Statistical Analysis Plan

Study alias & e-track number(s): FLU Q-PAN H5N1=AS03-023 (116938)

Detailed Title:	A phase II observer-blind, multicentre, dose-ranging study of children 6 to less than 36 months of age who are to be primed with a 2-dose series of GSK Biologicals' AS03-adjuvanted A/Indonesia/05/2005 (H5N1) vaccine
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Scope:	All data pertaining to the above study.
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The complete statistical analysis plan (**SAP**) and results presentation is divided into 2 parts: the first part detailing the analyses to be performed (known as SAP, current document) and a second part, annex (-es) (called **TFL**) describing the flow and format of tables, figures and listings to be annexed to the Study Report (SR).

LIST OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse Event of Special Interest
ANCOVA	Analysis of Covariance
AS03	AS03 is an Adjuvant System containing α-tocopherol and squalene in an oil and water emulsion
ATP	According-To-Protocol
CD40L	Cluster Differentiation-40 Ligand
CI	Confidence Interval
CTR	Clinical Trial Register
D_{GMT}	Desirability (Immunogenicity index)
D_R	Desirability (Fever index)
DOB	Date Of Birth
EL.U/ml	ELISA unit per milliliter
Eli Type	Internal GSK database code for type of elimination code
GMT	Geometric mean antibody titer
GSK	GlaxoSmithKline
HA	Hemagglutinin
HI	Hemagglutination Inhibition
ICS	Intracellular Cytokine Staining
IU/ml	International units per milliliter
iSRC	Internal Safety Review Committee
IFNγ	Interferon γ
IL-2	Interleukin-2
LL	Lower Limit of the confidence interval
MAEs	Medically Attended Adverse Events

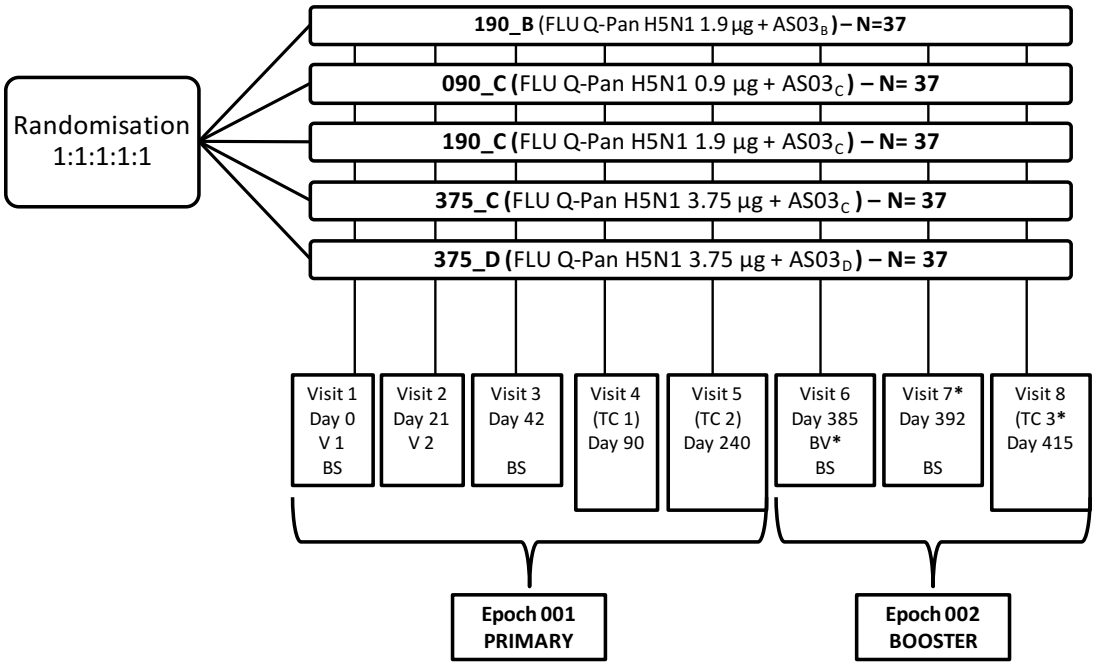
MedDRA	Medical Dictionary for Regulatory Activities
MGI	Mean Geometric Increase
MN	Microneutralization
N.A.	Not Applicable
pIMDs	Potential Immune-Mediated Diseases
PT	Preferred Term
R&D	Research and Development
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SCR	Seroconversion Rate
SD	Standard Deviation
SR	Study Report
SPR	Seroprotection Rate
SMQs	Standardized MedDRA Queries
TFL	Tables Figures and Listing template annexed to SAP
TFN-α	Tumor necrosis factor-α
VRR	Vaccine Response Rate
TVC	Total vaccinated cohort
UL	Upper Limit of the confidence interval

1. DOCUMENT HISTORY

Date	Description	Protocol Version
12-JAN-2017	Version 1	Amendment 1 Final: 20 January 2016

2. STUDY DESIGN

2.1. Design Overview



D=Day; TC = telephone contact, V = Primary Vaccination, *BV = *Unadjuvanted* booster vaccination *(3.75 µg HA)*;
BS = Blood sample,
FLU Q-Pan H5N1 = A/Indonesia/05/2005 hemagglutinin antigen

Experimental design: Phase II, observer blind, randomized, multi-center, multi-country study, parallel groups.

Duration of the study: approximately 415 days after vaccination on Day 0.

- The Primary Epoch encompasses data collected from Visit 1 (Day 0) through Visit 5-TC 2 (Day240) and ending at the start of Visit 6 (Day 385).
- The Booster Epoch encompasses data collected from Visit 6 (Day 385) through Visit 8-TC 3 (Day 415).

Sampling schedule:

- All study groups: Day 0 (before vaccination); Day 42 (post primary course); Day 385 (persistence) and Day 392 (booster response).

Vaccination schedule:

- All subjects are to receive an AS03 adjuvanted H5N1 vaccine given as a two-dose primary series at a 21 day interval.
- All subjects are to receive a 3.75 µg HA, unadjuvanted H5N1 vaccine as a booster dose at Day 385.

2.2. Study groups

Approximately 37 subjects are planned to be enrolled in each of the 5 following study groups:

Study groups and epochs foreseen in the study

Study groups	Number of subjects	Epochs	
		Epoch 001 (Primary Series)	Epoch 002 (Booster)
190_B	37	●	●
090_C	37	●	●
190_C	37	●	●
375_C	37	●	●
375_D	37	●	●

Study groups and treatments foreseen in the study

Treatment name	Vaccine/Product name (Formulation)	Study Groups				
		190_B	090_C	190_C	375_C	375_D
1.9 mcg H5N1 HA + AS03B	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)	●				
	AS03A (AS03 47.44 mg/mL , final vaccine dose contains 5.93 mg tocopherol)	●				
0.9 mcg H5N1 HA + AS03C	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)		●			
	AS03A (AS03 47.44 mg/mL, final vaccine dose contains 2.97 mg tocopherol)		●			
1.9 mcg H5N1 HA + AS03C	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)			●		
	AS03B (AS03 23.72 mg/mL; final vaccine dose contains 2.97 mg tocopherol			●		
3.75 mcg H5N1 HA + AS03C	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 30 µg/mL)				●	
	AS03B (AS03 23.72 mg/mL; final vaccine dose contains 2.97 mg tocopherol				●	
3.75 mcg H5N1 HA + AS03D	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 30 µg/mL)					●
	AS03C (AS03 11.86 mg/mL; final vaccine dose contains 1.48 mg tocopherol)					●
3.75 mcg H5N1 HA *	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)	●	●	●	●	●

*Booster vaccination to subjects in all study groups

2.3. Group description

The following group names will be used for the statistical analyses at Day 42 and Day 415:

Group order in tables	Group label in tables	Group definition for footnote
1	190_B	1.9 µg H5N1 HA antigen adjuvanted with $AS03_B$
2	090_C	0.9 µg H5N1 HA antigen adjuvanted with $AS03_C$
3	190_C	1.9 µg H5N1 HA antigen adjuvanted with $AS03_C$
4	375_C	3.75 µg H5N1 HA antigen adjuvanted with $AS03_C$
5	375_D	3.75 µg H5N1 HA antigen adjuvanted with $AS03_D$

3. OBJECTIVES

3.1. Co-Primary Objectives

3.1.1. Primary Doses

- To assess the performance of alternative dosing regimens for primary immunization with Q-Pan H5N1 vaccine using an immunogenicity-fever index that considers:
  - immunogenicity by HI assay against vaccine-homologous virus 21 days after the second priming dose, and
  - fever scores after the first and second priming doses.
- To assess the performance of alternative dosing regimens for primary immunization with Q-Pan H5N1 vaccine using an immunogenicity-fever index that considers:
  - immunogenicity by MN assay against vaccine-homologous virus 21 days after the second priming dose and
  - fever scores after the first and second priming doses.

The reference dose for each of these assessments will be 1.9 µg HA with $AS03_B$ (half the approved adult dose).

3.1.2. Booster Dose

- To assess the performance of dosing regimens for booster immunization with Q-Pan H5N1 vaccine considering:
  - immune response by HI assay against vaccine-homologous virus 7 days after a 12-month booster dose of 3.75 µg HA Q-Pan H5N1 unadjuvanted antigen
- To assess the performance of dosing regimens for booster immunization with Q-Pan H5N1 vaccine considering:
  - immune response by MN assay against vaccine-homologous virus 7 days after a 12-month booster dose of 3.75 µg HA Q-Pan H5N1 unadjuvanted antigen

3.2. Secondary objectives

- To describe the HI immune response to the vaccine-homologous virus 21 days after the second dose for each dosing regimen
- To assess the performance of alternative dosing regimens for primary immunization with Q-Pan H5N1 vaccine considering persistence of immune response by HI and MN assay at Day 385 in terms of persistence index.

- To assess the performance of the H5N1 vaccine regimens in terms of vaccine-homologous HI and MN antibody titers on Days 0, 42, 385 and Day 392.
- To assess the immunogenicity of the H5N1 vaccine regimens in terms of vaccine-heterologous HI antibody titers on Days 0, 42, 385 and Day 392.
- To assess vaccine induced cell-mediated immune responses on Days 0, 42, 385 and Day 392.
- To describe reactogenicity and safety of the different priming regimens in terms of solicited (7-days after each vaccination) and unsolicited (21 days after each vaccination) adverse events (AEs).
- To describe safety of the unadjuvanted booster dose in terms of solicited (7 days post boost) and unsolicited (30 days post boost) AEs.
- To describe safety in terms of medically attended AEs (MAEs), potential immune-mediated diseases (pIMDs), and serious adverse events (SAEs), adverse events of special interest (AESIs), during the entire study period.

4. ENDPOINTS

4.1. Primary Endpoints

4.1.1. Immunogenicity-fever indices

Separate HI and MN immunogenicity-fever indices will be constructed based on the following endpoints:

- Humoral immune response in terms of vaccine-homologous HI antibody for each group:
  - LL (lower limit) of 95% CI of GMT group ratio at Day 42 using 1.9 µg HA with $AS03_B$ as reference.
- Humoral immune response in terms of vaccine-homologous MN antibodies for each group:
  - LL of 95% CI GMT group ratio at Day 42 using 1.9 µg HA with $AS03_B$ as reference.
- Fever measurement (≥38°C) post dose 1 and dose 2:
  - For each subject, a fever index will be calculated using temperature measurements 3-days post Dose 1 (D0-D2) and 3-days post Dose 2 (D21-D23).

For details regarding construction of these indices, refer to Section 6.1.2.1 (Statistical Methods- Analysis of immunogenicity).

4.1.2. Immune response to a booster dose

Following booster dose administered at Day 385 the following will be evaluated:

- For immune response in terms of HI antibodies against vaccine-homologous antigen
  - Mean Geometric Increase (MGI) at Day 392 relative to Day 385.
- For the immune response in terms of MN antibodies against vaccine-homologous antigen
  - MGI at Day 392 relative to Day 385.

4.2. Secondary Endpoints

Immunogenicity

- For humoral immune response in terms of HI antibodies against vaccine-homologous/heterologous antigens post-primary immunization, the following aggregate variables will be calculated for each group:
  - Seroconversion rates (SCR) at Day 42
  - Seroprotection rates (SPR) at Day 42
  - MGI at Days 42 relative to Day 0.
- For humoral immune response in terms of vaccine-homologous MN antibody post the primary immunization, following aggregate variables will be calculated for each group:
  - MGI at Day 385 relative to Day 0.
- For humoral immune response in terms of HI antibodies against vaccine-homologous/heterologous antigens (at Days 0, 42 and 385 post the primary immunization, at Day 392 (7 days post booster dose)), the following aggregate variables will be calculated for each group:
  - Seropositivity rates at Days 0, 42, 385 and Day 392
  - Seroconversion rates (SCR) at Day 385 (relative to Day 0) and Day 392 (relative to Days 0 and 385)
  - Seroprotection rates (SPR) at Days 0, 385 and Day 392
  - Geometric Mean Titer (GMT) at Days 0, 42, 385 and Day 392
  - MGI at Day 385 (relative to Day 0), MGI at Day 392 (relative to Days 0 and 385).

- For humoral immune response in terms of vaccine-homologous MN antibody, the following aggregate variables will be calculated for each group:
  - Seropositivity rates at Days 0, 42, 385 and Day 392
  - GMT at Days 0, 42, 385 and Day 392
  - Vaccine response rate (VRR) at Days 42, 385 (relative to Day 0) and Day 392 (relative to Days 0 and 385).
- For CMI in terms of frequencies of antigen-specific cells (CD4+/CD8+) at Days 0, 42, 385 and 392:
  - Frequencies of cytokine CD4+/CD8+ T- cells per million CD4+/CD8+ cells producing two or more markers within CD40L, IL-2, TNF-α, IFN-γ upon in vitro stimulation using A/Indonesia/05/2005 (H5N1) split virus as determined by intracellular cytokine staining (ICS) in a sub-cohort of approximately 20 subjects per group at Days 0, 42, 385 and Day 392.

Reactogenicity /Safety:

- Solicited local and general AEs
  - Occurrence of each solicited local AEs during a 7-day follow-up period (i.e., day of vaccination and 6 subsequent days) after any vaccination.
    - Percentage, intensity and duration of solicited local AEs during a 7-day follow-up period (Day 0-Day 6) after any vaccination.
  - Occurrence of each solicited general AEs during a 7-day follow-up period (i.e., day of vaccination and 6 subsequent days) after any vaccination.
    - Percentage, intensity, duration and relationship to vaccination of solicited general AEs during a 7-day follow-up period (Day 0-Day 6) after any vaccination.
- Unsolicited adverse events (AEs)
  - For the primary series: occurrence and relationship to vaccination of unsolicited AEs within 21 days after each vaccine dose.
    - Percentage, intensity and relationship to vaccination of unsolicited AEs during a 21-day follow-up period (Day 0-Day 20) after each vaccine dose.
  - For the booster dose [unadjuvanted]: occurrence and relationship to vaccination of unsolicited AEs within 30 days after vaccination
    - Percentage, intensity and relationship to vaccination of unsolicited AEs during a 30-day follow-up period.
  - Occurrence and relationship to vaccination of (MAEs) during the entire study period.

- Percentage and relationship to vaccination of MAEs during the entire study period.

– Occurrence and relationship to vaccination of pIMDs, SAEs, AESIs during the entire study period.

- Percentage and relationship to vaccination of pIMDs, SAEs, AESIs during the entire study period.

5. STUDY POPULATION

5.1.1. Total Vaccinated Cohort

The total vaccinated cohort (TVC) will include all subjects who received at least one dose of vaccine (TVC for booster safety analysis will include all the subjects who received the booster dose):

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

The TVC analyses will be performed per investigational vaccine actually administered at the first dose.

The analysis of safety will be performed on the TVC.

5.1.2. Cohort for according-to-protocol (ATP) immunogenicity analyses

The ATP cohort for immunogenicity analyses will include all vaccinated/eligible subjects:

- who have received all study vaccine dose(s) (3-doses for booster immunogenicity analysis, 2-doses for other analyses) per protocol treatment assignment;
- for whom the randomization code is unbroken during the relevant analysis interval;
- who have not received any investigational or non-registered product (drug or vaccine) other than the study vaccine(s) during the relevant analysis interval;
- who have not received any non-study vaccine during the relevant analysis interval who have not received any immunoglobulins and/or any blood products during the relevant analysis interval
- for whom there was no chronic administration of immunosuppressants) during the relevant analysis interval.
- who develop a physician-confirmed infection with an A/Indonesia/5/2005 (H5N1)-like influenza virus during the relevant analysis interval.

- who have results available for the relevant assay (HI and MN) for all blood samples to be collected during the relevant analysis intervals for ATP-Day 42; for ATP-Day 385 (persistence); for ATP-Day 392 post booster dose (ATP-booster).

For the final analysis, an Adapted ATP- cohort for immunogenicity will be used. The Adapted ATP cohort for immunogenicity analysis will include all vaccinated/eligible subjects who have results available for the relevant assay (HI and MN) for all blood samples to be collected during the relevant analysis intervals for ATP-Day 42; for ATP-Day 385 (persistence); for ATP-Day 392 post booster dose (ATP-booster).

5.2. Cohort for analysis of the immunogenicity-fever score

The analysis cohort for combined immunogenicity and safety of the two-dose primary series will include all subjects in the ATP cohort for immunogenicity analysis at Day 42 for whom temperature measurements are available during the first 3 days after both vaccine doses 1 and 2.

Subjects without immunogenicity results at Day 42 or for whom one or more daily temperature measurements are missing during the first 3 days after either vaccine dose [dose 1 or dose 2] will be excluded from the immunogenicity/fever index calculation for the primary series.

The list of applicable elimination codes for each cohort can be found in the study specific form FORM-9000036402-13 Criteria for eliminating subjects from the analyses and filled in CARS: Clinical R&D/FLU Q-PAN/Studies/H5N1-AS03-023 (116938)/11 Statistics/11.01 Statistics Oversight/11.01.01 Statistical Analysis Plan.

Cohort	Elimination codes	Eli Type
ATP cohort for Day 42 analysis of the immunogenicity	1030.1040,1050,1060,1070,1080, 1090, 2010, 2040, 2050, 2060, 2070, 2080, 2090, 2100, 2500.	PR
ATP Day 392 post-booster dose.	1030.1040,1050,1060,1070,1080, 1090, 2010, 2040, 2050, 2060, 2070, 2080, 2090, 2100, 2500.	MA (including PR)

6. STATISTICAL METHODS

6.1.1. Analysis of demographics

The analysis of demography will be performed on the TVC and on the ATP for immunogenicity.

Demographic characteristics (age at first study vaccination in months; gender; ethnicity) of all subjects will be tabulated by study group and overall using descriptive statistics:

- Frequency tables will be generated for categorical variables such as ethnicity.
- Mean, range, median, and standard deviation will be provided for continuous data such as age.

The distribution of subjects enrolled among the study sites (center) will be tabulated as a whole and per group.

In addition, the following table will be performed for Clinical Trial Register (CTR) posting:

– Percentage of Enrolled subjects by country will be tabulated by group and pooled vaccine groups.

6.1.2. Analysis of immunogenicity

The primary analysis will be based on the ATP cohort for analysis of immunogenicity. If, in any study group, the percentage of vaccinated subjects with serological results excluded from the ATP cohort for analysis of immunogenicity is 5% or more, a second analysis based on the TVC will be performed to complement the ATP analysis.

6.1.2.1. Immunogenicity/fever indices (Co-primary objectives)

An analysis of covariance model (ANCOVA) will be fitted on the $\log_{10}$ transformed HI and MN antibody responses at Day 42, with the vaccine group as a fixed independent variable, adjusted by the $\log_{10}$ transformed pre-vaccination titer and age.

For HI and MN separately, an immunogenicity index (D_{GMT}) will be constructed using a desirability function based on the computed GMT group ratio (alternative dose regimen to reference =1.9 µg HA with $AS03_B$) and the 95% CI.

D_{GMT} will be the LL of the 95% CI for GMT group ratio

- If the LL of the 95% CI for GMT group ratio is less than 0.25 (i.e., 4- fold less than that of the reference group), then D_{GMT} =0.
- If the LL of the 95% CI for GMT group ratio is greater than 1 (comparison group has higher GMT value than the reference group), D_{GMT} =1.

note D_{GMT} =1 for the reference group

The fever index (D_R) will be calculated according to body temperature measurements performed from Days 0-2 after each dose

- Any temperature < 38°C (100.4 F) will be assigned a value of 0. Any temperature > 40.5°C will be assigned a value of 40.5).
- The highest possible temperature value per subject is 243 (6 x 40.5°C; i.e. for 3 days after dose 1 and dose 2);
- The lowest possible temperature value per subject is 0 (all measurements < 38.0°C (100.4 F) for 3 days after dose 1 and dose 2).
- For each subject, a temperature index will be constructed as follows: (243 minus the sum of recorded temperature values for 3 days after dose 1 and dose 2)/243. The average temperature measurement for each vaccine group will be calculated as the D_R. A lower index value D_R indicates a less desirable regimen in terms of reactogenicity.

An immunogenicity-fever index (D) at Day 42 will be computed for each group

$$D = \sqrt{D_{GMT} \times D_R}$$

This index (D) will range between 0 and 1 (0 = not desirable; 1 = highly desirable) and the same weight (0.5) is assigned for immunogenicity index and reactogenicity (fever) index.

Two immunogenicity-fever indices will be separately calculated for HI and MN. Each will be used to rank the different dosing regimens as a tool to guide dosing regimen selection.

6.1.2.2. Immune Response to a booster dose

There will be two separate evaluations, performed on evaluable subjects (ATP cohort-booster) following a booster dose:

- Point estimates and 95% CIs for MGIs relative to Day 385 will be computed for vaccine-homologous antibody titers assessed by HI at Day 392 for each vaccine regimen
- Point estimates and 95% CIs for MGIs relative to Day 385 will be computed for vaccine-homologous antibody titers assessed by MN at Day 392 for each vaccine regimen

6.1.2.3. Analysis for immunogenicity (secondary objective)

- Point estimates and 95% CIs for MGIs relative to Day 0 will be computed for vaccine-homologous antibody titers assessed by MN at Day 385 for each vaccine regimen.

- Using vaccine-homologous and vaccine-heterologous antibody titers assessed by HI, the point estimates for SCR, SPR, MGI, and the associated 95% CIs will be computed at 21 days after the second dose.
- Using vaccine-homologous and vaccine-heterologous antibody titers assessed by HI, the point estimates and 95% CIs for GMTs and seropositivity rates (at all timepoints), and SPRs, SCRs and MGIs at Day 385 will be computed for each vaccine regimen.
- Point estimates and 95% CIs for seropositivity, GMTs and VRRs for vaccine-homologous MN titers will be computed for all appropriate timepoints.

All comparisons will be descriptive.

The ATP immunogenicity cohorts (Day 42, persistence or booster) will be used for the related analysis of immunogenicity.

The reverse cumulative distribution curves (RCCs) for HI antibodies against A/Indonesia/05/2005 (H5N1)-like influenza virus at Days 0, 42, 385 and 392 will be performed.

6.1.2.4. Analysis for CMI

- Cell Mediated Immunity (CMI) parameters at Day 0, 42 , 385 and 392 will be evaluated in TVC:
  - Antigen-specific CD4+/CD8+ T Cells identified as CD4/CD8+ T- cells producing two or more markers within CD40L, IL-2, TNF-α, IFN-γ upon *in vitro* stimulation using A/Indonesia/05/2005 (H5N1) split virus,
  - The frequency of the response for CD4+/CD8+T-cells stained with probes for various cytokines and activation marker (IFN-γ, TNF-α, IL-2, CD40L) and elicited by vaccine components measured in a sub-cohort of approximately 20 subjects per group at Days 0, 42, 385 and 392 will be described according to the technical specifications provided by R&D (Clinical Data – Information Sheet).

6.1.3. Analysis for safety

- Safety data will be analyzed based on the TVC.
- Analysis will be of subject incidence rates of solicited and unsolicited adverse (AEs) events, by solicited local and general AEs terms, and, for unsolicited AEs, by MedDRA preferred term and system organ class. Safety data will be summarized for all subjects by treatment group. The incidence of solicited local and general AEs occurring during 7 days after vaccination will be tabulated with exact 95% CI for each treatment group. The same calculations will be performed for AEs of any intensity, those with intensity Grade ≥ 2, and those with intensity of Grade 3, as well

as for solicited general events assessed as related to vaccination. All solicited local AEs are considered to be related to vaccination.

- The percentage of subjects with at least one report of an unsolicited AE classified by MedDRA (System Organ Class and Preferred Term) 21 days after each primary vaccination will be tabulated with exact 95% CI for each treatment group. The same tabulation will be performed for Grade 3 unsolicited AEs and for unsolicited AEs that are considered by the investigator to be possibly or probably related to vaccination.
- The percentage of subjects with at least one report of an unsolicited AE classified by MedDRA (System Organ Class and Preferred Term) 30 days after the booster vaccination will be tabulated with exact 95% CI. The same tabulation will be performed for Grade 3 unsolicited AEs and for unsolicited AEs that are considered by the investigator to be possibly or probably related to vaccination.
- The proportion of subjects who begin at least one new concomitant medication during the first 42 days after primary vaccination will be calculated with 95% CI.
- MAEs, AESIs, SAEs, and pIMDs will be summarized through the entire study period. In addition, serious AEs and withdrawals due to AE(s) will be described in detail.

The pIMD listings will include pIMDs reported by the Investigator and those identified from the clinical database search using a list of pre-specified pIMD PTs.

The AESIs listings will include AESIs identified from the clinical database search using a list of pre-specified AESIs PTs and SMQs. The AESIs will include the events listed below and the MedDRA PT or SMQ used to identify reports of these events is provided in parentheses:

- Anaphylaxis (narrow SMQs “Anaphylactic reaction” and “Angioedema”)
- Bell’s palsy (MedDRA PT “VIIth nerve palsy”)
- Convulsion (Narrow SMQ “Convulsions”)
- Demyelination (narrow SMQ “Demyelination”)
- Encephalitis (narrow SMQ “Non-infectious encephalitis”)
- Guillain-Barré syndrome (narrow SMQ “Guillain-Barré syndrome”)
- Neuritis (MedDRA PT “Neuritis”)
- Vasculitis (narrow SMQ “Vasculitis”)

7. STATISTICAL CALCULATIONS

7.1. Derived and transformed data

7.1.1. Demography

For a given subject and a given demographic variable, missing measurement will *not be replaced* except for incomplete date of birth (DOB) data.

7.1.1.1. Age at vaccination

Age will be calculated as the number of months between the DOB and the date of vaccination.

In some countries, to ensure that the collection of DOB will not jeopardise the privacy of personally identifiable information, only a partial DOB (MMYYYY) will be collected.

Therefore, the 15th of the month will be used to replace the missing date.

In case the day and the months are missing, the date will be replaced by the June 30th of the year.

7.1.2. Immunogenicity

The cut-off value for antibody titer is defined by the laboratory before the analysis. **A seronegative** subject is a subject whose antibody titer is below the cut-off value, and conversely; **a seropositive** subject is one whose antibody titer is greater than or equal to the cut-off value. For this study:

- It is assumed that **HI titers** of < 1:10 will be considered below the cut-off. For the calculations of GMT, HI titers of < 10 will be considered to take the value 5.
- It is also assumed that **MN titers** of < 1:28 will be considered below the cut-off. For the calculations of GMT, MN titers of < 28 will be considered to take the value 14.

Geometric Mean Titer (GMT) calculations are performed by taking the anti-log of the mean of the log (base 10) transformed inverse titers (the number X would denote the inverse of a titer expressed as "1: X"). Antibody titers below the cut-off of the assay are given an arbitrary value of half the cut-off for the purpose of GMT calculation.

Mean Geometric Increase (MGI) is defined as the geometric mean of the within-subject ratios of the post-vaccination reciprocal HI titer to the pre-vaccination HI titer.

Seroconversion Rate (SCR) is defined as the incidence rate of vaccines who have either a pre-vaccination titer recorded as <1:10 for HI and a post-vaccination reciprocal titer ≥ 40 or a pre-vaccination reciprocal titer ≥10 and at least a 4-fold increase in post-vaccination reciprocal titer.

Incidence Rate of HI Reciprocal Titers ≥ 40 (SPR), defined as the percentage of all vaccinees with a serum reciprocal HI antibody titer ≥ 40 post-vaccination, a level of HI antibodies that may correlate with benefit in protection against influenza.

Vaccine Response Rate (VRR) by MN is defined as the post-vaccination reciprocal titer of vaccines that have at least 4-fold increase compared with their pre-vaccination reciprocal titer. Antibody titers below the cut-off of the assay are given an arbitrary value of half the cut-off for the purpose of VRR calculation.

Handling of missing immunogenicity data: for a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, analyses will exclude subjects with missing or non-evaluable measurements.

7.1.3. Reactogenicity and safety

Incidence rates of AEs will be calculated as the number of subjects who experience the event, divided by the number of subjects in the safety analysis cohort (the TVC).

Handling of missing safety data:

- **Solicited AEs**: For a given subject and the analysis of solicited AEs within 7 days post-vaccination (Days 0 through 6), missing or non-evaluable measurements will not be replaced.
    - The analysis of the solicited AEs will include only vaccinated subjects and doses with documented safety data (i.e., symptom screen/sheet completed, including the response 'Yes' or 'No' to the solicited symptom page).
    - Subjects who answered "Yes" in the solicited symptom page, but had all symptoms missing are considered as having no solicited symptoms.
    - Subjects who answered 'Yes' to the presence of a specific symptom but partially recorded the daily measurement (e.g. intensity missing for Day 3) over the considered solicited period will be included in the summaries and classified according to their maximum observed daily recording over the solicited period.
    - Subjects who answered 'Yes' to the presence of a specific symptom but recorded no daily measurement over the considered solicited period will not be counted in the summary of symptoms by grade/category but will be part of the summary corresponding to the 'All' category for that symptom.

- **Unsolicited AEs**: For the analysis of unsolicited AEs/MAEs/SAEs/pIMDs/AESIs, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.
- **Concomitant medication and vaccination**: For the analysis of concomitant medications and vaccinations, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.

7.1.3.1. Counting rule

Event	N used for deriving % per subject for vaccination phase
Solicited general symptom	All subjects with at least one solicited general symptom documented as either present or absent (i.e. symptom screen completed)
Solicited local symptom	All subjects with at least one solicited local symptom documented as either present or absent (i.e. symptom screen completed)
Unsolicited symptom MAEs/SAEs/pIMDs/AESIs	All subjects with study vaccine administered
Concomitant medication	All subjects with study vaccine administered
Concomitant vaccination	All subjects with study vaccine administered

7.1.3.2. Coding of grading for Solicited symptoms

The maximum intensity of local injection site redness or swelling will be scored at GSK Biologicals as follows:

0 : ≤ 20 mm
1 : > 20 to 50 mm
2 : > 50 to 100 mm
3 : > 100 mm

Body temperatures will be scored at GSK Biologicals as follows:

0 < 38.0°C (< 100.4°F)
1 ≥ 38.0 – 38.4°C (≥100.4 – 101.2°F)
2 ≥ 38.5 – 38.9°C (≥ 101.3 – 102.1°F)
3 ≥ 39.0 - 40°C (≥ 102.2 – 104.0°F)
4 >40.0°C (>104.0°F)

Fever is defined as temperature ≥ 38.0°C/100.4°F. The preferred route for recording temperature in this study is axillary.

For clinicaltrials.gov and EudraCT posting purposes, a summary of subjects with all combined solicited (regardless of their duration) and unsolicited adverse events will be provided. Solicited adverse events will be coded by MedDRA as per the following codes:

Solicited symptom	Lower level code	Lower level term
Pain at injection site	10022086	Injection site pain
Redness at injection site	10022098	Injection site redness
Swelling at injection site	10053425	Injection site swelling
Fever	10016558	Fever
Irritability/Fussiness	10057224	Irritability postvaccinal
Loss of appetite	10003028	Appetite lost
Drowsiness	10013649	Drowsiness

7.1.3.3. Onset day

The onset day for an event will be the number of days between the most recent vaccination and the start of the event. Events occurring on the day of vaccination will have an onset day of 0.

7.1.3.4. Duration of events

The duration of an event will be the number of days (not necessarily consecutive) with symptom during the solicited follow-up period.

7.2. Methodology for computing CI

All CI will be 2 sided 95% CI.

- The exact 95% CIs for a proportion within a group will be calculated from Proc StatXact [Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. *Biometrika.* 1934; 26:404-413].
- The 95% CIs for GMT are obtained within each group separately. The 95% CI for the mean of lot-transformed titer is first obtained assuming that log-transformed titers 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 titer.
- The group GMT ratio will be obtained using an ANCOVA model on the logarithm-transformed titers. The ANCOVA model will include the vaccine group as fixed effect and the pre-vaccination $\log_{10}$ titer and, age as regressor. The GMT ratio and its 95% CI will be derived as exponential-transformation of the corresponding group contrast in the model.

7.3. Data presentation description:

The following decimal description from the decision rules will be used for the demography, immunogenicity and safety/reactogenicity.

Display Table	Parameters	Number of decimal digits
Demographic characteristics	Mean, median age	1
Demographic characteristics	SD (age)	1
Immunogenicity	Ratio of GMT	2
Reactogenicity	Mean, Min, Q1, Median, Q3, Max for duration	1
All summaries	% of count, including LL & UL of CI	1
All summaries	% of ratio, including LL & UL of CI	1

7.4. Interpretation of analyses

In this study, all the comparative analyses will be descriptive with the aim to rank alternative dosing regimens with respect to immunogenicity and reactogenicity at Day 42, persistence at Day 385, and anamnestic response to plain antigen booster dose at Day 392. In order to evaluate all the effects at the same scale (values between 0-1), a persistence parameter and a booster effect parameter will be calculated for HI and MN:

- Persistence index = (MGI – 4)/MGI for each group.
- Boostability index= (MGI – 4)/MGI for each group.

If the MGI value is less than 4, the index will be set to 0, the immunogenicity- fever, persistence and boostability indices will be ranked for each vaccine group.

Given the complexity of possible outcomes, an algorithm for dose selection will **not** be proposed.

Dose selection will be based on Day 42 immunogenicity and fever as key indicator of reactogenicity, persistence of the immune response and anamnestic response to unadjuvanted antigen as well as any additional safety concerns. Additional practical matters related to the estimated incremental benefit versus the complexity of a pediatric specific dosing regimen, should it not be a fraction of the adult dose, will also be weighed for dose selection as this could lead to increased complexity from a manufacturing and delivery perspective in a pandemic response setting.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

8.1.1. ISRC analyses

iSRC (independent Safety Review Committee) analyses based on TVC are currently performed for the purposes of safety data review. These analyses were described in detail in a separate SAP/TFL associated to iSRC Charter.

8.1.2. Analysis at Day 42

An analysis will be performed on data collected through the Day 42 visit. Elements will include:

- An analysis of cleaned immunogenicity and solicited AEs data collected through the Day 42 visit will be conducted.
- Analyses of unsolicited AEs reported up to the Day 42 visit and cleaned in so far as is possible will be carried out.
- Analyses of MAEs, AESIs, SAEs, pIMDs and withdrawals due to AEs collected up to the Day 42 visit will be carried out.
- Results will be presented in a Day 42 statistical report. Access to individual treatment codes will be restricted to the designated statisticians in charge of the analysis. No individual listings or data with the subjects' identifying information will be disseminated. Listings of final data will be provided with the Day 415 report.

8.1.3. Final analysis

A final data analysis will be performed at the end of study (Day 415) of all primary and secondary endpoints based on the clean data, including evaluations of:

- demography and baseline characteristics
- immunogenicity
- solicited AE data (Day 0-6) after each vaccination
- unsolicited AEs reported up to the Day 42 visit (21 days after each dose), as well as 30 days after the Day 385 booster dose
- concomitant medications reported up to the Day 42 visit, as well as 30 days after the Day 385 booster dose

- MAEs, AESIs, SAEs, pIMDs and withdrawals due to AEs collected throughout the entire study.

Results of the final analysis, as well as individual data listings, will be presented in a final, integrated clinical study report (CSR).

8.2. SDD Stored analysis

Description	Analysis ID (SDD sub-folder)	Disclosure Purpose	*Study Headline Summary (SHS)* requiring expedited communication to Upper Management (Yes/No)	Reference for TFL
iSRC 1 to iSRC 11 iSRC 12 to iSRC 17	E1_02 to E1_12 E1_13 to E1_18	Internal	No	SAP/TFL associated to the Charter
Day 42 Analysis	E1_13	CTR	Yes	TFL for Day 42 and Final Analysis
Final Analysis	E1_01	Study Report (SR) CTR	Yes	TFL for Day 42 and Final Analysis

8.3. Statistical considerations for interim analyses

Not applicable

9. CHANGES FROM PLANNED ANALYSES

– The reverse cumulative distribution curves (RCCs) for HI antibodies against A/Indonesia/05/2005 (H5N1) –like influenza virus at Days 0, 42, 385 and 392 will be performed.
– For clintrial.gov and EudraCT posting purposes, a summary of subjects with all combined solicited (regardless of their duration) and unsolicited AEs will be provided.

10. REFERENCES

The exact 95% CIs for a proportion within a group will be calculated from Proc StatXact [Clopper, 1934*].

Proc StatXact will be used to derive the standardised asymptotic 95% CI for the group difference in proportions [Robert, 1998, method six]. The standardised asymptotic method used within GSK Biologicals is the method six.

Damaso S, Dewé, W, and al. *Selection of a vaccine Formulation in Clinical Development: Study designs and Statistical analysis*, GlaxoSmithKline, **Point to Consider (PtC),** August 2014.

Walthere Dewé, Christelle Durand, Sandie Marion, Lidia Oostvogels, Jeanne-Marie, Devaster, Marc Fourneau: *A multi-criteria decision making approach to identify a vaccine formulation,* Journal of Biopharmaceutical Statistics**,** 2015.

* Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. Biometrika, 1934; 26:404-413;

Statistical Analysis Plan

Study alias & e-track number(s): FLU Q-PAN H5N1=AS03-023 (116938)

Detailed Title:	A phase II observer-blind, multicentre, dose-ranging study of children 6 to less than 36 months of age who are to be primed with a 2-dose series of GSK Biologicals' AS03-adjuvanted A/Indonesia/05/2005 (H5N1) vaccine
SAP version	Amendment 1
SAP date	13-APR-2017
Scope:	All data pertaining to the above study.
Co-ordinating author:	PPD (Project Statistician)
Other author(s):	
Adhoc reviewers:	PPD (CRDL) PPD (CEPL) PPD (Lead Statistician) PPD (Scientific Writer) PPD (Safety Physician) PPD (Regulatory) PPD (Lead Stat Analyst) PPD (Stat Analyst) CTR Team (Vaccine Web Disclosure)
Approved by:	PPD KIM (CRDL) PPD (CEPL) PPD (Lead Statistician) PPD (Project Statistician) PPD (Scientific Writer)

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The complete statistical analysis plan (**SAP**) and results presentation is divided into 2 parts: the first part detailing the analyses to be performed (known as SAP, current document) and a second part, annex (-es) (called **TFL**) describing the flow and format of tables, figures and listings to be annexed to the Study Report (SR).

LIST OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse Event of Special Interest
ANCOVA	Analysis of Covariance
AS03	AS03 is an Adjuvant System containing α-tocopherol and squalene in an oil and water emulsion
ATP	According-To-Protocol
CD40L	Cluster Differentiation-40 Ligand
CI	Confidence Interval
CTR	Clinical Trial Register
D_{GMT}	Desirability (Immunogenicity index)
D_R	Desirability (Fever index)
DOB	Date Of Birth
EL.U/ml	ELISA unit per milliliter
Eli Type	Internal GSK database code for type of elimination code
GMT	Geometric mean antibody titer
GSK	GlaxoSmithKline
HA	Hemagglutinin
HI	Hemagglutination Inhibition
ICS	Intracellular Cytokine Staining
IU/ml	International units per milliliter
iSRC	Internal Safety Review Committee
IFNγ	Interferon γ
IL-2	Interleukin-2
LL	Lower Limit of the confidence interval
MAEs	Medically Attended Adverse Events

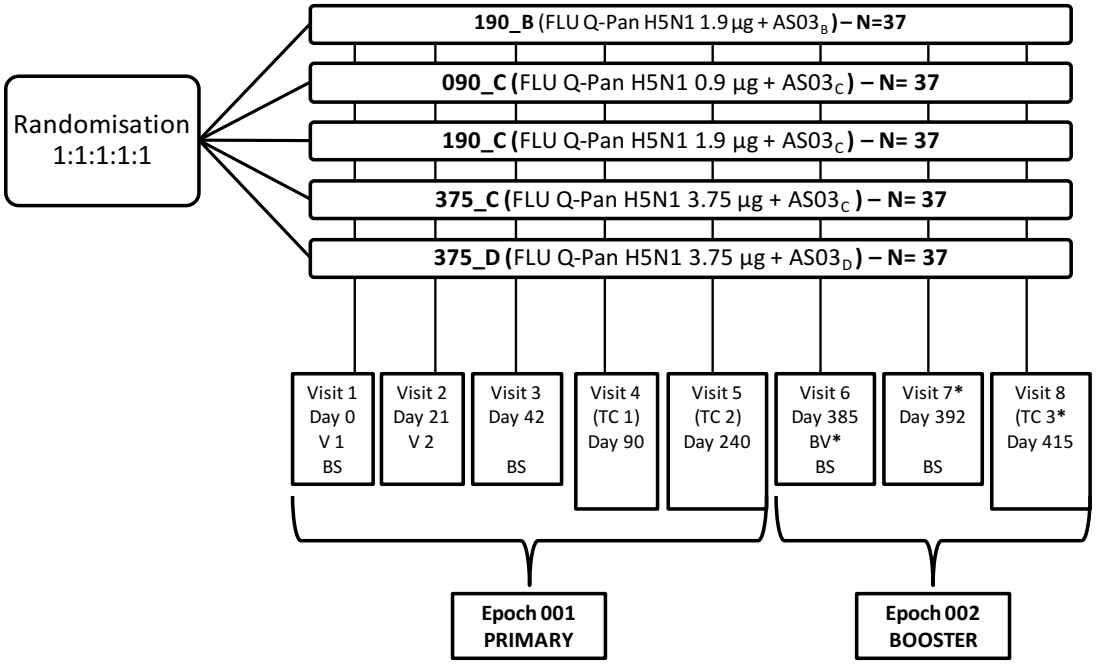
MedDRA	Medical Dictionary for Regulatory Activities
MGI	Mean Geometric Increase
MN	Microneutralization
N.A.	Not Applicable
pIMDs	Potential Immune-Mediated Diseases
PT	Preferred Term
R&D	Research and Development
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SCR	Seroconversion Rate
SD	Standard Deviation
SR	Study Report
SPR	Seroprotection Rate
SMQs	Standardized MedDRA Queries
TFL	Tables Figures and Listing template annexed to SAP
TNF-α	Tumor necrosis factor-α
VRR	Vaccine Response Rate
TVC	Total vaccinated cohort
UL	Upper Limit of the confidence interval

1. DOCUMENT HISTORY

Date	Description	Protocol Version
12-JAN-2017	Version 1	Amendment 1 Final: 20 January 2016
13-APR-2017	Version 2	Amendment 2 Final : 27-March 2017

2. STUDY DESIGN

2.1. Design Overview



D=Day; TC = telephone contact, V = Primary Vaccination, *BV = *Unadjuvanted* booster vaccination *(3.75 µg HA)*;
BS = Blood sample,
FLU Q-Pan H5N1 = A/Indonesia/05/2005 hemagglutinin antigen

Experimental design: Phase II, observer blind, randomized, multi-center, multi-country study, parallel groups.

Duration of the study: approximately 415 days after vaccination on Day 0.

- The Primary Epoch encompasses data collected from Visit 1 (Day 0) through Visit 5-TC 2 (Day240) and ending at the start of Visit 6 (Day 385).

- The Booster Epoch encompasses data collected from Visit 6 (Day 385) through Visit 8-TC 3 (Day 415).

Sampling schedule:

- All study groups: Day 0 (before vaccination); Day 42 (post primary course); Day 385 (persistence) and Day 392 (booster response).

Vaccination schedule:

- All subjects are to receive an AS03 adjuvanted H5N1 vaccine given as a two-dose primary series at a 21 day interval.
- All subjects are to receive a 3.75 µg HA, unadjuvanted H5N1 vaccine as a booster dose at Day 385.

2.2. Study groups

Approximately 37 subjects are planned to be enrolled in each of the 5 following study groups:

Study groups and epochs foreseen in the study

Study groups	Number of subjects	Epochs	
		Epoch 001 (Primary Series)	Epoch 002 (Booster)
190_B	37	●	●
090_C	37	●	●
190_C	37	●	●
375_C	37	●	●
375_D	37	●	●

Study groups and treatments foreseen in the study

Treatment name	Vaccine/Product name (Formulation)	Study Groups				
		190_B	090_C	190_C	375_C	375_D
1.9 mcg H5N1 HA + AS03B	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)	●				
	AS03A (AS03 47.44 mg/mL , final vaccine dose contains 5.93 mg tocopherol)	●				
0.9 mcg H5N1 HA + AS03C	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)		●			
	AS03A (AS03 47.44 mg/mL, final vaccine dose contains 2.97 mg tocopherol)		●			
1.9 mcg H5N1 HA + AS03C	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)			●		
	AS03B (AS03 23.72 mg/mL; final vaccine dose contains 2.97 mg tocopherol			●		
3.75 mcg H5N1 HA + AS03C	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 30 µg/mL)				●	
	AS03B (AS03 23.72 mg/mL; final vaccine dose contains 2.97 mg tocopherol				●	
3.75 mcg H5N1 HA + AS03D	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 30 µg/mL)					●
	AS03C (AS03 11.86 mg/mL; final vaccine dose contains 1.48 mg tocopherol)					●
3.75 mcg H5N1 HA *	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)	●	●	●	●	●

*Booster vaccination to subjects in all study groups

2.3. Group description

The following group names will be used for the statistical analyses at Day 42 and Day 415:

Group order in tables	Group label in tables	Group definition for footnote
1	190_B	1.9 µg H5N1 HA antigen adjuvanted with $AS03_B$
2	090_C	0.9 µg H5N1 HA antigen adjuvanted with $AS03_C$
3	190_C	1.9 µg H5N1 HA antigen adjuvanted with $AS03_C$
4	375_C	3.75 µg H5N1 HA antigen adjuvanted with $AS03_C$
5	375_D	3.75 µg H5N1 HA antigen adjuvanted with $AS03_D$

3. OBJECTIVES

3.1. Co-Primary Objectives

3.1.1. Primary Doses

- To assess the performance of alternative dosing regimens for primary immunization with Q-Pan H5N1 vaccine using an immunogenicity-fever index that considers:
  - immunogenicity by HI assay against vaccine-homologous virus 21 days after the second priming dose, and
  - fever scores after the first and second priming doses.
- To assess the performance of alternative dosing regimens for primary immunization with Q-Pan H5N1 vaccine using an immunogenicity-fever index that considers:
  - immunogenicity by MN assay against vaccine-homologous virus 21 days after the second priming dose and
  - fever scores after the first and second priming doses.

The reference dose for each of these assessments will be 1.9 µg HA with $AS03_B$ (half the approved adult dose).

3.1.2. Booster Dose

- To assess the performance of dosing regimens for booster immunization with Q-Pan H5N1 vaccine considering:
  - immune response by HI assay against vaccine-homologous virus 7 days after a 12-month booster dose of 3.75 µg HA Q-Pan H5N1 ***unadjuvanted*** antigen
- To assess the performance of dosing regimens for booster immunization with Q-Pan H5N1 vaccine considering:
  - immune response by MN assay against vaccine-homologous virus 7 days after a 12-month booster dose of 3.75 µg HA Q-Pan H5N1 ***unadjuvanted*** antigen

3.2. Secondary objectives

- To describe the HI immune response to the vaccine-homologous virus 21 days after the second dose for each dosing regimen
- To assess the performance of alternative dosing regimens for primary immunization with Q-Pan H5N1 vaccine considering persistence of immune response by HI and MN assay at Day 385 in terms of persistence index.

- To assess the performance of the H5N1 vaccine regimens in terms of vaccine-homologous HI and MN antibody titers on Days 0, 42, 385 and Day 392.
- To assess the immunogenicity of the H5N1 vaccine regimens in terms of vaccine-heterologous HI ***and MN*** antibody titers on Days 0, 42, 385 and Day 392.
- To assess vaccine induced cell-mediated immune responses on Days 0, 42, 385 and Day 392.
- To describe reactogenicity and safety of the different priming regimens in terms of solicited (7-days after each vaccination) and unsolicited (21 days after each vaccination) adverse events (AEs).
- To describe safety of the unadjuvanted booster dose in terms of solicited (7 days post boost) and unsolicited (30 days post boost) AEs.
- To describe safety in terms of medically attended AEs (MAEs), potential immune-mediated diseases (pIMDs), and serious adverse events (SAEs), adverse events of special interest (AESIs), during the entire study period.

4. ENDPOINTS

4.1. Primary Endpoints

4.1.1. Immunogenicity-fever indices

Separate HI and MN immunogenicity-fever indices will be constructed based on the following endpoints:

- Humoral immune response in terms of vaccine-homologous HI antibody for each group:
  - LL (lower limit) of 95% CI of GMT group ratio at Day 42 using 1.9 µg HA with $AS03_B$ as reference.
- Humoral immune response in terms of vaccine-homologous MN antibodies for each group:
  - LL of 95% CI GMT group ratio at Day 42 using 1.9 µg HA with $AS03_B$ as reference.
- Fever measurement (≥38°C) post dose 1 and dose 2:
  - For each subject, a fever index will be calculated using temperature measurements 3-days post Dose 1 (D0-D2) and 3-days post Dose 2 (D21-D23).

For details regarding construction of these indices, refer to Section 6.1.2.1 (Statistical Methods- Analysis of immunogenicity).

4.1.2. Immune response to a booster dose

Following booster dose administered at Day 385 the following will be evaluated:

- For immune response in terms of HI antibodies against vaccine-homologous antigen
  - Mean Geometric Increase (MGI) at Day 392 relative to Day 385.
- For the immune response in terms of MN antibodies against vaccine-homologous antigen
  - MGI at Day 392 relative to Day 385.

4.2. Secondary Endpoints

Immunogenicity

- For humoral immune response in terms of HI antibodies against vaccine-homologous/heterologous antigens post-primary immunization, the following aggregate variables will be calculated for each group:
  - Seroconversion rates (SCR) at Day 42
  - Seroprotection rates (SPR) at Day 42
  - MGI at Days 42 relative to Day 0.
- For humoral immune response in terms of vaccine-homologous MN antibody post the primary immunization, following aggregate variables will be calculated for each group:
  - MGI at Day 385 relative to Day 0.
- For humoral immune response in terms of HI antibodies against vaccine-homologous/heterologous antigens (at Days 0, 42 and 385 post the primary immunization, at Day 392 (7 days post booster dose)), the following aggregate variables will be calculated for each group:
  - Seropositivity rates at Days 0, 42, 385 and Day 392
  - Seroconversion rates (SCR) at Day 385 (relative to Day 0) and Day 392 (relative to Days 0 and 385)
  - Seroprotection rates (SPR) at Days 0, 385 and Day 392
  - Geometric Mean Titer (GMT) at Days 0, 42, 385 and Day 392
  - MGI at Day 385 (relative to Day 0), MGI at Day 392 (relative to Days 0 and 385).

- For humoral immune response in terms of vaccine-homologous***/heterologous*** MN antibody, the following aggregate variables will be calculated for each group:
  - Seropositivity rates at Days 0, 42, 385 and Day 392
  - GMT at Days 0, 42, 385 and Day 392
  - Vaccine response rate (VRR) at Days 42, 385 (relative to Day 0) and Day 392 (relative to Days 0 and 385).
- For CMI in terms of frequencies of antigen-specific cells (CD4+/CD8+) at Days 0, 42, 385 and 392:
  - Frequencies of cytokine CD4+/CD8+ T- cells per million CD4+/CD8+ cells producing two or more markers within CD40L, IL-2, TNF-α, IFN-γ upon in vitro stimulation using A/Indonesia/05/2005 (H5N1) split virus as determined by intracellular cytokine staining (ICS) in a sub-cohort of approximately 20 subjects per group at Days 0, 42, 385 and Day 392.

Reactogenicity /Safety:

- Solicited local and general AEs
  - Occurrence of each solicited local AEs during a 7-day follow-up period (i.e., day of vaccination and 6 subsequent days) after any vaccination.
    - Percentage, intensity and duration of solicited local AEs during a 7-day follow-up period (Day 0-Day 6) after any vaccination.
  - Occurrence of each solicited general AEs during a 7-day follow-up period (i.e., day of vaccination and 6 subsequent days) after any vaccination.
    - Percentage, intensity, duration and relationship to vaccination of solicited general AEs during a 7-day follow-up period (Day 0-Day 6) after any vaccination.
- Unsolicited adverse events (AEs)
  - For the primary series: occurrence and relationship to vaccination of unsolicited AEs within 21 days after each vaccine dose.
    - Percentage, intensity and relationship to vaccination of unsolicited AEs during a 21-day follow-up period (Day 0-Day 20) after each vaccine dose.
  - For the booster dose [unadjuvanted]: occurrence and relationship to vaccination of unsolicited AEs within 30 days after vaccination
    - Percentage, intensity and relationship to vaccination of unsolicited AEs during a 30-day follow-up period.
  - Occurrence and relationship to vaccination of (MAEs) during the entire study period.

- Percentage and relationship to vaccination of MAEs during the entire study period.

– Occurrence and relationship to vaccination of pIMDs, SAEs, AESIs during the entire study period.

- Percentage and relationship to vaccination of pIMDs, SAEs, AESIs during the entire study period.

5. STUDY POPULATION

5.1.1. Total Vaccinated Cohort

The total vaccinated cohort (TVC) will include all subjects who received at least one dose of vaccine (TVC for booster safety analysis will include all the subjects who received the booster dose):

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

The TVC analyses will be performed per investigational vaccine actually administered at the first dose.

The analysis of safety will be performed on the TVC.

5.1.2. Cohort for according-to-protocol (ATP) immunogenicity analyses

The ATP cohort for immunogenicity analyses will include all vaccinated/eligible subjects:

- who have received all study vaccine dose(s) (3-doses for booster immunogenicity analysis, 2-doses for other analyses) per protocol treatment assignment;
- for whom the randomization code is unbroken during the relevant analysis interval;
- who have not received any investigational or non-registered product (drug or vaccine) other than the study vaccine(s) during the relevant analysis interval;
- who have not received any non-study vaccine during the relevant analysis interval who have not received any immunoglobulins and/or any blood products during the relevant analysis interval
- for whom there was no chronic administration of immunosuppressants) during the relevant analysis interval.
- who develop a physician-confirmed infection with an A/Indonesia/5/2005 (H5N1)-like influenza virus during the relevant analysis interval.

- who have results available for the relevant assay (HI and MN) for all blood samples to be collected during the relevant analysis intervals for ATP-Day 42; for ATP-Day 385 (persistence); for ATP-Day 392 post booster dose (ATP-booster).

For the final analysis, an Adapted ATP- cohort for immunogenicity will be used. The Adapted ATP cohort for immunogenicity analysis will include all vaccinated/eligible subjects who have results available for the relevant assay (HI and MN) for all blood samples to be collected during the relevant analysis intervals for ATP-Day 42; for ATP-Day 385 (persistence); for ATP-Day 392 post booster dose (ATP-booster).

5.2. Cohort for analysis of the immunogenicity-fever score

The analysis cohort for combined immunogenicity and safety of the two-dose primary series will include all subjects in the ATP cohort for immunogenicity analysis at Day 42 for whom temperature measurements are available during the first 3 days after both vaccine doses 1 and 2.

Subjects without immunogenicity results at Day 42 or for whom one or more daily temperature measurements are missing during the first 3 days after either vaccine dose [dose 1 or dose 2] will be excluded from the immunogenicity/fever index calculation for the primary series.

The list of applicable elimination codes for each cohort can be found in the study specific form FORM-9000036402-13 Criteria for eliminating subjects from the analyses and filled in CARS: Clinical R&D/FLU Q-PAN/Studies/H5N1-AS03-023 (116938)/11 Statistics/11.01 Statistics Oversight/11.01.01 Statistical Analysis Plan.

Cohort	Elimination codes	Eli Type
ATP cohort for Day 42 analysis of the immunogenicity	1030.1040,1050,1060,1070,1080, 1090, 2010, 2040, 2050, 2060, 2070, 2080, 2090, 2100, 2500.	PR
ATP Day 392 post-booster dose.	1030.1040,1050,1060,1070,1080, 1090, 2010, 2040, 2050, 2060, 2070, 2080, 2090, 2100, 2500.	MA (including PR)

6. STATISTICAL METHODS

6.1.1. Analysis of demographics

The analysis of demography will be performed on the TVC and on the ATP for immunogenicity.

Demographic characteristics (age at first study vaccination in months; gender; ethnicity) of all subjects will be tabulated by study group and overall using descriptive statistics:

- Frequency tables will be generated for categorical variables such as ethnicity.
- Mean, range, median, and standard deviation will be provided for continuous data such as age.

The distribution of subjects enrolled among the study sites (center) will be tabulated as a whole and per group.

In addition, the following table will be performed for Clinical Trial Register (CTR) posting:

– Percentage of Enrolled subjects by country will be tabulated by group and pooled vaccine groups.

6.1.2. Analysis of immunogenicity

The primary analysis will be based on the ATP cohort for analysis of immunogenicity. If, in any study group, the percentage of vaccinated subjects with serological results excluded from the ATP cohort for analysis of immunogenicity is 5% or more, a second analysis based on the TVC will be performed to complement the ATP analysis.

6.1.2.1. Immunogenicity/fever indices (Co-primary objectives)

An analysis of covariance model (ANCOVA) will be fitted on the log_{10} transformed HI and MN antibody responses at Day 42, with the vaccine group as a fixed independent variable, adjusted by the log_{10} transformed pre-vaccination titer and age.

For ***vaccine homologous*** HI and MN separately, an immunogenicity index (D_{GMT}) will be constructed using a desirability function based on the computed GMT group ratio (alternative dose regimen to reference =1.9 µg HA with $AS03_B$) and the 95% CI.

D_{GMT} will be the LL of the 95% CI for GMT group ratio

- If the LL of the 95% CI for GMT group ratio is less than 0.25 (i.e., 4- fold less than that of the reference group), then D_{GMT} =0.
- If the LL of the 95% CI for GMT group ratio is greater than 1 (comparison group has higher GMT value than the reference group), D_{GMT} =1.

note D_{GMT} =1 for the reference group

The fever index (D_R) will be calculated according to body temperature measurements performed from Days 0-2 after each dose

- Any temperature < 38°C (100.4 F) will be assigned a value of 0. Any temperature > 40.5°C will be assigned a value of 40.5).
- The highest possible temperature value per subject is 243 (6 x 40.5°C; i.e. for 3 days after dose 1 and dose 2);
- The lowest possible temperature value per subject is 0 (all measurements < 38.0°C (100.4 F) for 3 days after dose 1 and dose 2).
- For each subject, a temperature index will be constructed as follows: (243 minus the sum of recorded temperature values for 3 days after dose 1 and dose 2)/243. The average temperature measurement for each vaccine group will be calculated as the D_R. A lower index value D_R indicates a less desirable regimen in terms of reactogenicity.

An immunogenicity-fever index (D) at Day 42 will be computed for each group

$$D = \sqrt{D_{GMT} \times D_R}$$

This index (D) will range between 0 and 1 (0 = not desirable; 1 = highly desirable) and the same weight (0.5) is assigned for immunogenicity index and reactogenicity (fever) index.

Two immunogenicity-fever indices will be separately calculated for HI and MN ***assay against vaccine-homologous virus***. Each will be used to rank the different dosing regimens as a tool to guide dosing regimen selection.

6.1.2.2. Immune Response to a booster dose

There will be two separate evaluations, performed on evaluable subjects (ATP cohort-booster) following a booster dose:

- Point estimates and 95% CIs for MGIs relative to Day 385 will be computed for vaccine-homologous antibody titers assessed by HI at Day 392 for each vaccine regimen
- Point estimates and 95% CIs for MGIs relative to Day 385 will be computed for vaccine-homologous antibody titers assessed by MN at Day 392 for each vaccine regimen

6.1.2.3. Analysis for immunogenicity (secondary objective)

- Point estimates and 95% CIs for MGIs relative to Day 0 will be computed for vaccine-homologous antibody titers assessed by MN at Day 385 for each vaccine regimen.

- Using vaccine-homologous and vaccine-heterologous antibody titers assessed by HI, the point estimates for SCR, SPR, MGI, and the associated 95% CIs will be computed at 21 days after the second dose.
- Using vaccine-homologous and vaccine-heterologous antibody titers assessed by HI, the point estimates and 95% CIs for GMTs and Seropositivity rates (at all timepoints), and SPRs, SCRs and MGIs at Day 385 will be computed for each vaccine regimen.
- Point estimates and 95% CIs for Seropositivity, GMTs and VRRs for vaccine-homologous/***heterologous*** MN titers will be computed for all appropriate timepoints.

All comparisons will be descriptive.

The ATP immunogenicity cohorts (Day 42, persistence or booster) will be used for the related analysis of immunogenicity.

The reverse cumulative distribution curves (RCCs) for HI antibodies against A/Indonesia/05/2005 (H5N1)-like influenza virus at Days 0, 42, 385 and 392 will be performed.

6.1.2.4. Analysis for CMI

- Cell Mediated Immunity (CMI) parameters at Day 0, 42 , 385 and 392 will be evaluated in TVC:
  - Antigen-specific CD4+/CD8+ T Cells identified as CD4/CD8+ T- cells producing two or more markers within CD40L, IL-2, TNF-α, IFN-γ upon ***in vitro*** stimulation using A/Indonesia/05/2005 (H5N1) split virus,
  - The frequency of the response for CD4+/CD8+T-cells stained with probes for various cytokines and activation marker (IFN-γ, TNF-α, IL-2, CD40L) and elicited by vaccine components measured in a sub-cohort of approximately 20 subjects per group at Days 0, 42, 385 and 392 will be described according to the technical specifications provided by R&D (Clinical Data – Information Sheet).

6.1.3. Analysis for safety

- Safety data will be analyzed based on the TVC.
- Analysis will be of subject incidence rates of solicited and unsolicited adverse events (AEs), by solicited local and general AEs terms, and, for unsolicited AEs, by MedDRA preferred term and system organ class. Safety data will be summarized for all subjects by treatment group. The incidence of solicited local and general AEs occurring during 7 days after vaccination will be tabulated with exact 95% CI for each treatment group. The same calculations will be performed for AEs of any intensity, those with intensity Grade ≥ 2, and those with intensity of Grade 3, as well

as for solicited general events assessed as related to vaccination. All solicited local AEs are considered to be related to vaccination.

- The percentage of subjects with at least one report of an unsolicited AE classified by MedDRA (System Organ Class and Preferred Term) 21 days after each primary vaccination will be tabulated with exact 95% CI for each treatment group. The same tabulation will be performed for Grade 3 unsolicited AEs and for unsolicited AEs that are considered by the investigator to be possibly or probably related to vaccination.
- The percentage of subjects with at least one report of an unsolicited AE classified by MedDRA (System Organ Class and Preferred Term) 30 days after the booster vaccination will be tabulated with exact 95% CI. The same tabulation will be performed for Grade 3 unsolicited AEs and for unsolicited AEs that are considered by the investigator to be possibly or probably related to vaccination.
- The proportion of subjects who begin at least one new concomitant medication during the first 42 days after primary vaccination will be calculated with 95% CI.
- MAEs, AESIs, SAEs, and pIMDs will be summarized through the entire study period. In addition, serious AEs and withdrawals due to AE(s) will be described in detail.

The pIMD listings will include pIMDs reported by the Investigator and those identified from the clinical database search using a list of pre-specified pIMD PTs.

The AESIs listings will include AESIs identified from the clinical database search using a list of pre-specified AESIs PTs and SMQs. The AESIs will include the events listed below and the MedDRA PT or SMQ used to identify reports of these events is provided in parentheses:

- Anaphylaxis (narrow SMQs “Anaphylactic reaction” and “Angioedema”)
- Bell’s palsy (MedDRA PT “VIIth nerve palsy”)
- Convulsion (Narrow SMQ “Convulsions”)
- Demyelination (narrow SMQ “Demyelination”)
- Encephalitis (narrow SMQ “Non-infectious encephalitis”)
- Guillain-Barré syndrome (narrow SMQ “Guillain-Barré syndrome”)
- Neuritis (MedDRA PT “Neuritis”)
- Vasculitis (narrow SMQ “Vasculitis”)

7. STATISTICAL CALCULATIONS

7.1. Derived and transformed data

7.1.1. Demography

For a given subject and a given demographic variable, missing measurement will not be replaced except for incomplete date of birth (DOB) data.

7.1.1.1. Age at vaccination

Age will be calculated as the number of months between the DOB and the date of vaccination.

In some countries, to ensure that the collection of DOB will not jeopardise the privacy of personally identifiable information, only a partial DOB (MMYYYY) will be collected.

Therefore, the 15th of the month will be used to replace the missing date.

In case the day and the months are missing, the date will be replaced by the June 30th of the year.

7.1.2. Immunogenicity

The cut-off value for antibody titer is defined by the laboratory before the analysis. **A seronegative** subject is a subject whose antibody titer is below the cut-off value, and conversely; **a seropositive** subject is one whose antibody titer is greater than or equal to the cut-off value. For this study:

- It is assumed that **HI titers** of < 1:10 will be considered below the cut-off. For the calculations of GMT, HI titers of < 10 will be arbitrarily assigned the value 5.
- It is also assumed that **MN titers** of < 1:28 will be considered below the cut-off. For the calculations of GMT, MN titers of < 28 will be arbitrarily the value 14.

Geometric Mean Titer (GMT) calculations are performed by taking the anti-log of the mean of the log (base 10) transformed inverse titers (the number X would denote the inverse of a titer expressed as "1: X"). Antibody titers below the cut-off of the assay are given an arbitrary value of half the cut-off for the purpose of GMT calculation.

Mean Geometric Increase (MGI) for HI is defined as the geometric mean of the within-subject ratios of the post-vaccination reciprocal HI titer to the ***pre-vaccination*** HI titer.

Seroconversion Rate (SCR) is defined as the incidence rate of vaccines who have either a pre-vaccination titer recorded as <1:10 for HI and a post-vaccination reciprocal titer ≥

40 or a pre-vaccination reciprocal titer ≥10 and at least a 4-fold increase in post-vaccination reciprocal titer.

Incidence Rate of HI Reciprocal Titers ≥ 40 (SPR), defined as the percentage of all vaccinees with a serum reciprocal HI antibody titer ≥ 40 post-vaccination, a level of HI antibodies that may correlate with benefit in protection against influenza.

Vaccine Response Rate (VRR) by MN is defined as the post-vaccination reciprocal titer of ***vaccines*** that have at least 4-fold increase compared with their pre-vaccination reciprocal titer. Antibody titers below the cut-off of the assay are given an arbitrary value of half the cut-off for the purpose of VRR calculation.

Handling of missing immunogenicity data: for a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, analyses will exclude subjects with missing or non-evaluable measurements.

7.1.3. Reactogenicity and safety

Incidence rates of AEs will be calculated as the number of subjects who experience the event, divided by the number of subjects in the safety analysis cohort (the TVC).

Handling of missing safety data:

- **Solicited AEs**: For a given subject and the analysis of solicited AEs within 7 days post-vaccination (Days 0 through 6), missing or non-evaluable measurements will not be replaced.
  - The analysis of the solicited AEs will include only vaccinated subjects and doses with documented safety data (i.e., symptom screen/sheet completed, including the response 'Yes' or 'No' to the solicited symptom page).
  - Subjects who answered "Yes" in the solicited symptom page, but had all symptoms missing are considered as having no solicited symptoms.
  - Subjects who answered 'Yes' to the presence of a specific symptom but partially recorded the daily measurement (e.g. intensity missing for Day 3) over the considered solicited period will be included in the summaries and classified according to their maximum observed daily recording over the solicited period.
  - Subjects who answered 'Yes' to the presence of a specific symptom but recorded no daily measurement over the considered solicited period will not be counted in the summary of symptoms by grade/category but will be part of the summary corresponding to the 'All' category for that symptom.

- **Unsolicited AEs**: For the analysis of unsolicited AEs/MAEs/SAEs/pIMDs/AESIs, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.
- **Concomitant medication and vaccination**: For the analysis of concomitant medications and vaccinations, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.

7.1.3.1. Counting rule

Event	N used for deriving % per subject for vaccination phase
Solicited general symptom	All subjects with at least one solicited general symptom documented as either present or absent (i.e. symptom screen completed)
Solicited local symptom	All subjects with at least one solicited local symptom documented as either present or absent (i.e. symptom screen completed)
Unsolicited symptom MAEs/SAEs/pIMDs/AESIs	All subjects with study vaccine administered
Concomitant medication	All subjects with study vaccine administered
Concomitant vaccination	All subjects with study vaccine administered

7.1.3.2. Coding of grading for Solicited symptoms

The maximum intensity of local injection site redness or swelling will be scored at GSK Biologicals as follows:

0 : ≤ 20 mm
1 : > 20 to 50 mm
2 : > 50 to 100 mm
3 : > 100 mm

Body temperatures will be scored at GSK Biologicals as follows:

0 < 38.0°C (< 100.4°F)
1 ≥ 38.0 – 38.4°C (≥100.4 – 101.2°F)
2 ≥ 38.5 – 38.9°C (≥ 101.3 – 102.1°F)
3 ≥ 39.0 - 40°C (≥ 102.2 – 104.0°F)
4 >40.0°C (>104.0°F)

Fever is defined as temperature ≥ 38.0°C/100.4°F. The preferred route for recording temperature in this study is axillary.

For clinicaltrials.gov and EudraCT posting purposes, a summary of subjects with all combined solicited (regardless of their duration) and unsolicited adverse events will be provided. Solicited adverse events will be coded by MedDRA as per the following codes:

Solicited symptom	Lower level code	Lower level term
Pain at injection site	10022086	Injection site pain
Redness at injection site	10022098	Injection site redness
Swelling at injection site	10053425	Injection site swelling
Fever	10016558	Fever
Irritability/Fussiness	10057224	Irritability post vaccinal
Loss of appetite	10003028	Appetite lost
Drowsiness	10013649	Drowsiness

7.1.3.3. Onset day

The onset day for an event will be the number of days between the most recent vaccination and the start of the event. Events occurring on the day of vaccination will have an onset day of 0.

7.1.3.4. Duration of events

The duration of an event will be the number of days (not necessarily consecutive) with symptom during the solicited follow-up period.

7.2. Methodology for computing CI

All CI will be 2 sided 95% CI.

- The exact 95% CIs for a proportion within a group will be calculated from Proc StatXact [Clopper, 1934,].
- The 95% CIs for GMT are obtained within each group separately. The 95% CI for the mean of lot-transformed titer is first obtained assuming that log-transformed titers 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 titer.
- The group GMT ratio will be obtained using an ANCOVA model on the logarithm-transformed titers. The ANCOVA model will include the vaccine group as fixed effect and the pre-vaccination $\log_{10}$ titer and, age as regressor. The GMT ratio and its 95% CI will be derived as exponential-transformation of the corresponding group contrast in the model.

7.3. Data presentation description:

The following decimal description from the decision rules will be used for the demography, immunogenicity and safety/reactogenicity.

Display Table	Parameters	Number of decimal digits
Demographic characteristics	Mean, median age	1
Demographic characteristics	SD (age)	1
Immunogenicity	Ratio of GMT	2
Reactogenicity	Mean, Min, Q1, Median, Q3, Max for duration	1
All summaries	% of count, including LL & UL of CI	1
All summaries	% of ratio, including LL & UL of CI	1

7.4. Interpretation of analyses

In this study, all the comparative analyses will be descriptive with the aim to rank alternative dosing regimens with respect to immunogenicity and reactogenicity at Day 42, persistence at Day 385, and anamnestic response to unadjuvanted antigen booster dose at Day 392. In order to evaluate all the effects at the same scale (values between 0-1), a persistence parameter and a booster effect parameter will be calculated for HI and MN:

- Persistence index = (MGI – 4)/MGI for each group.
- Boostability index= (MGI – 4)/MGI for each group.

If the MGI value is less than 4, the index will be set to 0, the immunogenicity- fever, persistence and boostability indices will be ranked for each vaccine group.

Given the complexity of possible outcomes, an algorithm for dose selection will **not** be proposed.

Dose selection will be based on Day 42 immunogenicity and fever as key indicator of reactogenicity, persistence of the immune response and anamnestic response to unadjuvanted antigen as well as any additional safety concerns. Additional practical matters related to the estimated incremental benefit versus the complexity of a pediatric specific dosing regimen, should it not be a fraction of the adult dose, will also be weighed

for dose selection as this could lead to increased complexity from a manufacturing and delivery perspective in a pandemic response setting.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

8.1.1. ISRC analyses

iSRC (independent Safety Review Committee) analyses based on TVC are currently performed for the purposes of safety data review. These analyses were described in detail in a separate SAP/TFL associated to iSRC Charter.

8.1.2. Analysis at Day 42

An analysis will be performed on data collected through the Day 42 visit. Elements will include:

- An analysis of cleaned immunogenicity and solicited AEs data collected through the Day 42 visit will be conducted.
- Analyses of unsolicited AEs reported up to the Day 42 visit and cleaned in so far as is possible will be carried out.
- Analyses of MAEs, AESIs, SAEs, pIMDs and withdrawals due to AEs collected up to the Day 42 visit will be carried out.
- Results will be presented in a Day 42 statistical report. Access to individual treatment codes will be restricted to the designated statisticians in charge of the analysis. No individual listings or data with the subjects' identifying information will be disseminated. Listings of final data will be provided with the Day 415 report.

8.1.3. Final analysis

A final data analysis will be performed at the end of study (Day 415) of all primary and secondary endpoints based on the clean data, including evaluations of:

- demography and baseline characteristics
- immunogenicity
- solicited AE data (Day 0-6) after each vaccination
- unsolicited AEs reported up to the Day 42 visit (21 days after each dose), as well as 30 days after the Day 385 booster dose

- concomitant medications reported up to the Day 42 visit, as well as 30 days after the Day 385 booster dose
- MAEs, AESIs, SAEs, pIMDs and withdrawals due to AEs collected throughout the entire study.

Results of the final analysis, as well as individual data listings, will be presented in a final, integrated clinical study report (CSR).

8.2. SDD Stored analysis

Description	Analysis ID (SDD sub-folder)	Disclosure Purpose	*Study Headline Summary (SHS)* requiring expedited communication to Upper Management (Yes/No)	Reference for TFL
iSRC 1 to iSRC 11 iSRC 12 to iSRC 17	E1_02 to E1_12 E1_13 to E1_18	Internal	No	SAP/TFL associated to the Charter
Day 42 Analysis	E1_13	CTR	Yes	TFL for Day 42 and Final Analysis
Final Analysis	E1_01	Study Report (SR) CTR	Yes	TFL for Day 42 and Final Analysis

8.3. Statistical considerations for interim analyses

Not applicable

9. CHANGES FROM PLANNED ANALYSES

– The reverse cumulative distribution curves (RCCs) for HI antibodies against A/Indonesia/05/2005 (H5N1) –like influenza virus at Days 0, 42, 385 and 392 will be performed.
– For clintrial.gov and EudraCT posting purposes, a summary of subjects with all combined solicited (regardless of their duration) and unsolicited AEs will be provided.

10. REFERENCES

The exact 95% CIs for a proportion within a group will be calculated from Proc StatXact [Clopper, 1934*].

Proc StatXact will be used to derive the standardised asymptotic 95% CI for the group difference in proportions [Robert, 1998, method six]. The standardised asymptotic method used within GSK Biologicals is the method six.

Damaso S, Dewé, W, and al. *Selection of a vaccine Formulation in Clinical Development: Study designs and Statistical analysis*, GlaxoSmithKline, **Point to Consider (P-t-C),** August 2014.

Walthere Dewé, Christelle Durand, Sandie Marion, Lidia Oostvogels, Jeanne-Marie, Devaster, Marc Fourneau: *A multi-criteria decision making approach to identify a vaccine formulation,* Journal of Biopharmaceutical Statistics**,** 2015.

* Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. Biometrika, 1934; 26:404-413;

GlaxoSmithKline	**Statistical Analysis Plan**
Detailed Title:	A phase II observer-blind, multicentre, dose-ranging study of children 6 to less than 36 months of age who are to be primed with a 2-dose series of GSK Biologicals' AS03-adjuvanted A/Indonesia/05/2005 (H5N1) vaccine
eTrack study number and Abbreviated	116938 (FLU Q-PAN H5N1=AS03-023)
Scope:	All data pertaining to the above study.
Date of Statistical Analysis Plan	Amendment 2 (01-Mar-2018) Amendment 1 (13-Apr-2017) Version 1.0 (12-Jan-2017)
Co-ordinating author:	PPD (Lead BioStatistician)
Adhoc reviewers:	PPD (CRDL) PPD (Manager Scientific Writer) PPD (Lead Stat Analyst) PPD (Stat Analyst)
Approved by:	PPD (CRDL) PPD (Lead Statistician) PPD (Manager Scientific Writer)

APP 9000058193 Statistical Analysis Plan Template (Effective date: 14 April 2017)

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The complete statistical analysis plan (**SAP**) and results presentation is divided into 2 parts: the first part detailing the analyses to be performed (known as SAP, current document) and a second part, annex (-es) (called **TFL**) describing the flow and format of tables, figures and listings to be annexed to the Study Report (SR).

LIST OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse Event of Special Interest
ANCOVA	Analysis of Covariance
AS03	AS03 is an Adjuvant System containing α-tocopherol and squalene in an oil and water emulsion
ATP	According-To-Protocol
CD40L	Cluster Differentiation-40 Ligand
CI	Confidence Interval
CTR	Clinical Trial Register
D_{GMT}	Desirability (Immunogenicity index)
DOB	Date Of Birth
D_R	Desirability (Fever index)
EL.U/ml	ELISA unit per milliliter
Eli Type	Internal GSK database code for type of elimination code
GMT	Geometric mean antibody titer
GSK	GlaxoSmithKline
HA	Hemagglutinin
HI	Hemagglutination Inhibition
ICS	Intracellular Cytokine Staining
IFNγ	Interferon γ
IL-2	Interleukin-2
iSRC	Internal Safety Review Committee
IU/ml	International units per milliliter
LL	Lower Limit of the confidence interval
MAEs	Medically Attended Adverse Events
MedDRA	Medical Dictionary for Regulatory Activities
MGI	Mean Geometric Increase
MN	Microneutralization

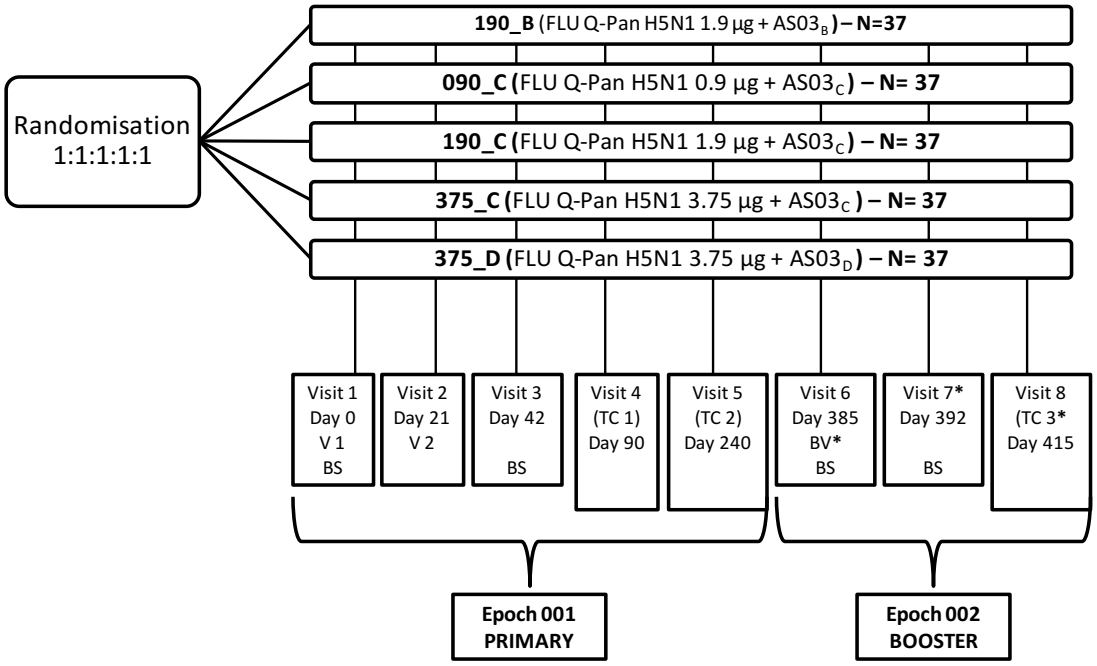
N.A.	Not Applicable
pIMDs	Potential Immune-Mediated Diseases
PT	Preferred Term
R&D	Research and Development
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SCR	Seroconversion Rate
SD	Standard Deviation
SMQs	Standardized MedDRA Queries
SPR	Seroprotection Rate
SR	Study Report
TFL	Tables Figures and Listing template annexed to SAP
TNF-α	Tumor necrosis factor-α
TVC	Total vaccinated cohort
UL	Upper Limit of the confidence interval
VRR	Vaccine Response Rate

1. DOCUMENT HISTORY

Date	Description	Protocol Version
12-JAN-2017	Version 1	Amendment 1: 20 January 2016
13-APR-2017	Amendment 1	Amendment 2 : 27-March 2017
01-MAR-2018	Amendment 2	Amendment 2 : 27-March 2017

2. STUDY DESIGN

2.1. Design Overview



D=Day; TC = telephone contact, V = Primary Vaccination, *BV = *Unadjuvanted b*ooster vaccination *(3.75 µg HA)*; BS = Blood sample,
FLU Q-Pan H5N1 = A/Indonesia/05/2005 hemagglutinin antigen

- **Experimental design**: Phase II, observer blind, randomized, multi-center, multi-country study, parallel groups.
- **Duration of the study**: approximately 415 days after vaccination on Day 0.
  - The Primary Epoch encompasses data collected from Visit 1 (Day 0) through Visit 5-TC 2 (Day240) and ending at the start of Visit 6 (Day 385).
  - The Booster Epoch encompasses data collected from Visit 6 (Day 385) through Visit 8-TC 3 (Day 415).

Sampling schedule:

- All study groups: Day 0 (before vaccination); Day 42 (post primary course); Day 385 (persistence) and Day 392 (booster response).

Vaccination schedule:

- All subjects are to receive an AS03 adjuvanted H5N1 vaccine given as a two-dose primary series at a 21 day interval.
- All subjects are to receive a 3.75 μg HA, unadjuvanted H5N1 vaccine as a booster dose at Day 385.

2.2. Study groups

Approximately 37 subjects are planned to be enrolled in each of the 5 following study groups:

Study groups	Number of subjects	Epochs	
		Epoch 001 (Primary Series)	Epoch 002 (Booster)
190_B	37	●	●
090_C	37	●	●
190_C	37	●	●
375_C	37	●	●
375_D	37	●	●

Treatment name	Vaccine/Product name (Formulation)	Study Groups				
		190_B	090_C	190_C	375_C	375_D
1.9 mcg H5N1 HA + AS03B	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)	●				
	AS03A (AS03 47.44 mg/mL , final vaccine dose contains 5.93 mg tocopherol)	●				
0.9 mcg H5N1 HA + AS03C	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)		●			
	AS03A (AS03 47.44 mg/mL, final vaccine dose contains 2.97 mg tocopherol)		●			
1.9 mcg H5N1 HA + AS03C	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)			●		
	AS03B (AS03 23.72 mg/mL; final vaccine dose contains 2.97 mg tocopherol			●		
3.75 mcg H5N1 HA + AS03C	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 30 µg/mL)				●	
	AS03B (AS03 23.72 mg/mL; final vaccine dose contains 2.97 mg tocopherol				●	
3.75 mcg H5N1 HA + AS03D	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 30 µg/mL)					●
	AS03C (AS03 11.86 mg/mL; final vaccine dose contains 1.48 mg tocopherol)					●
3.75 mcg H5N1 HA *	FLU-Q-PAN (A/Indonesia/05/2005 H5N1 HA, 15 µg/mL)	●	●	●	●	●

*Booster vaccination to subjects in all study groups

2.3. Group description

The following group names will be used for the statistical analyses at Day 42 and Day 415:

Group order in tables	Group label in tables	Group definition for footnote
1	190_B	1.9 µg H5N1 HA antigen adjuvanted with $AS03_B$
2	090_C	0.9 µg H5N1 HA antigen adjuvanted with $AS03_C$
3	190_C	1.9 µg H5N1 HA antigen adjuvanted with $AS03_C$
4	375_C	3.75 µg H5N1 HA antigen adjuvanted with $AS03_C$
5	375_D	3.75 µg H5N1 HA antigen adjuvanted with $AS03_D$

3. OBJECTIVES

3.1. Co-Primary Objectives

3.1.1. Primary Doses

- To assess the performance of alternative dosing regimens for primary immunization with Q-Pan H5N1 vaccine using an immunogenicity-fever index that considers:
  - immunogenicity by HI assay against vaccine-homologous virus 21 days after the second priming dose, and
  - fever scores after the first and second priming doses.
- To assess the performance of alternative dosing regimens for primary immunization with Q-Pan H5N1 vaccine using an immunogenicity-fever index that considers:
  - immunogenicity by MN assay against vaccine-homologous virus 21 days after the second priming dose and
  - fever scores after the first and second priming doses.

The reference dose for each of these assessments will be 1.9 µg HA with $AS03_B$ (half the approved adult dose).

3.1.2. Booster Dose

- To assess the performance of dosing regimens for booster immunization with Q-Pan H5N1 vaccine considering:
  - immune response by HI assay against vaccine-homologous virus 7 days after a 12-month booster dose of 3.75 µg HA Q-Pan H5N1 ***unadjuvanted*** antigen
- To assess the performance of dosing regimens for booster immunization with Q-Pan H5N1 vaccine considering:
  - immune response by MN assay against vaccine-homologous virus 7 days after a 12-month booster dose of 3.75 µg HA Q-Pan H5N1 ***unadjuvanted*** antigen

3.2. Secondary objectives

- To describe the HI immune response to the vaccine-homologous virus 21 days after the second dose for each dosing regimen
- To assess the performance of alternative dosing regimens for primary immunization with Q-Pan H5N1 vaccine considering persistence of immune response by HI and MN assay at Day 385 in terms of persistence index.
- To assess the performance of the H5N1 vaccine regimens in terms of vaccine-homologous HI and MN antibody titers on Days 0, 42, 385 and Day 392.
- To assess the immunogenicity of the H5N1 vaccine regimens in terms of vaccine-heterologous HI ***and MN*** antibody titers on Days 0, 42, 385 and Day 392.

- To assess vaccine induced cell-mediated immune responses on Days 0, 42, 385 and Day 392.
- To describe reactogenicity and safety of the different priming regimens in terms of solicited (7-days after each vaccination) and unsolicited (21 days after each vaccination) adverse events (AEs).
- To describe safety of the unadjuvanted booster dose in terms of solicited (7 days post boost) and unsolicited (30 days post boost) AEs.
- To describe safety in terms of medically attended AEs (MAEs), potential immune-mediated diseases (pIMDs), and serious adverse events (SAEs), adverse events of special interest (AESIs), during the entire study period.

4. ENDPOINTS

4.1. Primary Endpoints

4.1.1. Immunogenicity-fever indices

Separate HI and MN immunogenicity-fever indices will be constructed based on the following endpoints:

- Humoral immune response in terms of vaccine-homologous HI antibody for each group:
  - LL (lower limit) of 95% CI of GMT group ratio at Day 42 using 1.9 µg HA with $AS03_B$ as reference.
- Humoral immune response in terms of vaccine-homologous MN antibodies for each group:
  - LL of 95% CI GMT group ratio at Day 42 using 1.9 µg HA with $AS03_B$ as reference.
- Fever measurement (≥38°C) post dose 1 and dose 2:
  - For each subject, a fever index will be calculated using temperature measurements 3-days post Dose 1 (D0-D2) and 3-days post Dose 2 (D21-D23).

For details regarding construction of these indices, refer to Section 6.1.2.1 (Statistical Methods- Analysis of immunogenicity).

4.1.2. Immune response to a booster dose

Following booster dose administered at Day 385 the following will be evaluated:

- For immune response in terms of HI antibodies against vaccine-homologous antigen
  - Mean Geometric Increase (MGI) at Day 392 relative to Day 385.
- For the immune response in terms of MN antibodies against vaccine-homologous antigen
  - MGI at Day 392 relative to Day 385.

4.2. Secondary Endpoints

Immunogenicity

- For humoral immune response in terms of HI antibodies against vaccine-homologous/heterologous antigens post-primary immunization, the following aggregate variables will be calculated for each group:
  - Seroconversion rates (SCR) at Day 42
  - Seroprotection rates (SPR) at Day 42
  - MGI at Days 42 relative to Day 0.
- For humoral immune response in terms of vaccine-homologous MN antibody post the primary immunization, following aggregate variables will be calculated for each group:
  - MGI at Day 385 relative to Day 0.
- For humoral immune response in terms of HI antibodies against vaccine-homologous/heterologous antigens (at Days 0, 42 and 385 post the primary immunization, at Day 392 (7 days post booster dose)), the following aggregate variables will be calculated for each group:
  - Seropositivity rates at Days 0, 42, 385 and Day 392
  - Seroconversion rates (SCR) at Day 385 (relative to Day 0) and Day 392 (relative to Days 0 and 385)
  - Seroprotection rates (SPR) at Days 0, 385 and Day 392
  - Geometric Mean Titer (GMT) at Days 0, 42, 385 and Day 392
  - MGI at Day 385 (relative to Day 0), MGI at Day 392 (relative to Days 0 and 385).
- For humoral immune response in terms of vaccine-homologous***/heterologous*** MN antibody, the following aggregate variables will be calculated for each group:
  - Seropositivity rates at Days 0, 42, 385 and Day 392
  - GMT at Days 0, 42, 385 and Day 392

  - Vaccine response rate (VRR) at Days 42, 385 (relative to Day 0) and Day 392 (relative to Days 0 and 385).

- For CMI in terms of frequencies of antigen-specific cells (CD4+/CD8+) at Days 0, 42, 385 and 392:
  - Frequencies of cytokine CD4+/CD8+ T- cells per million CD4+/CD8+ cells producing two or more markers within CD40L, IL-2, TNF-α, IFN-γ upon in vitro stimulation using A/Indonesia/05/2005 (H5N1) split virus as determined by intracellular cytokine staining (ICS) in a sub-cohort of approximately 20 subjects per group at Days 0, 42, 385 and Day 392.

Reactogenicity /Safety:

- Solicited local and general AEs
  - Occurrence of each solicited local AEs during a 7-day follow-up period (i.e., day of vaccination and 6 subsequent days) after any vaccination.
    - Percentage, intensity and duration of solicited local AEs during a 7-day follow-up period (Day 0-Day 6) after any vaccination.
  - Occurrence of each solicited general AEs during a 7-day follow-up period (i.e., day of vaccination and 6 subsequent days) after any vaccination.
    - Percentage, intensity, duration and relationship to vaccination of solicited general AEs during a 7-day follow-up period (Day 0-Day 6) after any vaccination.
- Unsolicited adverse events (AEs)
  - For the primary series: occurrence and relationship to vaccination of unsolicited AEs within 21 days after each vaccine dose.
    - Percentage, intensity and relationship to vaccination of unsolicited AEs during a 21-day follow-up period (Day 0-Day 20) after each vaccine dose.
  - For the booster dose [unadjuvanted]: occurrence and relationship to vaccination of unsolicited AEs within 30 days after vaccination
    - Percentage, intensity and relationship to vaccination of unsolicited AEs during a 30-day follow-up period.
  - Occurrence and relationship to vaccination of (MAEs) during the entire study period.
    - Percentage and relationship to vaccination of MAEs during the entire study period.
  - Occurrence and relationship to vaccination of pIMDs, SAEs, AESIs during the entire study period.
    - Percentage and relationship to vaccination of pIMDs, SAEs, AESIs during the entire study period.

5. STUDY POPULATION

5.1.1. Total Vaccinated Cohort

The total vaccinated cohort (TVC) will include all subjects who received at least one dose of vaccine (TVC for booster safety analysis will include all the subjects who received the booster dose):

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

The TVC analyses will be performed per investigational vaccine actually administered at the first dose.

The analysis of safety will be performed on the TVC.

5.1.2. Cohort for according-to-protocol (ATP) immunogenicity analyses

The ATP cohort for immunogenicity analyses will include all vaccinated/eligible subjects:

- who have received all study vaccine dose(s) (3-doses for booster immunogenicity analysis, 2-doses for other analyses) per protocol treatment assignment;
- for whom the randomization code is unbroken during the relevant analysis interval;
- who have not received any investigational or non-registered product (drug or vaccine) other than the study vaccine(s) during the relevant analysis interval;
- who have not received any non-study vaccine during the relevant analysis interval who have not received any immunoglobulins and/or any blood products during the relevant analysis interval
- for whom there was no chronic administration of immunosuppressants) during the relevant analysis interval.
- who develop a physician-confirmed infection with an A/Indonesia/5/2005 (H5N1)-like influenza virus during the relevant analysis interval.
- who have results available for the relevant assay (HI and MN) for all blood samples to be collected during the relevant analysis intervals for ATP-Day 42; for ATP-Day 385 (persistence); for ATP-Day 392 post booster dose (ATP-booster).

For the final analysis, an Adapted ATP- cohort for immunogenicity will be used. The Adapted ATP cohort for immunogenicity analysis will include all vaccinated/eligible subjects who have results available for the relevant assay (HI and MN) for all blood samples to be collected during the relevant analysis intervals for ATP-Day 42; for ATP-Day 385 (persistence); for ATP-Day 392 post booster dose (ATP-booster).

5.2. Cohort for analysis of the immunogenicity-fever score

The analysis cohort for combined immunogenicity and safety of the two-dose primary series will include all subjects in the ATP cohort for immunogenicity analysis at Day 42 for whom temperature measurements are available during the first 3 days after both vaccine doses 1 and 2.

Subjects without immunogenicity results at Day 42 or for whom one or more daily temperature measurements are missing during the first 3 days after either vaccine dose [dose 1 or dose 2] will be excluded from the immunogenicity/fever index calculation for the primary series.

The list of applicable elimination codes for each cohort can be found in the study specific form FORM-9000036402-13 Criteria for eliminating subjects from the analyses and filled in CARS.

Cohort	Elimination codes	Eli Type
ATP cohort for Day 42 analysis of the immunogenicity	1030;1040;1050;1060;1070;1080; 1090; 2010; 2040; 2050; 2060; 2070; 2080; 2090; 2100; 2500.	PR
ATP - Day 385 (Persistence)	***1030;1040;1050;1060;1070;1080 ; 1090; 2010; 2040; 2050; 2060; 2070; 2080; 2090; 2100; 2500.***	***PE***
ATP Day 392 post-booster dose.	1030;1040;1050;1060;1070;1080; 1090; 2010; 2040; 2050; 2060; 2070; 2080; 2090; 2100; 2500.	MA (including PR)

6. STATISTICAL METHODS

6.1.1. Analysis of demographics

The analysis of demography will be performed on the TVC and on the ATP for immunogenicity.

Demographic characteristics (age at first study vaccination in months; gender; ethnicity) of all subjects will be tabulated by study group and overall using descriptive statistics:

- Frequency tables will be generated for categorical variables such as ethnicity.
- Mean, range, median, and standard deviation will be provided for continuous data such as age.

The distribution of subjects enrolled among the study sites (center) will be tabulated as a whole and per group.

In addition, the following table will be performed for Clinical Trial Register (CTR) posting:

- Percentage of Enrolled subjects by country will be tabulated by group and pooled vaccine groups.

6.1.2. Analysis of immunogenicity

The primary analysis will be based on the ATP cohort for analysis of immunogenicity. If, in any study group, the percentage of vaccinated subjects with serological results excluded from the ATP cohort for analysis of immunogenicity is 5% or more, a second analysis based on the TVC will be performed to complement the ATP analysis.

6.1.2.1. Immunogenicity/fever indices (Co-primary objectives)

An analysis of covariance model (ANCOVA) will be fitted on the $\log_{10}$ transformed HI and MN antibody responses at Day 42, with the vaccine group as a fixed independent variable, adjusted by the $\log_{10}$ transformed pre-vaccination titer and age.

For ***vaccine homologous*** HI and MN separately, an immunogenicity index (D_{GMT}) will be constructed using a desirability function based on the computed GMT group ratio (alternative dose regimen to reference =1.9 µg HA with $AS03_B$) and the 95% CI.

D_{GMT} will be the LL of the 95% CI for GMT group ratio

- If the LL of the 95% CI for GMT group ratio is less than 0.25 (i.e., 4- fold less than that of the reference group), then D_{GMT} =0.
- If the LL of the 95% CI for GMT group ratio is greater than 1 (comparison group has higher GMT value than the reference group), D_{GMT} =1.

  note D_{GMT} =1 for the reference group

The fever index (D_R) will be calculated according to body temperature measurements performed from Days 0-2 after each dose

- Any temperature < 38°C (100.4 F) will be assigned a value of 0. Any temperature > 40.5°C will be assigned a value of 40.5).
- The highest possible temperature value per subject is 243 (6 x 40.5°C; i.e. for 3 days after dose 1 and dose 2);
- The lowest possible temperature value per subject is 0 (all measurements < 38.0°C (100.4 F) for 3 days after dose 1 and dose 2).
- For each subject, a temperature index will be constructed as follows: (243 minus the sum of recorded temperature values for 3 days after dose 1 and dose 2)/243. The average temperature measurement for each vaccine group will be calculated as the D_R. A lower index value D_R indicates a less desirable regimen in terms of reactogenicity.

An immunogenicity-fever index (D) at Day 42 will be computed for each group

$$D = \sqrt{D_{GMT} \times D_R}$$

This index (D) will range between 0 and 1 (0 = not desirable; 1 = highly desirable) and the same weight (0.5) is assigned for immunogenicity index and reactogenicity (fever) index.

Two immunogenicity-fever indices will be separately calculated for HI and MN ***assay against vaccine-homologous virus***. Each will be used to rank the different dosing regimens as a tool to guide dosing regimen selection.

6.1.2.2. Immune Response to a booster dose

There will be two separate evaluations, performed on evaluable subjects (ATP cohort-booster) following a booster dose:

- Point estimates and 95% CIs for MGIs relative to Day 385 will be computed for vaccine-homologous antibody titers assessed by HI at Day 392 for each vaccine regimen
- Point estimates and 95% CIs for MGIs relative to Day 385 will be computed for vaccine-homologous antibody titers assessed by MN at Day 392 for each vaccine regimen

6.1.2.3. Analysis for immunogenicity (secondary objective)

- Point estimates and 95% CIs for MGIs relative to Day 0 will be computed for vaccine-homologous antibody titers assessed by MN at Day 385 for each vaccine regimen.
- Using vaccine-homologous and vaccine-heterologous antibody titers assessed by HI, the point estimates for SCR, SPR, MGI, and the associated 95% CIs will be computed at 21 days after the second dose.
- Using vaccine-homologous and vaccine-heterologous antibody titers assessed by HI, the point estimates and 95% CIs for GMTs and Seropositivity rates (at all timepoints), and SPRs, SCRs and MGIs at Day 385 will be computed for each vaccine regimen.
- Point estimates and 95% CIs for Seropositivity, GMTs and VRRs for vaccine-homologous/***heterologous*** MN titers will be computed for all appropriate timepoints.

All comparisons will be descriptive.

The ATP immunogenicity cohorts (Day 42, persistence or booster) will be used for the related analysis of immunogenicity.

The reverse cumulative distribution curves (RCCs) for HI antibodies against A/Indonesia/05/2005 (H5N1)-like influenza virus at Days 0, 42, 385 and 392 will be performed.

6.1.2.4. Analysis for CMI

- Cell Mediated Immunity (CMI) parameters at Day 0, 42 , 385 and 392 will be evaluated in TVC:
  - Antigen-specific CD4+/CD8+ T Cells identified as CD4/CD8+ T- cells producing two or more markers within CD40L, IL-2, TNF-α, IFN-γ upon ***in vitro*** stimulation using A/Indonesia/05/2005 (H5N1) split virus,
  - The frequency of the response for CD4+/CD8+T-cells stained with probes for various cytokines and activation marker (IFN-γ, TNF-α, IL-2, CD40L) and elicited by vaccine components measured in a sub-cohort of approximately 20 subjects per group at Days 0, 42, 385 and 392 will be described according to the technical specifications provided by R&D (Clinical Data – Information Sheet).

6.1.3. Analysis for safety

- Safety data will be analyzed based on the TVC.
- Analysis will be of subject incidence rates of solicited and unsolicited adverse events (AEs), by solicited local and general AEs terms, and, for unsolicited AEs, by MedDRA preferred term and system organ class. Safety data will be summarized for all subjects by treatment group. The incidence of solicited local and general AEs occurring during 7 days after vaccination will be tabulated with exact 95% CI for each treatment group. The same calculations will be performed for AEs of any

intensity, those with intensity Grade ≥ 2, and those with intensity of Grade 3, as well as for solicited general events assessed as related to vaccination. All solicited local AEs are considered to be related to vaccination.

- The percentage of subjects with at least one report of an unsolicited AE classified by MedDRA (System Organ Class and Preferred Term) 21 days after each primary vaccination will be tabulated with exact 95% CI for each treatment group. The same tabulation will be performed for Grade 3 unsolicited AEs and for unsolicited AEs that are considered by the investigator to be possibly or probably related to vaccination.
- The percentage of subjects with at least one report of an unsolicited AE classified by MedDRA (System Organ Class and Preferred Term) 30 days after the booster vaccination will be tabulated with exact 95% CI. The same tabulation will be performed for Grade 3 unsolicited AEs and for unsolicited AEs that are considered by the investigator to be possibly or probably related to vaccination.
- The proportion of subjects who begin at least one new concomitant medication during the first 42 days after primary vaccination will be calculated with 95% CI.
- MAEs, AESIs, SAEs, and pIMDs will be summarized through the entire study period. In addition, serious AEs and withdrawals due to AE(s) will be described in detail.

The pIMD listings will include pIMDs reported by the Investigator and those identified from the clinical database search using a list of pre-specified pIMD PTs.

The AESIs listings will include AESIs identified from the clinical database search using a list of pre-specified AESIs PTs and SMQs. The AESIs will include the events listed below and the MedDRA PT or SMQ used to identify reports of these events is provided in parentheses:

- Anaphylaxis (narrow SMQs “Anaphylactic reaction” and “Angioedema”)
- Bell’s palsy (MedDRA PT “VIIth nerve palsy”)
- Convulsion (Narrow SMQ “Convulsions”)
- Demyelination (narrow SMQ “Demyelination”)
- Encephalitis (narrow SMQ “Non-infectious encephalitis”)
- Guillain-Barré syndrome (narrow SMQ “Guillain-Barré syndrome”)
- Neuritis (MedDRA PT “Neuritis”)
- Vasculitis (narrow SMQ “Vasculitis”)

7. STATISTICAL CALCULATIONS

7.1. Derived and transformed data

7.1.1. Demography

For a given subject and a given demographic variable, missing measurement will not be replaced except for incomplete date of birth (DOB) data.

7.1.1.1. Age at vaccination

Age will be calculated as the number of months between the DOB and the date of vaccination.

In some countries, to ensure that the collection of DOB will not jeopardise the privacy of personally identifiable information, only a partial DOB (MMYYYY) will be collected.

Therefore, the 15th of the month will be used to replace the missing date.

In case the day and the months are missing, the date will be replaced by the June 30th of the year.

7.1.2. Immunogenicity

The cut-off value for antibody titer is defined by the laboratory before the analysis. **A seronegative** subject is a subject whose antibody titer is below the cut-off value, and conversely; **a seropositive** subject is one whose antibody titer is greater than or equal to the cut-off value. For this study:

- It is assumed that **HI titers** of < 1:10 will be considered below the cut-off. For the calculations of GMT, HI titers of < 10 will be arbitrarily assigned the value 5.
- It is also assumed that **MN titers** of < 1:28 will be considered below the cut-off. For the calculations of GMT, MN titers of < 28 will be arbitrarily the value 14.

Geometric Mean Titer (GMT) calculations are performed by taking the anti-log of the mean of the log (base 10) transformed inverse titers (the number X would denote the inverse of a titer expressed as "1: X"). Antibody titers below the cut-off of the assay are given an arbitrary value of half the cut-off for the purpose of GMT calculation.

Mean Geometric Increase (MGI) for HI is defined as the geometric mean of the within-subject ratios of the post-vaccination reciprocal HI titer to the ***pre-vaccination*** HI titer.

Seroconversion Rate (SCR) is defined as the incidence rate of vaccines who have either a pre-vaccination titer recorded as <1:10 for HI and a post-vaccination reciprocal titer ≥ 40 or a pre-vaccination reciprocal titer ≥10 and at least a 4-fold increase in post-vaccination reciprocal titer.

Incidence Rate of HI Reciprocal Titers ≥ 40 (SPR), defined as the percentage of all vaccinees with a serum reciprocal HI antibody titer ≥ 40 post-vaccination, a level of HI antibodies that may correlate with benefit in protection against influenza.

Vaccine Response Rate (VRR) by MN is defined as the post-vaccination reciprocal titer of ***vaccines*** that have at least 4-fold increase compared with their pre-vaccination reciprocal titer. Antibody titers below the cut-off of the assay are given an arbitrary value of half the cut-off for the purpose of VRR calculation.

Handling of missing immunogenicity data: for a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, analyses will exclude subjects with missing or non-evaluable measurements.

7.1.3. Reactogenicity and safety

Incidence rates of AEs will be calculated as the number of subjects who experience the event, divided by the number of subjects in the safety analysis cohort (the TVC).

Handling of missing safety data:

- **Solicited AEs**: For a given subject and the analysis of solicited AEs within 7 days post-vaccination (Days 0 through 6), missing or non-evaluable measurements will not be replaced.
  - The analysis of the solicited AEs will include only vaccinated subjects and doses with documented safety data (i.e., symptom screen/sheet completed, including the response 'Yes' or 'No' to the solicited symptom page).
  - Subjects who answered "Yes" in the solicited symptom page, but had all symptoms missing are considered as having no solicited symptoms.
  - Subjects who answered 'Yes' to the presence of a specific symptom but partially recorded the daily measurement (e.g. intensity missing for Day 3) over the considered solicited period will be included in the summaries and classified according to their maximum observed daily recording over the solicited period.
  - Subjects who answered 'Yes' to the presence of a specific symptom but recorded no daily measurement over the considered solicited period will not be counted in the summary of symptoms by grade/category but will be part of the summary corresponding to the 'All' category for that symptom.
- **Unsolicited AEs**: For the analysis of unsolicited AEs/MAEs/SAEs/pIMDs/AESIs, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.
- **Concomitant medication and vaccination**: For the analysis of concomitant medications and vaccinations, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.

7.1.3.1. Counting rule

Event	N used for deriving % per subject for vaccination phase
Solicited general symptom	All subjects with at least one solicited general symptom documented as either present or absent (i.e. symptom screen completed)
Solicited local symptom	All subjects with at least one solicited local symptom documented as either present or absent (i.e. symptom screen completed)
Unsolicited symptom MAEs/SAEs/pIMDs/AESIs	All subjects with study vaccine administered
Concomitant medication	All subjects with study vaccine administered
Concomitant vaccination	All subjects with study vaccine administered

7.1.3.2. Coding of grading for Solicited symptoms

The maximum intensity of local injection site redness or swelling will be graded at GSK Biologicals as follows:

0 : ≤ 20 mm
1 : > 20 to 50 mm
2 : > 50 to 100 mm
3 : > 100 mm

Body temperatures will be graded at GSK Biologicals as follows:

0 < 38.0°C (< 100.4°F)
1 ≥ 38.0 – 38.4°C (≥100.4 – 101.2°F)
2 ≥ 38.5 – 38.9°C (≥ 101.3 – 102.1°F)
3 ≥ 39.0 - 40°C (≥ 102.2 – 104.0°F)
4 >40.0°C (>104.0°F)

Fever is defined as temperature ≥ 38.0°C/100.4°F. The preferred route for recording temperature in this study is axillary.

- For clinicaltrials.gov and EudraCT posting purposes, a summary of subjects with all combined solicited (regardless of their duration) and unsolicited adverse events will be provided. Solicited adverse events will be coded by MedDRA as per the following codes:

Solicited symptom	Lower level code	Lower level term
Pain at injection site	10022086	Injection site pain
Redness at injection site	10022098	Injection site redness
Swelling at injection site	10053425	Injection site swelling
Fever	10016558	Fever
Irritability/Fussiness	10057224	Irritability post vaccinal
Loss of appetite	10003028	Appetite lost
Drowsiness	10013649	Drowsiness

7.1.3.3. Onset day

The onset day for an event will be the number of days between the most recent vaccination and the start of the event. Events occurring on the day of vaccination will have an onset day of 0.

7.1.3.4. Duration of events

The duration of an event will be the number of days (not necessarily consecutive) with symptom during the solicited follow-up period.

7.2. Methodology for computing CI

All CI will be 2 sided 95% CI.

- The exact 95% CIs for a proportion within a group will be calculated from Proc StatXact [Clopper CJ, Pearson ES, 1934].
- The 95% CIs for GMT are obtained within each group separately. The 95% CI for the mean of lot-transformed titer is first obtained assuming that log-transformed titers 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 titer.
- The group GMT ratio will be obtained using an ANCOVA model on the logarithm- transformed titers. The ANCOVA model will include the vaccine group as fixed effect and the pre-vaccination $\log_{10}$ titer and, age as regressor. The GMT ratio and its 95% CI will be derived as exponential-transformation of the corresponding group contrast in the model.

7.3. Data presentation description:

The following decimal description from the decision rules will be used for the demography, immunogenicity and safety/reactogenicity.

Display Table	Parameters	Number of decimal digits
Demographic characteristics	Mean, median age	1
Demographic characteristics	SD (age)	1
Immunogenicity	Ratio of GMT	2
Reactogenicity	Mean, Min, Q1, Median, Q3, Max for duration	1
All summaries	% of count, including LL & UL of CI	1
All summaries	% of ratio, including LL & UL of CI	1

7.4. Interpretation of analyses

In this study, all the comparative analyses will be descriptive with the aim to rank alternative dosing regimens with respect to immunogenicity and reactogenicity at Day 42, persistence at Day 385, and anamnestic response to unadjuvanted antigen booster dose at Day 392. In order to evaluate all the effects at the same scale (values between 0-1), a persistence parameter and a booster effect parameter will be calculated for HI and MN:

- Persistence index = (MGI – 4)/MGI for each group.
- Boostability index= (MGI – 4)/MGI for each group.

If the MGI value is less than 4, the index will be set to 0, the immunogenicity- fever, persistence and boostability indices will be ranked for each vaccine group.

Given the complexity of possible outcomes, an algorithm for dose selection will **not** be proposed.

Dose selection will be based on Day 42 immunogenicity and fever as key indicator of reactogenicity, persistence of the immune response and anamnestic response to unadjuvanted antigen as well as any additional safety concerns. Additional practical matters related to the estimated incremental benefit versus the complexity of a pediatric specific dosing regimen, should it not be a fraction of the adult dose, will also be weighed for dose selection as this could lead to increased complexity from a manufacturing and delivery perspective in a pandemic response setting.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

8.1.1. ISRC analyses

iSRC (independent Safety Review Committee) analyses based on TVC are currently performed for the purposes of safety data review. These analyses were described in detail in a separate SAP/TFL associated to iSRC Charter.

8.1.2. Analysis at Day 42

An analysis will be performed on data collected through the Day 42 visit. Elements will include:

- An analysis of cleaned immunogenicity and solicited AEs data collected through the Day 42 visit will be conducted.
- Analyses of unsolicited AEs reported up to the Day 42 visit and cleaned in so far as is possible will be carried out.
- Analyses of MAEs, AESIs, SAEs, pIMDs and withdrawals due to AEs collected up to the Day 42 visit will be carried out.
- Results will be presented in a Day 42 statistical report. Access to individual treatment codes will be restricted to the designated statisticians in charge of the analysis. No individual listings or data with the subjects' identifying information will be disseminated. Listings of final data will be provided with the Day 415 report.

8.1.3. Final analysis

A final data analysis will be performed at the end of study (Day 415) of all primary and secondary endpoints based on the clean data, including evaluations of:

- demography and baseline characteristics
- immunogenicity
- solicited AE data (Day 0-6) after each vaccination
- unsolicited AEs reported up to the Day 42 visit (21 days after each dose), as well as 30 days after the Day 385 booster dose
- concomitant medications reported up to the Day 42 visit, as well as 30 days after the Day 385 booster dose
- MAEs, AESIs, SAEs, pIMDs and withdrawals due to AEs collected throughout the entire study.

Results of the final analysis, as well as individual data listings, will be presented in a final, integrated clinical study report (CSR).

8.2. SDD Stored analysis

Description	Analysis ID (SDD sub-folder)	Disclosure Purpose	*Study Headline Summary (SHS)* requiring expedited communication to Upper Management (Yes/No)	Reference for TFL
iSRC 1 to iSRC 11 iSRC 12 to iSRC 17	E1_02 to E1_12 E1_13 to E1_18	Internal	No	SAP/TFL associated to the Charter
Day 42 Analysis	E1_13	CTR	Yes	TFL for Day 42 and Final Analysis
Final Analysis	E1_01	Study Report (SR) CTR	Yes	TFL for Day 42 and Final Analysis

8.3. Statistical considerations for interim analyses

Not applicable

9. CHANGES FROM PLANNED ANALYSES

- The reverse cumulative distribution curves (RCCs) for HI antibodies against A/Indonesia/05/2005 (H5N1) –like influenza virus at Days 0, 42, 385 and 392 will be performed.
- For clintrial.gov and EudraCT posting purposes, a summary of subjects with all combined solicited (regardless of their duration) and unsolicited AEs will be provided.

10. REFERENCES

The exact 95% CIs for a proportion within a group will be calculated from Proc StatXact [Clopper, 1934*].

Proc StatXact will be used to derive the standardised asymptotic 95% CI for the group difference in proportions [Robert, 1998, method six]. The standardised asymptotic method used within GSK Biologicals is the method six.

Damaso S, Dewé, W, and al. *Selection of a vaccine Formulation in Clinical Development: Study designs and Statistical analysis*, GlaxoSmithKline, **Point to Consider (P-t-C),** August 2014.

Walthere Dewé, Christelle Durand, Sandie Marion, Lidia Oostvogels, Jeanne-Marie, Devaster, Marc Fourneau: *A multi-criteria decision making approach to identify a vaccine formulation,* Journal of Biopharmaceutical Statistics**,** 2015.

* Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. Biometrika, 1934; 26:404-413;