09	Anti-H1 HA stalk ELISA	[…]	TBD
	H2 HA full length = Recombinant antigen based on ~~A/mallard/Netherlands/5/1999 (H2N9)~~ ***A/Japan/305/1957 (H2N2)*** HA	Anti- H2 HA full length ELISA	[…]	TBD
	[…]	[…]	[…]	[…]
Hemagglutination Inhibition (HI) assay				
Serum	[…]	HI assay	[…]	[…]
	Chimeric cH6/1N5 strain			***TBD***
	H5N8 virus strain			***TBD***
	H1N1 swine virus strains			***TBD***

Section 5.7.4.1 Immunological read-outs

Table 13 Immunological read-outs for humoral immunity and cell-mediated immunity

Blood sampling timepoint		Sub-cohort Name	No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Humoral immunity					
Visit 1 (Day 1) Visit 3 (Day 29) Visit 6 (Day 85) Visit 8 (Month 14) Visit 10 (Month 14 + 28 days) Visit 12 (Month 26)	PRE PId28 PIId28 M14 PIIId28 M26	All subjects	~470	[…]	[…]
				HI with cH5/1N1 and cH8/1N1 virus	P P
				HI with cH6/1N5, H5N8 and H1N1 swine virus strains	D P P D
Visit 1 (Day 1) Visit 3 (Day 29) Visit 6 (Day 85) Visit 8 (Month 14)	PRE PId28 PIId28 M14	All subjects	~470	HI with IIV4 H1N1 strain from 2017/2018 season	P P D
Visit 8 (Month 14) Visit 10 (Month 14 + 28 days) Visit 12 (Month 26)	M14 PIIId28 M26	All subjects	~470	HI with IIV4 H1N1 strain from 2018/2019 season	P P P P P D
				HI with cH11/1N1 virus	

Section 6.7.2 Concomitant medications/products/vaccines that may lead to the elimination of a subject from per-protocol analyses

- ***Administration of any influenza vaccine during the study period.***

Section 8.1.5 Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

[…]

In case of clinically significant grade 3 and above abnormal laboratory findings that cannot be reasonably explained (e.g. 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) for a repeat test to confirm the result.

Section 10.3 Tertiary endpoints (+ in synopsis)

- Levels of HI antibody to IIV4 H1N1**,** ~~and~~ chimeric vaccine strains***, chimeric cH6/1N5 strain, H5N8 virus strain and H1N1 swine virus strain***:

  The following aggregate variables will be calculated with 95% CI:

  – […]

- Assessment of the *in vivo* protective effect of the anti-stalk antibodies […]:

  – Survival over 14 days post-challenge (day of death/euthanasia for weight loss > 25% baseline body weight) in groups of ~~25~~ ***35*** mice**/serum pool/vaccine group/timepoint.

- ~~Mean~~ Weight loss (change from baseline over 14 days post-challenge) in groups of ~~25~~ ***35*** mice**/serum pool/vaccine group/timepoint.
- Lung virus titer in ~~plaque-forming units (pfu)/µg~~ ***$TCID_{50}$/mg*** (log10 fold change [Day 1 minus Day 85, Month 14 and Month 26]), within challenge group.
- ***Pre- and*** post-transfer titer of human IgG to cH6/1***N5**** by ELISA ***or HI***.
- ***Pre- and*** post-transfer titer of human IgG to H1N1 by ELISA ***or HI***.
- ***Pre- and post-transfer titer of human IgG to recombinant HA protein by ELISA.***

- ***Evaluation of the anti-H9 full length HA response by ELISA pre-and post-vaccination.***

Section 10.11.1 Sequence of analyses

Excluding the IDMC monitoring analyses, the analyses will be performed in a stepwise manner:

- Interim analyses will be performed when safety, reactogenicity and immunogenicity (including at least H1 anti-stalk ELISA ~~and MN~~) data from all subjects are available up to Day 85 and up to Month 14 + 28 days. […]

Appendix D Serum passive transfer/virus challenge experiment in BALB/c mice from adult subjects involved in the FLU D-SUIV-ADJ-001 study cohort

Objectives

This study is considered as part of exploratory read-outs in the clinical protocol.

1. The *in vivo* protective effect of transferring pooled adult human serum […] will be assessed in terms of the following endpoints:
   - 1A: Survival over 14 days post-challenge (day of death or euthanasia for weight loss > 25% baseline body weight) in groups of 10 to ~~25~~ ***35*** mice/human-group/timepoint (number of mice = most likely 25, to be decided upon the pathogenicity of the challenge virus in mice***; the exact sample size will be specified in an annex statistical report***).
   - 1B: ~~Mean~~ Weight loss (change from baseline over 14 days post-challenge) in groups of 10 to ~~25~~ ***35*** mice/human-group/timepoint.
   - 1C: Lung virus titer in ~~pfu/µg~~ ***$TCID_{50}$/mg*** ~~[log_{10} fold-change (Day 1 – Day 85), (Day 1 – Month 14), (Day 1 – Month 26)]~~ on Day 3 and/or Day 6 post-challenge in subset of 5 mice for each timepoint. […]

     Comparisons between FLU D-SUIV-ADJ-001 study groups and/or timepoints (Day 1, Day 85, Month 14 and Month 26) will be investigated.

2. The association between ***pre- or*** post-transfer ~~ELISA~~ ***human IgG binding antibody*** titer***s*** ~~of human IgG~~ to the challenge viruses (~~anti-stalk IgG~~) ***and the lung virus titer*** at Day 3 and/or Day 6 and the post-challenge outcome will be explored.
   - 2A: Measure of the ***pre- and*** post-transfer geometric mean ~~ELISA titer of human IgG~~ ***human IgG binding antibody specific*** to **cH6/1Nx** virus, ~~and human IgG to A/~~H1N1~~pdm09~~ virus ***or recombinant HA proteins*** in blood collected from mice receiving either one of the serum pools (Day 1, Day 85, Month 14 and Month 26) ~~upon challenge and at Day 3 and/or Day 6 post-transfer~~.
   - 2***B***~~C~~: ~~Correlation~~ ***Descriptive analyses will be conducted to detect possible associations*** between ***the antibody titers as measured in 2A and*** post-challenge endpoints ~~as listed above~~.
     a. Proportion of survival over 14 days post-challenge,
     b. ~~Mean~~ Weight loss,
     c. Geometric mean lung virus titer.

Protocol endpoints:

Objective	Endpoint
1A	~~Primary:~~ Survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 10 to ~~25~~ ***35*** mice*/serum pool/timepoint
1B	~~Primary: mean~~ Weight loss (change from baseline over 14 days post-challenge) in groups of 10 to ~~25~~ ***35*** mice*/serum pool/timepoint
1C	~~Exploratory:~~ Lung virus titer in ~~pfu/µg~~ ***$TCID_{50}$/mg*** (log_{10} fold change (D1 - D85), (D1 - M14) (D1 - M26), within challenge group
2A	~~Exploratory:~~ ***Pre- or*** post-transfer ~~ELISA titer~~ of human IgG ***binding antibody titers*** to cH6/1***Nx virus*** by ELISA ***or HI***, ~~Exploratory:~~ ***Pre- or*** post-transfer ~~ELISA titer~~ of human IgG ***binding antibody titers*** to ~~A/~~H1N1~~pdm09~~ ***virus*** by ELISA ***or HI*** ***Pre- or post-transfer of human IgG binding antibody titers to recombinant HA proteins by ELISA***

*If sufficient serum volumes are not available and depending on the challenge virus pathogenicity, the number of mice can be reduced to as low as 10 mice per timepoint and virus challenge

Methods

Viruses:

[…]

Both viruses will be pre-assessed for mouse lethality in LD_{50} experiments (~~with and without~~ ***in the*** presence of ***control*** adult human serum pools). The volume and route […]

Passive transfer experimental method:

- Twenty to 35 mice per timepoint […] (volume to be determined, within the range 150-250 µL): ~~(35 mice x 2 virus challenges x 4 timepoints = 280 mice in total per human group)~~. ***The exact sample size by challenge study will be specified in an annex statistical report.*** ~~However, depending on the lethality of the virus or if sufficient serum volumes are not available, the number of mice can be reduced to as low as 10 mice per timepoint and virus for the survival monitoring and 5 for the lung virus titer assays.~~ Also, most likely all the human group […] in accordance with the animal ethical 3R's principles. ***Pre-transfer pooled serum human IgG titers against HA proteins or influenza virus will be evaluated by ELISA as previously mentioned***.
- Two hours post serum transfer, the mice will be sedated and:
  - Blood will be collected for determination of post-transfer human IgG titers ***against HA proteins or influenza viruses*** by ELISA.
  - Then challenged with ~~5~~ ***10*** x LD_{50} delivered by the IN/IT route ~~(or a lower dose selected to provide a level of weight-loss and lethality using Day 1 serum that could be reduced by a 3-10 fold increased level of anti-HA stalk antibodies present in post-vaccination serum)~~.
- […]
- On Day 3 and/or Day 6 post-infection, 5 mice per timepoint/virus will be euthanized to assess viral lung titers ~~(5 mice per timepoint/virus x 2 harvesting days x 5 timepoints x 2 virus; 100 mice in total)~~. This will occur at Day 3 or Day 6 post-challenge, or both, depending on anticipated additional supportive pre-clinical data. ***Upon results, lung histopathology may be performed at selected timepoints on relevant groups.***

Statistical analytical plan:

~~Primary objective is defined as~~ ***Statistical analysis will be performed to evaluate*** the improved survival after challenge of any vaccine groups […], measured at each timepoint. Additional ~~exploratory testing~~ ***analysis*** might be added to the study~~, including potential correlation~~ ***to detect potential association*** between post-transfer titers and survival***, post-transfer titers and weight loss*** or ~~correlation~~ between lung-titers at Day 3 or Day 6 and survival.

The analyses and reporting of those data will be handled by the pre-clinical team according to their processes. No data will be incorporated in the clinical study report, but presented as an appendix.

Ethical statement:

The study protocol will be ethically reviewed and approved […]the GSK Biologicals' policy on the care, welfare and treatment of animals ~~(the country and animal facilities where the study where the study will be carried out remains to be defined at this stage)~~.

GlaxoSmithKline Biologicals SA

Vaccines R&D

Protocol Amendment 3

eTrack study number and Abbreviated Title	207543 (FLU D-SUIV-ADJ-001)
IND number	17602
EudraCT number	2017-001584-20
Amendment number:	Amendment 3
Amendment date:	07 December 2018
Coordinating author:	PPD, Scientific Writer (XPE Pharma & Science for GSK Biologicals)

Rationale/background for changes:

This protocol is amended for the following reasons:

- To specify that an additional interim analysis will be performed on available immunogenicity data when Phase I subjects eligible for booster vaccination have completed their Visit 10 (Month 14 + 28 days timepoint).
- To clarify that laboratory parameters (hematology and biochemistry) reported as clinically significant adverse events have to be graded according the Food and Drug Administration Guidance for Industry "*Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*".
- To update the wording of the objective (and corresponding endpoint) related to testing of anti-Group 2 HA by ELISA.
- To add the description of the anti-H9 HA full length ELISA testing.

In addition, the list of contributing authors has been updated.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Cover page

Contributing authors	• PPD Clinical Research & Development Lead***s***
	• PPD, Clinical Trial Supply Manager***s***
	• PPD**,** Global Regulatory Affairs representative***s***

Section 2.3 Tertiary objectives (+ in synopsis)

- To explore the ~~anti-H3~~ ***anti-Group 2 HA*** stalk response (***e.g., H3, H4, H10, …***) (~~i.e. influenza A group 2~~).

Section 5.7.3 Laboratory assays

Table 9 Humoral immunity

System	Component (Strain or Antigen Description)	Method	Kit/ Manufacturer	Unit	Cut-off*
ELISAs					
Serum	H18 HA full length = […]	Anti-H18 HA […]	In-house assay	ELISA units per mL (EU/mL)	TBD
	H9 HA full length = Recombinant antigen based on A/chicken/Hong Kong/G9/1997 HA	***Anti-H9 HA full length ELISA***			***TBD***
	N1 NA = […]	Anti-N1 NA ELISA			TBD

Table 12 Molecular Biology (PCR tests)

Component	Kit/ Manufacturer	Method	Unit	Laboratory
Nasal swab samples				
[…]	[…]	[…]	[…]	GSK Biologicals* or designated laboratory
Human Influenza A virus subtype H1 (Flu A-H1) Human Influenza A virus subtype H3 (Flu A-H3)	In-house	RT-PCR	Qualitative assay (positive/negative)	
RSV A virus (RSV A) RSV B virus (RSV B)	In-house	~~Quantitative or~~ Qualitative RT-PCR	Copies/mL or pos/neg	

Section 5.7.4.1 Immunological read-outs

Table 13 Immunological read-outs for humoral immunity and cell-mediated immunity

Blood sampling timepoint		Sub-cohort Name	No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Humoral immunity					
Visit 1 (Day 1) Visit 3 (Day 29) […]	PRE PId28 […]	All subjects	~470	Anti-H18 HA full length ELISA	P P
				Anti-H9 HA full length ELISA	P P
Visit 1 (Day 1) Visit 3 (Day 29) […]	PRE PId28 […]	All subjects	~470	Anti-N1 NA ELISA	P P
				HI with cH5/1N1 and cH8/1N1 virus	PP D
				HI with cH6/1N5, H5N8 and H1N1 swine virus strains	PPD
Visit 1 (Day 1) Visit 3 (Day 29) […]	PRE PId28 […]	All subjects	~470	HI with IIV4 H1N1 strain from 2017/2018 season	PPD
Visit 8 (Month 14) Visit 10 (Month 14 + 28 days) Visit 12 (Month 26)	M14 PIIId28 M26	All subjects	~470	HI with IIV4 H1N1 strain from 2018/2019 season	PPD
				HI with cH11/1N1 virus	PPD

Section 8.1.5 Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

In absence of diagnosis, abnormal laboratory findings […] (refer to Sections 8.1.1 and 8.1.2). ***The grading of laboratory parameters will be based on the Food and Drug Administration (FDA) Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (refer to Appendix C).*** Clinically significant abnormal laboratory findings […]

Section 8.3.3.2.1 Assessment of intensity

[…]

Note that the grading of laboratory parameters will be based on the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (refer to Section 8.1.5 and Appendix C).

Section 10.3 Tertiary endpoints

- Evaluation of the ~~anti-H3~~ ***anti-Group 2 HA*** stalk response (***e.g., H3, H4, H10, …***) by ELISA and/or MN assay pre-and post-vaccination

Section 10.11.1 Sequence of analyses

- Interim analyses will be performed when safety, reactogenicity and immunogenicity (including at least H1 anti-stalk ELISA) data from all subjects are available up to Day 85 and up to Month 14 + 28 days*****. The GSK statistician/statistical analyst […]

  ****Note that for this timepoint, an additional interim analysis will be performed on all available immunogenicity data when Phase I subjects eligible for booster vaccination have completed their Visit 10 according to the allowed interval.***

GlaxoSmithKline Biologicals SA

Vaccines R&D

Protocol Amendment 4

eTrack study number and Abbreviated Title	207543 (FLU D-SUIV-ADJ-001)
IND number	17602
EudraCT number	2017-001584-20
Amendment number:	Amendment 4
Amendment date:	11 July 2019
Coordinating author:	PPD, Scientific Writer (Modis Life Sciences for GSK Biologicals)

Rationale/background for changes:

Following review of the Day 85 interim analysis and the additional interim analysis performed on available immunogenicity data from Phase I subjects who had completed their Visit 10 (Month 14 + 28 days), the Sponsor made the decision not to pursue the clinical development of the investigational supra-seasonal influenza vaccine. Based on these interim analyses results, some assays will not be performed or developed as initially planned, either because limited or no response is expected to be observed, or because the assay results are no longer relevant in light of the decision not to pursue the development of the investigational supra-seasonal influenza vaccine based on the chimeric hemagglutinin technology.

The changes are as follows:

- The cell-mediated immune response will not be assessed at Month 14 and later timepoints. The blood sampling for cell-mediated immunity at Visit 12 (Month 26) has been removed.
- The hemagglutination inhibition assay (HI) will not be performed on the inactivated influenza quadrivalent vaccine H1N1 component, cH11/1N1, cH6/1N5, H5N8 and H1N1 swine flu strains. For cH5/1N1 and cH8/1N1, the HI assay will only be performed until Visit 6 (Day 85).
- The micro-neutralisation (MN) assay will only be performed for cH6/1N5 and H1N1 until Visit 6 (Day 85). MN assay will not be performed for H5N8.
- The anti-group 2 hemagglutinin (HA) stalk response by ELISA will not be performed.
- The immune response in terms of anti-neuraminidase antibodies will not be assessed.

- The passive transfer experiment in mice will not be performed for the Month 14 and Month 26 timepoints. The blood collection at Month 26 for passive transfer has been removed.
- The anti-H9 full length HA ELISA will not be performed.
- Anti-stalk antibody functionality will not be further investigated, except for the antibody-dependent cell-mediated cytotoxicity until the interim analysis at Day 85.
- Occurrence of RT-PCR-confirmed influenza cases endpoint was removed.

In case samples have already been taken but not tested yet, they will be stored for future research.

In addition, the list of contributing authors has been updated.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Cover page

Coordinating author	• PPD, Scientific Writer (~~XPE Pharma & Science~~ ***Modis Life Sciences*** for GSK Biologicals)
Contributing authors	• PPD ***, Expert Statistician*** • […] • PPD, Study Delivery Lead***s***

Section 1.2.2 Rationale for the study design (+ in synopsis)

[…] Finally, the cell-mediated immune response ~~after each vaccination~~ and the protective effect *in vivo* of the anti-stalk antibodies will be explored. Passive surveillance will be put in place in order to capture the occurrence of ~~RT-PCR-confirmed influenza cases~~ ***influenza-like illnesses*** during the entire study period.

Section 2.3 Tertiary objectives (+ in synopsis)

- To explore the cell-mediated immune responses (B-cells and T-cells) ~~after each vaccination~~.
- To explore the immune response against ~~the IIV4 H1N1~~, the HA head of cH5/1N1 ***and*** cH8/1N1~~, cH11/1N1, the chimeric cH6/1N5 strain, H5N8 virus strain and H1N1 swine virus~~ strain by hemagglutination inhibition (HI) assay.
- ~~To explore the anti-Group 2 HA stalk response (e.g., H3, H4, H10, …).~~
- ~~To explore the immune response in terms of anti-NA antibodies after each vaccination.~~
- ~~To evaluate the occurrence of RT-PCR-confirmed influenza cases during the entire study period.~~
- […]

- To develop ~~and validate~~ assays for evaluation/characterization of the humoral and cellular immune responses to the investigational vaccines.
- ~~To explore the humoral immune response in term of anti-H9 full length HA serum antibodies.~~
- To explore anti-stalk antibody functionality**,** ~~(~~e.g., antibody-dependent cell-mediated cytotoxicity (ADCC),~~ complement dependent lysis (CDL), antibody dependent cellular phagocytosis (ADCP) or glycoform analysis assays)~~.

Section 3 Study design overview (+ in synopsis)

Figure 1 Study design overview

*If a subject presents signs and symptoms of influenza-like illness (ILI) […] to test for influenza and/or other respiratory pathogens by RT-PCR ***if deemed necessary, or for storage***.

[…]

- **Sampling schedule**
  - […]
  - Blood samples for passive transfer experiment in animals will be drawn from all subjects at […], Month 14 (Visit 8)***** ~~and Month 26 (Visit 12)~~.
  - Blood samples for cell-mediated immunity (CMI) assessment will be drawn from a sub-cohort of 225 subjects at […], Month 14 (Visit 8)*****, Month 14 + 7 days (Visit 9)*****, and Month 14 + 28 days (Visit 10)***** ~~and Month 26 (Visit 12)~~.
  - During the entire study period, nasal and throat swabs will be collected […] by RT-PCR ***if deemed necessary, or stored for future research***.

    ****Note that samples already collected for these timepoints by the time of Protocol Amendment 4 implementation at site will not be tested and will be stored, unless deemed necessary based on medical review of the cases.***

Section 4.1 Number of subjects/centres

Table 4 Sub-cohort

Sub-cohort name	Description	Estimated number of subjects
Cell-mediated immunity (CMI) testing	Additional blood sample (~40 mL) to be taken at Visits 1, 2, 3, 5, 6, 8, 9 ***and*** 10 ~~and 12~~ for CMI testing. […]	Approximately 225 The sub-cohort will consist […].

Section 5.5 Outline of study procedures

Table 5 List of study procedures for Phase I subjects

Epoch	Screening	[…]	Booster					In case of ILI**
Type of contact	Screening Visit*		Visit 8	Visit 9	Visit 10	Visit 11	**Visit 12**	
Timepoints	Pre-Day 1		M14	M14+7D	M14+28D	M20	**M26**	
Sampling timepoints	Screening		M14	PIIId7	PIIId28	M20	**M26**	
Blood sampling for passive transfer (~2.5 mL)			●				-●-	

Table 6 List of study procedures for Phase II subjects

Epoch	[…]	Booster					In case of ILI**
Type of contact		Visit 8	Visit 9	Visit 10	Visit 11	**Visit 12**	
Timepoints		M14	M14+7D	M14+28D	M20	**M26**	
Sampling timepoints		M14	PIIId7	PIIId28	M20	**M26**	
Blood sampling for CMI response (~40 mL)#		●	●	●		-●-	
Blood sampling for passive transfer (~2.5 mL)		●				-●-	

Section 5.7.2 Biological samples

Table 8 Biological samples

Sample type	Quantity	Unit	Timepoint	Sub-cohort name**
Blood	Approximately 20	mL	Visits 1, 6, 8 ~~and 12~~	All subjects
	Approximately 5.5*	mL	Visits 2, 4, 5, 9	
	Approximately 17.5	mL	Visits 3, 7, 10, 11***, 12***	
Blood	Approximately 40	mL	Visits 1, 2, 3, 5, 6, 8, 9, 10 ~~and 12~~	In addition for the CMI sub-cohort

Section 5.7.3 Laboratory assays

Molecular biology (PCR tests)

At the onset of an ILI episode, […]. ~~Each~~ ***N***asal and throat swab specimen***s*** will be tested by RT-PCR for influenza and/or other respiratory virus infections […]***, if deemed necessary, or will be stored for future research***.

Table 9 Humoral immunity

Component (Strain or Antigen Description)	Method	Kit/ Manufacturer	Unit	Cut-off~~*~~
ELISAs				
cH6/1 HA = […]	Anti-H1 HA stalk ELISA	In-house assay	ELISA units per mL (EU/mL)	~~TBD~~ ***66***
H2 HA full length = […]	Anti-H2 HA full length ELISA			~~TBD~~ ***22***
H18 HA full length = […]	Anti-H18 HA full length ELISA			~~TBD~~ ***43***
~~H9 HA full length = Recombinant antigen based on A/chicken/Hong Kong/G9/1997 HA~~	~~Anti-H9 HA full length ELISA~~			~~TBD~~
~~**N1 NA** = Recombinant antigen based on A/California/04/2009 (H1N1) NA~~	~~Anti-N1 NA ELISA~~			~~TBD~~
Microneutralization (MN) assays				
cH6/1N5 virus: […]	Anti-H1 HA stalk MN Assay	In-house assay	1/DIL (IC_{50})	~~TBD~~ ***20***
H1N1 swine influenza virus: A/Swine/Jiangsu/40/2011 (H1N1)	Anti-heterosubtypic HA Group 1 virus MN Assay			~~TBD~~ ***20***
IIV4 H1N1 strains[$]	Anti-heterosubtypic HA Group 1 virus MN Assay			~~TBD~~ ***20***
Hemagglutination Inhibition (HI) assay				
~~**IIV4 H1N1 strains**[$]~~	HI assay	In-house assay	1/DIL	~~TBD~~
Chimeric vaccine strains: cH5/1N1 ***and*** cH8/1N1 ~~and cH11/1N1~~				~~TBD~~ ***10***
~~Chimeric cH6/1N5 strain~~				~~TBD~~
~~H5N8 virus strain~~				~~TBD~~
~~H1N1 swine virus strains~~				~~TBD~~

[…]

~~*Cut-off value will be determined following set-up/qualification data.~~
**Refer to APPENDIX B for the laboratory addresses.
***GSK Biologicals laboratory refers to […]
~~$ IIV4 H1N1 strains will depend on the World Health Organization recommendation for the 2017/2018 season (Dose 1 at Day 1) and the 2018/2019 season (booster dose at Month 14).~~

Section 5.7.4.1 Immunological read-outs

Table 13 Immunological read-outs for humoral immunity and cell-mediated immunity

Blood sampling timepoint		Sub-cohort Name	No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Humoral immunity					
Visit 1 (Day 1) Visit 3 (Day 29) […]	PRE PId28 […]	All subjects	~470	[…]	[…]
				~~Anti-H9 HA full length ELISA~~	P P
Visit 1 (Day 1) Visit 3 (Day 29) Visit 6 (Day 85) ~~Visit 8 (Month 14)~~ ~~Visit 10 (Month 14 + 28 days)~~ ~~Visit 12 (Month 26)~~	PRE PId28 PIId28 ~~M14~~ ~~PIIId28~~ ~~M26~~	All subjects	~470	Anti-H1 HA stalk MN assay	P P
				~~Anti-heterosubtypic HA Group 1 virus MN assay (H5N8)~~	P P
				Anti-heterosubtypic HA Group 1 virus MN assay (H1N1 swine)	P P D
				Anti-heterosubtypic HA Group 1 virus MN assay (IIV4 H1N1 strains)	P P D
				~~Anti-N1 NA ELISA~~	P P
				HI with cH5/1N1 and cH8/1N1 virus	P P D
				~~HI with cH6/1N5, H5N8 and H1N1 swine virus strains~~	P P
~~Visit 1 (Day 1)~~ ~~Visit 3 (Day 29)~~ ~~Visit 6 (Day 85)~~ ~~Visit 8 (Month 14)~~	~~PRE~~ ~~PId28~~ ~~PIId28~~ ~~M14~~	~~All subjects~~	~~~470~~	~~HI with IIV4 H1N1 strain from 2017/2018 season~~	D P P D
~~Visit 8 (Month 14)~~ ~~Visit 10 (Month 14 + 28 days)~~ ~~Visit 12 (Month 26)~~	~~M14~~ ~~PIIId28~~ ~~M26~~	~~All subjects~~	~~~470~~	~~HI with IIV4 H1N1 strain from 2018/2019 season~~	P P
				~~HI with cH11/1N1 virus~~	P P D
Cell-mediated immunity					
Visit 1 (Day 1) Visit 3 (Day 29) Visit 6 (Day 85) ~~Visit 8 (Month 14)~~ ~~Visit 10 (Month 14 + 28 days)~~ ~~Visit 12 (Month 26)~~	PRE PId28 PIId28 ~~M14~~ ~~PIIId28~~ ~~M26~~	CMI sub-cohort*	~225	T-cell response by ICS assay	P P D
Visit 1 (Day 1) […] ~~Visit 8 (Month 14)~~ ~~Visit 9 (Month 14 + 7 days)~~ ~~Visit 10 (Month 14 + 28 days)~~ ~~Visit 12 (Month 26)~~	PRE […] ~~M14~~ ~~PIIId7~~ ~~PIIId28~~ ~~M26~~	CMI sub-cohort*	~225	B memory cells by ELISPOT	P P D
Visit 1 (Day 1) […] ~~Visit 8 (Month 14)~~ ~~Visit 9 (Month 14 + 7 days)~~	PRE […] ~~M14~~ ~~PIIId7~~	CMI sub-cohort*	~225	Plasmablast detection to HA by flow cytometry	P P D

Section 5.7.4.3 Molecular biology

Table 15 Read-outs for molecular biology tests

Sampling timepoint		Number of subjects	Component
Type of contact and timepoint	Sampling timepoint		
Assessment visit	Unscheduled	~~All nasal and throat swabs (~~***E***vent-driven~~)~~	Influenza A virus (Flu A) Influenza B virus (Flu B) […]

Section 10.2 Secondary endpoints (+ in synopsis)

Anti-H1 stalk immune response measured by ELISA and by MN assay ~~after each dose~~:

[…]

- Levels of anti-H1 stalk antibody titers by MN assay ~~post-each vaccination~~.

  The following aggregate variables will be calculated for the above parameters with 95% CI:

  – Seropositivity rates and GMTs at Days 1, 29 ***and*** 85~~, Month 14, Month 14 + 28 days and Month 26~~.

- […]
- Levels of antibody titers by MN assay for ~~H5N8,~~ H1N1 swine influenza and IIV4 H1N1 vaccine strains.

  The following aggregate variables will be calculated for the above parameters with 95% CI:

  – Seropositivity rates and GMTs at Days 1, 29 ***and*** 85~~, Month 14, Month 14 + 28 days and Month 26~~.

Section 10.3 Tertiary endpoints (+ in synopsis)

- Evaluation of CMI parameters in terms of frequencies of:

  – Antigen-specific CD4+/CD8+ T-cells […] at Days 1, 29 ***and*** 85~~, Month 14, Month 14 + 28 days and Month 26~~.

  – B-memory cells […] at Days 1, 8, 29, 64 ***and*** 85~~, Month 14, Month 14 + 7 days, Month 14 + 28 days and Month 26~~.

  – Plasmablasts […] at Days 1, 8 ***and*** 64~~, Month 14, Month 14 + 7 days~~.

- Levels of HI antibody to ~~IIV4 H1N1,~~ chimeric vaccine strains ***cH5/1N1 and cH8/1N1***, ~~chimeric cH6/1N5 strain, H5N8 virus strain and H1N1 swine virus strain~~:

  The following aggregate variables will be calculated with 95% CI:

  – Seropositivity rates and GMTs at Days 1, 29 ***and*** 85~~, Month 14, Month 14 + 28 days and Month 26~~.

  – […]

  - Seroconversion rate (SCR) at Days 29 ***and*** 85~~, Month 14, Month 14 + 28 days and Month 26~~.

- ~~Evaluation of the anti-Group 2 HA stalk response (e.g., H3, H4, H10, …) by ELISA and/or MN assay pre-and post-vaccination.~~
- ~~Levels of anti-N1 NA antibody by ELISA at Days 1, 29, 85, Month 14, Month 14 + 28 days and Month 26.~~
- ~~Occurrence of RT-PCR-confirmed influenza cases during the entire study period.~~
- Assessment of the *in vivo* protective effect of the anti-stalk antibodies when transferring Day 1 ***and*** Day 85~~, Month 14 and Month 26~~ pooled serum […]:
  - Lung virus titer in $TCID_{50}$/mg (log_{10} fold change [Day 1 minus Day 85~~, Month 14 and Month 26~~]), within challenge group.
- ~~Evaluation of the anti-H9 full length HA response by ELISA pre-and post-vaccination.~~

Section 10.8 Analysis of safety

[…]

- The percentage of subjects with episode(s) of ILI ~~(any, RT-PCR-confirmed)~~ will be summarized by group with exact 95% CI.

Section 10.9.1.2 CMI assessment

For each study group, ***at Days 1, 8, 29, 64 and 85*** ~~at each timepoint where a blood sample result is available from subjects in the CMI sub-cohort~~, the frequency of specific CD4+/CD8+ T-cells, B-memory cells and plasmablasts will be summarised using descriptive statistics.

Appendix D Serum passive transfer/virus challenge experiment in BALB/c mice from adult subjects involved in the FLU D-SUIV-ADJ-001 study cohort

Objectives

1. The *in vivo* protective effect of transferring pooled adult human serum from subjects from the FLU D-SUIV-ADJ-001 study cohort to mice […]:
   - 1C: Lung virus titer in $TCID_{50}$/mg on Day 3 and/or Day 6 post-challenge […].

     Comparisons between FLU D-SUIV-ADJ-001 study groups and/or timepoints (Day 1 ***and*** Day 85~~, Month 14 and Month 26~~) will be investigated.
2. The association between pre- or post-transfer human IgG binding antibody titers […]
   - 2A: Measure of the pre- and post-transfer geometric mean human IgG binding antibody specific to cH6/1Nx virus[…] (Day 1 ***and*** Day 85~~, Month 14 and Month 26~~).

Protocol endpoints:

Objective	Endpoint
[…]	[…]
1C	Lung virus titer in $TCID_{50}$/mg (log_{10} fold change (D1 - D85)~~, (D1 - M14) (D1 - M26)~~), within challenge group

Human serum pools:

Serum pools will be created using serum samples […] and the timepoints Day 1 ***and*** Day 85, ~~Month 14 and Month 26~~ from the FLU D-SUIV-ADJ-001 cohort.