Statistical Analysis Plan

Study alias & e-track number(s): NTHI MCAT-001 (201281)

Detailed Title:	A Phase I, randomised, observer-blind, placebo-controlled, multi-centre study to evaluate the safety, reactogenicity and immunogenicity of GSK Biologicals' GSK3277511A investigational vaccine when administered intramuscularly according to a 0, 2 months schedule in adults
SAP version	Version 1
SAP date	25MAR2016
Scope:	All data pertaining to the above study.
Co-ordinating author:	PPD
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The complete statistical analysis plan 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 SR.

LIST OF ABBREVIATIONS

AE	Adverse event
AECOPD	Acute exacerbation of COPD
ALT	Alanine aminotransferase
ATP	According-To-Protocol
ANCOVA	Analysis of covariance
CI	Confidence Interval
CMI	Cell-mediated immunity
COPD	Chronic obstructive pulmonary disease
CRF	Case Report Form
CTRS	Clinical Trial Registry
EL.U/ml	ELISA unit per milliliter
ELISA	Enzyme-linked immunosorbent assay
Eli Type	Internal GSK database code for type of elimination code
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
FU	Internal GSK database code for Follow-up analysis (elimination codes) link to FORM-BIO-CLIN-9004-05 Criteria for eliminating subjects from the analyses
GMC	Geometric mean antibody concentration
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GSK	GlaxoSmithKline
H. influenzae	Haemophilus influenzae
ICF	Informed consent form
ICS	Intracellular cytokine staining
IU/ml	International units per milliliter
LL	Lower Limit of the confidence interval

MA	Internal GSK database code for Main analysis (elimination codes) link to FORM-BIO-CLIN-9004-05 Criteria for eliminating subjects from the analyses
MATEX	MATerial EXcellence
M. catarrhalis	Moraxella catarrhalis
MedDRA	Medical Dictionary for Regulatory Activities
N.A.	Not Applicable
NTHI	Non-Typeable *Haemophilus influenzae*
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PD	Protein D
PE	Protein E
PI	Prescribing information
PilA	Type IV pili subunit of non-typeable Haemophilus influenzae
pIMD	Potential immune-mediated disease
PT	Preferred term
SAE	Serious adverse event
S. aureus	Staphylococcus aureus
SAP	Statistical Analysis Plan
SBIR	GSK Biological's Internet Randomization System
SD	Standard Deviation
SR	Study Report
STGG	Skim milk, tryptone, glucose, and glycerin transport medium
SYN	Synopsis
TFL	Tables Figures and Listing template annexed to SAP
TVC	Total vaccinated cohort
UL	Upper Limit of the confidence interval
UspA2	Ubiquitous surface protein A2 of Moraxella catarrhalis
VE	Vaccine efficacy

1. DOCUMENT HISTORY

Date	Description	Protocol Version
11-MAR-2016	Version 1	Amendment 1 – 30JUL2015

2. STUDY DESIGN

The following group names will be used for the statistical analyses:

Group order in tables	Group label in tables	Group definition for footnote	Pooled Groups label in tables	Pooled definition for footnote
1	10-10-10	2 doses of non-adjuvanted NTHI/Mcat vaccine containing 10 mcg of PD, PE-PilA and UspA2		
2	10-10-10-AS	2 doses of AS01E-adjuvanted NTHI/Mcat vaccine containing 10 mcg of PD, PE-PilA and UspA2		
3	10-10-3-AS	2 doses of AS01E-adjuvanted NTHI/Mcat vaccine containing 10 mcg of PD, PE-PilA and 3 mcg of UspA2		
4	PLACE1	2 doses of saline solution – Step 1	Placebo	2 doses of saline solution
5	PLACE2	2 doses of saline solution – Step 2	Placebo	

3. OBJECTIVES

3.1. Primary objective

- To evaluate the safety and reactogenicity profile of the NTHI-Mcat investigational vaccines.

3.2. Secondary objective

- To evaluate the humoral and cellular immune response of the NTHI-Mcat investigational vaccines.

3.3. Tertiary objective

- To collect blood samples for assay development/validation and/or for evaluation/characterisation of the humoral and cellular immune responses to components of either the NTHI-Mcat investigational vaccines and/or of other respiratory pathogens.

4. ENDPOINTS

4.1. Primary endpoints

- Occurrence of each solicited local and general AE, during a 7-day follow-up period (*i.e.* day of vaccination and 6 subsequent days) post-Dose 1 and post-Dose 2, in all subjects, in all vaccine groups.
- Occurrence of any unsolicited AEs, during a 30-day follow-up period (i.e. day of vaccination and 29 subsequent days) post-Dose 1 and post-Dose 2, in all subjects, in all vaccine groups.
- Occurrence of haematological and biochemical laboratory abnormalities, after vaccination, in all subjects, in all vaccine groups:
  - Any haematological (RBC, WBC and differential count, platelets count and haemoglobin level) or biochemical (ALT, AST and creatinine) laboratory abnormality on Day 7, Day 60, Day 67, Day 210 and Day 420.
- Occurrence of any SAE, occurring from first vaccination (Day 0) to study conclusion (Day 420) in all subjects, in all vaccine groups.
- Occurrence of any pIMD occurring from first vaccination (Day 0) to study conclusion (Day 420) in all subjects, in all vaccine groups.

4.2. Secondary endpoints

- Humoral immune response to the components of the NTHI-Mcat vaccine formulations, on Day 0, Day 30, Day 60, Day 90, Day 210 and Day 420, in all subjects, in all vaccine groups:
  - Anti-PD, anti-PE, anti-PilA and anti-UspA2 antibody concentrations.
- Cell-mediated immune response to components of the NTHI-Mcat vaccine formulations, on Day 0, Day 60, Day 90, Day 210 and Day 420, in a sub-cohort of subjects, in all vaccine groups:
  - Frequency of specific $CD4^+/CD8^+$ T-cells measured on cryopreserved peripheral blood mononuclear cells (PBMCs) and identified by flow cytometry intracellular cytokine staining (ICS) expressing two or more markers (such as IL-2, IL-13, IL-17, IFN-γ, TNF-α and CD40L).

5. STUDY POPULATION

The following subject cohorts will be evaluated.

5.1.1. Total vaccinated cohort

The total vaccinated cohort (TVC) will include all subjects with at least one vaccine administration documented:

- 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 analysis will be performed per treatment actually administered at Dose 1.

5.1.2. According-to-protocol cohort

The ATP cohorts will be defined by epoch and will consist of all subjects from the TVC who will comply with eligibility criteria, study procedures up to the end of the epoch and had immunogenicity results in the epoch.

5.1.2.1. According-to-protocol cohort for analysis of immunogenicity (Epoch 001)

The ATP cohort for immunogenicity will include all subjects in the TVC:

- Who met all eligibility criteria.
- For whom the administration route and site of the vaccines was according to protocol.
- Who complied with the vaccination schedule.
- Who did not receive a concomitant medication/ product/vaccine leading to the elimination from the ATP analysis up the one month post-Dose 2 visit (Visit 6, Day 90).
- Who did not present with an intercurrent medical condition leading to elimination from the ATP analysis up to the one month post-Dose 2 visit (Visit 6, Day 90).
- Who complied with the immunogenicity blood sample timings at 1 month post-Dose 2 visit (Visit 6, Day 90).
- For whom post-Dose 2 immunogenicity results are available for at least one assay.

5.1.2.2. According-to-protocol cohort for analysis of persistence of immunogenicity (Epoch 002)

The ATP cohort for persistence of immunogenicity will include all evaluable subjects, i.e., those who were included in the ATP cohort for immunogenicity, or were excluded from this cohort solely because they had no blood samples taken or because of non-compliance with blood sample timings up to the one month post-Dose 2 visit (Visit 6, Day 90), and:

- Who did not receive a concomitant medication/ product/vaccine leading to elimination from the ATP analysis for immunogenicity.
- Who did not present with an intercurrent medical condition leading to elimination from the ATP analysis for immunogenicity.
- Who complied with at least one of the blood sample timings after the one month post-last vaccination visit (Visit 6, Day 90).
- For whom persistence immunogenicity results are available for at least one assay in at least one of the two persistence time points (Visit 7, Day 210 and Visit 8, Day 420).

The list of applicable elimination codes for each cohort can be found in the study specific form FORM-BIO-CLIN-9004-05 Criteria for eliminating subjects from the analyses.

Cohort	Elimination codes	Eli Type
ATP cohort for analysis of immunogenicity	1010-2500	MA
ATP cohort for analysis of persistence of immunogenicity	1010-2500	FU

6. STATISTICAL METHODS

6.1. Analysis up to Visit 6 (Day 90)

The final analyses will be descriptive and will be presented by study group, with pooled data from the two steps for the placebo groups.

6.1.1. Analysis of demographics

Demographic characteristics (age at the first dose in years, gender and race), cohort description and other characteristics such as smoking/exposure history status, pulmonary function test baseline results, height, weight and previous vaccination with a pneumococcal vaccine or an influenza vaccine will be summarized by group using descriptive statistics:

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

- The distribution of subjects enrolled among the study sites will be tabulated as a whole and per group.
- Withdrawal status will be summarised by group using descriptive statistics:
  - The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal.
- The number of subjects enrolled into the study as well as the number of subjects excluded from ATP analyses will be tabulated.

6.1.2. Analysis of safety

The primary analysis will be performed on the TVC and, if in any vaccine group and at any time point the percentage of vaccinated subjects excluded from the ATP cohort is at least 10%, a second analysis will be performed on the ATP cohort to complement the TVC.

The percentage of subjects with at least one **local AE** (solicited and unsolicited), with at least one **general AE** (solicited and unsolicited) and with any AE during the 7-day or 30-day follow-up period will be tabulated after Dose 1 and Dose 2 and overall with exact 95% CI. The same computations will be done for Grade 3 AEs, any AEs causally related to vaccination and any Grade 3 AEs causally related to vaccination.

The percentage of subjects/doses reporting each individual solicited local (any grade, Grade 3) and general (any grade, Grade 3, any causally related to vaccination and any Grade 3 causally related to vaccination) AE during the 7-days (Day 0 to Day 6) follow-up period will be tabulated for each group as follows:

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

The exact 95% CIs will be calculated assuming independence between doses. For fever, additional analyses will be performed by 0.5°C increments.

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

For each group and for each **haematology and biochemistry parameter**:

- The percentage of subjects having haematology and biochemistry results below or above the normal laboratory ranges will be tabulated by time point.
- The maximum grading from Screening up to Visit 6 (Day 90) will be tabulated (grades will be based on local laboratory normal ranges and derived from FDA Guidance to Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". Those laboratory parameters not included in the FDA grading scale will not be graded).

The number of subjects who experienced at least one **SAE** or any **pIMDs** from first vaccination up to 30 days post-Dose 2 will be reported.

The percentage of subjects/dose using **concomitant medication/ product** (any medication/ product, any antipyretic and any antipyretic taken prophylactically, respectively) during the 30-day follow-up period (Day 0 – Day 29) will be summarised per group for each dose and overall per dose.

The number of subjects who experienced any **AE leading to study withdrawal**, or any **SAE related to study participation or concurrent GSK medication/ vaccination,** from first vaccination up to 30 days post-Dose 2 will be reported.

Pregnancy reports from first vaccination up to 30 days post-Dose 2 and pregnancy outcomes will be described in detail.

6.1.3. Analysis of immunogenicity

The primary analysis will be performed on the ATP cohort for immunogenicity and if, in any study group, the percentage of vaccinated subjects with serological results excluded from the ATP cohort is at least 10%, a second analysis will be performed on the TVC.

6.1.3.1. Humoral immune response

Within group evaluation

For each group, at each time point during which blood samples are collected for humoral immune response and for each assay:

- Seropositivity rate and their exact 95% CI will be tabulated.
- GMCs and their 95% CI will be calculated.
- Antibody concentrations distribution will be investigated using Reverse Cumulative Curves.

These analyses will also be performed for each level of the following minimisation factors: age (50-59 years vs. 60-70 years), smoking status (current vs. former smoker), FEV_1/FCV ratio ($\geq$0.7 vs. <0.7).

Between groups evaluation (only for Step 2)

Comparative analyses will be exploratory with the aim to characterise the difference between the 10-10-10-AS and 10-10-3-AS groups in humoral immune response.

The difference in terms of GMCs will be evaluated, one month post-Dose 2, by computing the 95% CIs of the GMC ratio between groups for Step 2 by using a one-way ANCOVA model on the logarithm10 transformation of the concentrations/ titres. The ANCOVA model will include the group category, the age category, the smoking status and FEV_1/FVC ($\geq$ 0.7 or < 0.7) and the pre-Dose 1 concentration (as covariate) as fixed effects. The groups will be considered significantly different if the 95% CI for the GMC ratio between the two groups does not contain the value 1.

However, these differences should be interpreted with caution considering that there will be no adjustment for multiplicity of endpoints.

6.1.3.2. CMI response

The frequency of specific $CD4^+$/ $CD8^+$ T-cells will be summarised (mean, SD, minimum, Q1, median, Q3, and maximum) by group, at each time point during which blood samples are collected for CMI (descriptive statistics).

Each data will be obtained by subtracting the summary of the background value to each summary value. Values less than or equal to zero will be set to 1 for the purpose of geometric mean calculation and graphical representation.

6.1.3.3. Summary score to support dose selection

The calculation of a summary score for both active groups of Step 2 (i.e. 10-10-10-AS and 10-10-3-AS) will be proposed in order to help the formulation selection. The group with the highest summary score should potentially be the one considered for selection provided it has an acceptable safety and CMI profile. However note that a difference of less than 0.02 between 2 summary scores will not be sufficient to consider that a formulation should be ranked higher than/preferred to the other. Unacceptable safety profile encompasses cases such as high occurrence of grade 3 events, occurrence of specific serious adverse event. Unacceptable CMI profile encompasses cases with a pronounced Th_2 response in which there is an isolated elevated IL-13 response, in particular without a response in terms of IFN-γ. The discarding of a group from the selection because of specific safety or CMI reason will be substantiated in the CSR.

If the group(s) ultimately selected for use in upcoming studies for COPD patients is(/are) not the one with the highest summary scores at post dose 2 and/or post dose 3, the disqualification of the group(s) with higher summary scores should be adequately justified in the CSR.

The summary scores will be calculated based on the subjects from the ATP cohort.

For each group *g*, the summary score will summarize how well the group is performing compared to the average active group in terms of the following immunogenicity and CMI results. Before defining the summary score several quantities are introduced (for *XX* being PD, PE, or PilA):

- GM_{XX_g} : the 30 days post last vaccination GMC of Anti-*XX* of group g
- $AvGM_{XX}$: the 30 days post last vaccination GMC of Anti-*XX* of all subjects in any active group
- GM_{CMIXX_g} : the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of group g
- $AvGM_{CMIXX}$ the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of all subjects in any active group

These measures will be transformed in order to be contained between 0 and 1 via the following formulae:

$$D_{XX_g} = 1 / (1 + \mathrm{Exp}((AvGM_{XX} - GM_{XX_g})/AvGM_{XX}))$$

$$D_{CMIXX_g} = 1 / (1 + \mathrm{Exp}((AvGM_{CMIXX} - GM_{CMIXX_g})/AvGM_{CMIXX}))$$

The following groups will be evaluated at Post-dose 2:

- 10-10-10-AS summary score Post-dose 2

- 10-10-3-AS summary score Post-dose 2

The average GMCs used to calculate the summary scores will be an average of all vaccinated subjects from an active group at post dose 2.

The summary score for group g will be computed by taking the weighted geometric mean of the several D measures as follows:

$$D_g = \sqrt[15]{D_{PDg}^4 \times D_{PEg}^4 \times D_{PilAg}^4 \times D_{CMIPDg}^1 \times D_{CMIPEg}^1 \times D_{CMIPilAg}^1}$$

A group with a D score of ½ corresponds exactly at the average level of all subjects in the active groups. The highest the value of the summary score for a group, the better the group is performing in terms of immunogenicity and CMI compared to the others.

Differences of less than 0.02 in terms of summary measures will not be considered sufficient to establish a clear hierarchy between 2 groups.

6.2. Analysis up to Visit 8 (Day 420)

The final analyses will be descriptive and will be presented by study group, with pooled data from the two steps for the placebo groups.

6.2.1. Analysis of demographics

Withdrawal status will be summarised by group using descriptive statistics:

The number of subjects enrolled into the study as well as the number of subjects excluded from ATP analyses will be tabulated.

The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal.

6.2.2. Analysis of safety

The primary analysis will be performed on the TVC and, if in any vaccine group and at any time point the percentage of vaccinated subjects excluded from the ATP cohort is at least 10%, a second analysis will be performed on the ATP cohort to complement the TVC.

The percentage of subjects with at least one **local AE** (solicited and unsolicited), with at least one **general AE** (solicited and unsolicited) and with any AE during the 7-day or 30-day follow-up period will be tabulated after Dose 1 and Dose 2 and overall with exact

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For each group and for each **haematology and biochemistry parameter**:

- The percentage of subjects having haematology and biochemistry results below or above the normal laboratory ranges will be tabulated by time point.
- The maximum grading from Screening up to Visit 8 (Day 420) will be tabulated (grades will be based on local laboratory normal ranges and derived from FDA Guidance to Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". Those laboratory parameters not included in the FDA grading scale will not be graded).

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These analyses will also be performed for each level of the following minimisation factors: age (50-59 years vs. 60-70 years), smoking status (current vs. former smoker), FEV_1/FCV ratio ($\geq$0.7 vs. <0.7).

Between groups evaluation (only for Step 2)

Comparative analyses will be exploratory with the aim to characterise the difference between the 10-10-10-AS and 10-10-3-AS groups in humoral immune response.

The difference in terms of GMCs will be evaluated, one month post-Dose 2, by computing the 95% CIs of the GMC ratio between groups for Step 2 by using a one-way ANCOVA model on the logarithm10 transformation of the concentrations/ titres. The ANCOVA model will include the group category, the age category, the smoking status and FEV_1/FVC ($\geq$ 0.7 or < 0.7) and the pre-Dose 1 concentration (as co-variable) as fixed effects. The groups will be considered significantly different if the 95% CI for the GMC ratio between the two groups does not contain the value 1.

However, these differences should be interpreted with caution considering that there will be no adjustment for multiplicity of endpoints.

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Each data will be obtained by subtracting the summary of the background value to each summary value. Values less than or equal to zero will be set to 1 for the purpose of geometric mean calculation and graphical representation.

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The calculation of a summary score for both active groups of Step 2 (i.e. 10-10-10-AS and 10-10-3-AS) will be proposed in order to help the formulation selection. The group(s) with the highest summary score(s) should potentially be the one(s) considered for selection provided it (they) has (ve) an acceptable safety and CMI profile. However note that a difference of less than 0.02 between 2 summary scores will not be sufficient to consider that a formulation should be ranked higher than/preferred to the other. Unacceptable safety profile encompasses cases such as high occurrence of grade 3 events, occurrence of specific serious adverse event. Unacceptable CMI profile encompasses cases with a pronounced Th_2 response in which there is an isolated elevated IL-13 response, in particular without a response in terms of IFN-γ. The discarding of a group from the selection because of specific safety or CMI reason will be substantiated in the CSR.

If the group(s) ultimately selected for use in upcoming studies for COPD patients is(/are) not the one with the highest summary scores at post dose 2 and/or post dose 3, the disqualification of the group(s) with higher summary scores should be adequately justified in the CSR.

The summary scores will be calculated based on the subjects from the ATP cohort.

For each group *g*, the summary score will summarize how well the group is performing compared to the average active group in terms of the following immunogenicity and CMI results. Before defining the summary score several quantities are introduced (for *XX* being PD, PE, or PilA):

- GM_{XX_g} : the 30 days post last vaccination GMC of Anti-*XX* of group g
- $AvGM_{XX}$: the 30 days post last vaccination GMC of Anti-*XX* of all subjects in any active group
- GM_{CMIXX_g} : the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of group g

- AvGM$_{CMIXX}$ the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of all subjects in any active group

These measures will be transformed in order to be contained between 0 and 1 via the following formulae:

$$D_{XXg} = 1 / (1 + \text{Exp}((\text{AvGM}_{XX} - \text{GM}_{XXg})/\text{AvGM}_{XX}))$$

$$D_{CMIXXg} = 1 / (1 + \text{Exp}((\text{AvGM}_{CMIXX} - \text{GM}_{CMIXXg})/\text{AvGM}_{CMIXX}))$$

The following groups will be evaluated at Post-dose 2:

- 10-10-10-AS summary score Post-dose 2
- 10-10-3-AS summary score Post-dose 2

The average GMCs used to calculate the summary scores will be an average of all vaccinated subject from an active group at post dose 2.

The summary score for group g will be computed by taking the weighted geometric mean of the several D measures as follows:

$$D_g = \sqrt[15]{D_{PDg}^4 \times D_{PEg}^4 \times D_{PilAg}^4 \times D_{CMIPDg}^1 \times D_{CMIPEg}^1 \times D_{CMIPilAg}^1}$$

A group with a D score of ½ corresponds exactly at the average level of all subjects in the active groups. The highest the value of the summary score for a group, the better the group is performing in terms of immunogenicity and CMI compared to the others.

Differences of less than 0.02 in terms of summary measures will not be considered sufficient to establish a clear hierarchy between 2 groups. Therefore several groups may be considered to be classified as the best ones.

Although these comparisons are exploratory and should be interpreted with caution considering that there is no adjustment for multiplicity, a statistical significance on at least one GMC of the selected antigen is expected to confirm the potential use of the selected active group in future studies.

7. STATISTICAL CALCULATIONS

The study groups will be defined by treatment actually administered at Dose 1.

Demography

- For a given subject and a given demographic variable, missing measurement will not be replaced.
- Age: Age at the reference activity, computed as the number of units between the date of birth and the reference activity In case of partial dates of any of these 2 dates:
  - 15th of month, If only the day is missing
  - 30th of June, if day and months are missing

Immunogenicity

- For a given subject and the analysis of a given immunogenicity measurement, missing or un-evaluable measurements will not be replaced.
- A seronegative subject is defined as a subject whose antibody concentration is below the assay cut-off value.
- A seropositive subject is defined as a subject whose antibody concentration is greater than or equal to the assay cut-off value.
- Antibody concentrations below the assay cut-off will be given an arbitrary value of half the assay cut-off for the purpose of geometric mean concentration (GMC) calculation.
- Calculation of the GMCs will be performed by taking the anti-logarithm in base 10 (anti-log10) of the mean of the log10 concentration/titre transformations.
- All confidence intervals (CIs) computed will be two-sided 95% CIs.
- The assay cut-off is the value under which there is no quantifiable result available. For an assay with a specific 'cut_off' , numerical immuno result is derived from a character field (rawres):
  - If rawres is 'NEG' or '-' or '(-)', numeric result= cutt_off/2,
  - if rawres is 'POS' or '+' or '(+)', numeric result = cut_off,
  - if rawres is '< value' and value<=cut_off, numeric result =cut_off/2,
  - if rawres is '< value' and value>cut_off, numeric result =value,
  - if rawres is '> value' and value<cut_off, numeric result =cut_off/2,
  - if rawres is '> value' and value>=cut_off, numeric result =value,
  - if rawres is '<= value' or '>= value' and value<cut_off, numeric result =cut_off/2,
  - if rawres is '<= value' or '>= value' and value>=cut_off, numeric result =value,
  - if rawres is a value < cut_off, numeric result = cut_off/2,

- if rawres is a value >= cut_off, numeric result = rawres,
- else numeric result is left blank.

Safety/reactogenicity

- For **solicited symptoms**, the analysis will exclude subjects with missing or un-evaluable measurements (e.g. total analysis of solicited symptoms will include all vaccinated subjects with documented solicited symptom sheets).
- For the **unsolicited symptoms** and concomitant medications/ products/ vaccinations, subjects who did not report unsolicited symptoms/concomitant medications/ products/ vaccinations will be treated as subjects without unsolicited symptoms or concomitant medications/ products/ vaccinations, respectively.
- Note that for all tables described in this section, the way the percentage of subjects will be derived will depend on the event analysed (see table below for details). As a result, the N value will differ from one table to another.

Event	N used for deriving % per subject
Concomitant vaccination	All subjects with study vaccine administered
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	All subjects with study vaccine administered
Concomitant medication	All subjects with study vaccine administered

7.1. Data presentation description

The following decimal description will be used for the demography and safety/ reactogenicity.

Display Table	Parameters	Number of decimal digits
All summaries	% of count, including LL & UL of CI	1
All summaries	% of difference, including LL & UL of CI	2
Demographic characteristics	Mean, median age	1
Demographic characteristics	SD (age)	2
Immunogenicity	GMT ratio	2

The following data presentation description will be used for the immunogenicity analyses.

Component	GMC/T	Assay		Assay cut-off	Number of decimal digits		Tick marks on RCC's	
					GMC/T	GMC/T ratio		
		method	Unit				Low value	High value
Anti-PD	GMC	ELISA	EL.U/mL	100	1	2	1	10000
Anti-PE	GMC	ELISA	EL.U/mL	8	1	2	1	10000
Anti-PilA	GMC	ELISA	EL.U/mL	7	1	2	1	10000
Anti-UspA2	GMC	ELISA	EL.U/mL	18	1	2	1	10000

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

- The analysis of data up to Visit 6 (Day 90, end of Epoch 001) will be performed in a first step. This analysis will include:
  - The final analysis of all immunogenicity results (including CMI), solicited AEs post-Dose 1 and post-Dose 2,
  - The assessment of unsolicited AEs up to 30 days post-Dose 1 and post-Dose 2, and of SAEs and pIMDs up to 30 days post-Dose 2 on as cleaned as possible data. No individual listings will be written at this stage.

  This analysis will be documented in a statistical report. At this point, the GSK statistician will be unblinded (i.e. will have access to the individual subject treatment assignments).
- The analysis of Epoch 002 (from Visit 7 [Day 210] up to Visit 8 [Day 420]) will be performed in a second step, once those data will be available and cleaned. This analysis will include:
  - The final analysis of all immunogenicity results (including CMI) up to Visit 8 (Day 420).
  - SAEs and pIMDs up to Visit 8 (Day 420) on cleaned data.

  In addition, all previous analyses will be re-produced based on cleaned data at this point.

  Individual listings will only be provided at this stage.

Description	Analysis ID	Disclosure Purpose
Analysis up to Visit 6 (Day 90)	E1_02	Internal
Final analysis (up to day 450)	E1_01	CTRS & Clinical Study Report

8.2. Statistical considerations for interim analyses

Not applicable.

9. CHANGES FROM PLANNED ANALYSES

None.

10. REFERENCES

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].

Statistical Analysis Plan

Study alias & e-track number(s): NTHI MCAT-001 (201281)

Detailed Title:	A Phase I, randomised, observer-blind, placebo-controlled, multi-centre study to evaluate the safety, reactogenicity and immunogenicity of GSK Biologicals' GSK3277511A investigational vaccine when administered intramuscularly according to a 0, 2 months schedule in adults
SAP version	Amendment 1
SAP date	27JUN2016
Scope:	All data pertaining to the above study.
Co-ordinating author:	PPD
Other author(s):	
Adhoc reviewers:	PPD, Global Regulatory Lead (RA) PPD, PPD, Safety Representatives CTRS team
Approved by:	PPD, Clinical Research & Development Lead PPD, Lead Statistician PPD, Scientific Writer PPD, Statistician

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The complete statistical analysis plan 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 SR.

LIST OF ABBREVIATIONS

AE	Adverse event
AECOPD	Acute exacerbation of COPD
ALT	Alanine aminotransferase
ATP	According-To-Protocol
ANCOVA	Analysis of covariance
CI	Confidence Interval
CMI	Cell-mediated immunity
COPD	Chronic obstructive pulmonary disease
CRF	Case Report Form
CTRS	Clinical Trial Registry
EL.U/ml	ELISA unit per milliliter
ELISA	Enzyme-linked immunosorbent assay
Eli Type	Internal GSK database code for type of elimination code
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
FU	Internal GSK database code for Follow-up analysis (elimination codes) link to FORM-BIO-CLIN-9004-05 Criteria for eliminating subjects from the analyses
GMC	Geometric mean antibody concentration
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GSK	GlaxoSmithKline
H. influenzae	Haemophilus influenzae
ICF	Informed consent form
ICS	Intracellular cytokine staining
IU/ml	International units per milliliter
LL	Lower Limit of the confidence interval

MA	Internal GSK database code for Main analysis (elimination codes) link to FORM-BIO-CLIN-9004-05 Criteria for eliminating subjects from the analyses
MATEX	MATerial EXcellence
M. catarrhalis	Moraxella catarrhalis
MedDRA	Medical Dictionary for Regulatory Activities
N.A.	Not Applicable
NTHI	Non-Typeable *Haemophilus influenzae*
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PD	Protein D
PE	Protein E
PI	Prescribing information
PilA	Type IV pili subunit of non-typeable Haemophilus influenzae
pIMD	Potential immune-mediated disease
PT	Preferred term
SAE	Serious adverse event
S. aureus	Staphylococcus aureus
SAP	Statistical Analysis Plan
SBIR	GSK Biological's Internet Randomization System
SD	Standard Deviation
SR	Study Report
STGG	Skim milk, tryptone, glucose, and glycerin transport medium
SYN	Synopsis
TFL	Tables Figures and Listing template annexed to SAP
TVC	Total vaccinated cohort
UL	Upper Limit of the confidence interval
UspA2	Ubiquitous surface protein A2 of Moraxella catarrhalis
VE	Vaccine efficacy

1. DOCUMENT HISTORY

Date	Description	Protocol Version
11-MAR-2016	Version 1	Amendment 1 – 30JUL2015
27-JUN-2016	Amendment 1 – Anti-PD cut-off was updated from 100 EL.U/mL to 153 EL.U/mL following the development of the new anti-PD test.	Amendment 1 – 30JUL2015

2. STUDY DESIGN

The following group names will be used for the statistical analyses:

Group order in tables	Group label in tables	Group definition for footnote	Pooled Groups label in tables	Pooled definition for footnote
1	10-10-10	2 doses of non-adjuvanted NTHI/Mcat vaccine containing 10 mcg of PD, PE-PilA and UspA2		
2	10-10-10-AS	2 doses of AS01E-adjuvanted NTHI/Mcat vaccine containing 10 mcg of PD, PE-PilA and UspA2		
3	10-10-3-AS	2 doses of AS01E-adjuvanted NTHI/Mcat vaccine containing 10 mcg of PD, PE-PilA and 3 mcg of UspA2		
4	PLACE1	2 doses of saline solution – Step 1	Placebo	2 doses of saline solution
5	PLACE2	2 doses of saline solution – Step 2	Placebo	

3. OBJECTIVES

3.1. Primary objective

- To evaluate the safety and reactogenicity profile of the NTHI-Mcat investigational vaccines.

3.2. Secondary objective

- To evaluate the humoral and cellular immune response of the NTHI-Mcat investigational vaccines.

3.3. Tertiary objective

- To collect blood samples for assay development/validation and/or for evaluation/characterisation of the humoral and cellular immune responses to

components of either the NTHI-Mcat investigational vaccines and/or of other respiratory pathogens.

4. ENDPOINTS

4.1. Primary endpoints

- Occurrence of each solicited local and general AE, during a 7-day follow-up period (*i.e.* day of vaccination and 6 subsequent days) post-Dose 1 and post-Dose 2, in all subjects, in all vaccine groups.
- Occurrence of any unsolicited AEs, during a 30-day follow-up period (i.e. day of vaccination and 29 subsequent days) post-Dose 1 and post-Dose 2, in all subjects, in all vaccine groups.
- Occurrence of haematological and biochemical laboratory abnormalities, after vaccination, in all subjects, in all vaccine groups:
  - Any haematological (RBC, WBC and differential count, platelets count and haemoglobin level) or biochemical (ALT, AST and creatinine) laboratory abnormality on Day 7, Day 60, Day 67, Day 210 and Day 420.
- Occurrence of any SAE, occurring from first vaccination (Day 0) to study conclusion (Day 420) in all subjects, in all vaccine groups.
- Occurrence of any pIMD occurring from first vaccination (Day 0) to study conclusion (Day 420) in all subjects, in all vaccine groups.

4.2. Secondary endpoints

- Humoral immune response to the components of the NTHI-Mcat vaccine formulations, on Day 0, Day 30, Day 60, Day 90, Day 210 and Day 420, in all subjects, in all vaccine groups:
  - Anti-PD, anti-PE, anti-PilA and anti-UspA2 antibody concentrations.
- Cell-mediated immune response to components of the NTHI-Mcat vaccine formulations, on Day 0, Day 60, Day 90, Day 210 and Day 420, in a sub-cohort of subjects, in all vaccine groups:
  - Frequency of specific $CD4^+/CD8^+$ T-cells measured on cryopreserved peripheral blood mononuclear cells (PBMCs) and identified by flow cytometry intracellular cytokine staining (ICS) expressing two or more markers (such as IL-2, IL-13, IL-17, IFN-γ, TNF-α and CD40L).

5. STUDY POPULATION

The following subject cohorts will be evaluated.

5.1.1. Total vaccinated cohort

The total vaccinated cohort (TVC) will include all subjects with at least one vaccine administration documented:

- 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 analysis will be performed per treatment actually administered at Dose 1.

5.1.2. According-to-protocol cohort

The ATP cohorts will be defined by epoch and will consist of all subjects from the TVC who will comply with eligibility criteria, study procedures up to the end of the epoch and had immunogenicity results in the epoch.

5.1.2.1. According-to-protocol cohort for analysis of immunogenicity (Epoch 001)

The ATP cohort for immunogenicity will include all subjects in the TVC:

- Who met all eligibility criteria.
- For whom the administration route and site of the vaccines was according to protocol.
- Who complied with the vaccination schedule.
- Who did not receive a concomitant medication/ product/vaccine leading to the elimination from the ATP analysis up the one month post-Dose 2 visit (Visit 6, Day 90).
- Who did not present with an intercurrent medical condition leading to elimination from the ATP analysis up to the one month post-Dose 2 visit (Visit 6, Day 90).
- Who complied with the immunogenicity blood sample timings at 1 month post-Dose 2 visit (Visit 6, Day 90).
- For whom post-Dose 2 immunogenicity results are available for at least one assay.

5.1.2.2. According-to-protocol cohort for analysis of persistence of immunogenicity (Epoch 002)

The ATP cohort for persistence of immunogenicity will include all evaluable subjects, i.e., those who were included in the ATP cohort for immunogenicity, or were excluded from this cohort solely because they had no blood samples taken or because of non-compliance with blood sample timings up to the one month post-Dose 2 visit (Visit 6, Day 90), and:

- Who did not receive a concomitant medication/ product/vaccine leading to elimination from the ATP analysis for immunogenicity.
- Who did not present with an intercurrent medical condition leading to elimination from the ATP analysis for immunogenicity.
- Who complied with at least one of the blood sample timings after the one month post-last vaccination visit (Visit 6, Day 90).
- For whom persistence immunogenicity results are available for at least one assay in at least one of the two persistence time points (Visit 7, Day 210 and Visit 8, Day 420).

The list of applicable elimination codes for each cohort can be found in the study specific form FORM-BIO-CLIN-9004-05 Criteria for eliminating subjects from the analyses.

Cohort	Elimination codes	Eli Type
ATP cohort for analysis of immunogenicity	1010-2500	MA
ATP cohort for analysis of persistence of immunogenicity	1010-2500	FU

6. STATISTICAL METHODS

6.1. Analysis up to Visit 6 (Day 90)

The final analyses will be descriptive and will be presented by study group, with pooled data from the two steps for the placebo groups.

6.1.1. Analysis of demographics

Demographic characteristics (age at the first dose in years, gender and race), cohort description and other characteristics such as smoking/exposure history status, pulmonary function test baseline results, height, weight and previous vaccination with a pneumococcal vaccine or an influenza vaccine will be summarized by group using descriptive statistics:

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

- The distribution of subjects enrolled among the study sites will be tabulated as a whole and per group.
- Withdrawal status will be summarised by group using descriptive statistics:
  - The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal.
- The number of subjects enrolled into the study as well as the number of subjects excluded from ATP analyses will be tabulated.

6.1.2. Analysis of safety

The primary analysis will be performed on the TVC and, if in any vaccine group and at any time point the percentage of vaccinated subjects excluded from the ATP cohort is at least 10%, a second analysis will be performed on the ATP cohort to complement the TVC.

The percentage of subjects with at least one **local AE** (solicited and unsolicited), with at least one **general AE** (solicited and unsolicited) and with any AE during the 7-day or 30-day follow-up period will be tabulated after Dose 1 and Dose 2 and overall with exact 95% CI. The same computations will be done for Grade 3 AEs, any AEs causally related to vaccination and any Grade 3 AEs causally related to vaccination.

The percentage of subjects/doses reporting each individual solicited local (any grade, Grade 3) and general (any grade, Grade 3, any causally related to vaccination and any Grade 3 causally related to vaccination) AE during the 7-days (Day 0 to Day 6) follow-up period will be tabulated for each group as follows:

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

The exact 95% CIs will be calculated assuming independence between doses. For fever, additional analyses will be performed by 0.5°C increments.

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

For each group and for each **haematology and biochemistry parameter**:

- The percentage of subjects having haematology and biochemistry results below or above the normal laboratory ranges will be tabulated by time point.
- The maximum grading from Screening up to Visit 6 (Day 90) will be tabulated (grades will be based on local laboratory normal ranges and derived from FDA Guidance to Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". Those laboratory parameters not included in the FDA grading scale will not be graded).

The number of subjects who experienced at least one **SAE** or any **pIMDs** from first vaccination up to 30 days post-Dose 2 will be reported.

The percentage of subjects/dose using **concomitant medication/ product** (any medication/ product, any antipyretic and any antipyretic taken prophylactically, respectively) during the 30-day follow-up period (Day 0 – Day 29) will be summarised per group for each dose and overall per dose.

The number of subjects who experienced any **AE leading to study withdrawal**, or any **SAE related to study participation or concurrent GSK medication/ vaccination,** from first vaccination up to 30 days post-Dose 2 will be reported.

Pregnancy reports from first vaccination up to 30 days post-Dose 2 and pregnancy outcomes will be described in detail.

6.1.3. Analysis of immunogenicity

The primary analysis will be performed on the ATP cohort for immunogenicity and if, in any study group, the percentage of vaccinated subjects with serological results excluded from the ATP cohort is at least 10%, a second analysis will be performed on the TVC.

6.1.3.1. Humoral immune response

Within group evaluation

For each group, at each time point during which blood samples are collected for humoral immune response and for each assay:

- Seropositivity rate and their exact 95% CI will be tabulated.
- GMCs and their 95% CI will be calculated.
- Antibody concentrations distribution will be investigated using Reverse Cumulative Curves.

These analyses will also be performed for each level of the following minimisation factors: age (50-59 years vs. 60-70 years), smoking status (current vs. former smoker), FEV_1/FCV ratio ($\geq$0.7 vs. <0.7).

Between groups evaluation (only for Step 2)

Comparative analyses will be exploratory with the aim to characterise the difference between the 10-10-10-AS and 10-10-3-AS groups in humoral immune response.

The difference in terms of GMCs will be evaluated, one month post-Dose 2, by computing the 95% CIs of the GMC ratio between groups for Step 2 by using a one-way ANCOVA model on the logarithm10 transformation of the concentrations/ titres. The ANCOVA model will include the group category, the age category, the smoking status and FEV_1/FVC ($\geq$ 0.7 or < 0.7) and the pre-Dose 1 concentration (as covariate) as fixed effects. The groups will be considered significantly different if the 95% CI for the GMC ratio between the two groups does not contain the value 1.

However, these differences should be interpreted with caution considering that there will be no adjustment for multiplicity of endpoints.

6.1.3.2. CMI response

The frequency of specific $CD4^+$/ $CD8^+$ T-cells will be summarised (mean, SD, minimum, Q1, median, Q3, and maximum) by group, at each time point during which blood samples are collected for CMI (descriptive statistics).

Each data will be obtained by subtracting the summary of the background value to each summary value. Values less than or equal to zero will be set to 1 for the purpose of geometric mean calculation and graphical representation.

6.1.3.3. Summary score to support dose selection

The calculation of a summary score for both active groups of Step 2 (i.e. 10-10-10-AS and 10-10-3-AS) will be proposed in order to help the formulation selection. The group with the highest summary score should potentially be the one considered for selection provided it has an acceptable safety and CMI profile. However note that a difference of less than 0.02 between 2 summary scores will not be sufficient to consider that a formulation should be ranked higher than/preferred to the other. Unacceptable safety profile encompasses cases such as high occurrence of grade 3 events, occurrence of specific serious adverse event. Unacceptable CMI profile encompasses cases with a pronounced Th_2 response in which there is an isolated elevated IL-13 response, in particular without a response in terms of IFN-γ. The discarding of a group from the selection because of specific safety or CMI reason will be substantiated in the CSR.

If the group(s) ultimately selected for use in upcoming studies for COPD patients is(/are) not the one with the highest summary scores at post dose 2 and/or post dose 3, the disqualification of the group(s) with higher summary scores should be adequately justified in the CSR.

The summary scores will be calculated based on the subjects from the ATP cohort.

For each group *g*, the summary score will summarize how well the group is performing compared to the average active group in terms of the following immunogenicity and CMI results. Before defining the summary score several quantities are introduced (for *XX* being PD, PE, or PilA):

- GM_{XX_g} : the 30 days post last vaccination GMC of Anti-*XX* of group g
- $AvGM_{XX}$: the 30 days post last vaccination GMC of Anti-*XX* of all subjects in any active group
- GM_{CMIXX_g} : the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of group g
- $AvGM_{CMIXX}$ the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of all subjects in any active group

These measures will be transformed in order to be contained between 0 and 1 via the following formulae:

$$D_{XX_g} = 1 / (1 + \mathrm{Exp}((AvGM_{XX} - GM_{XX_g})/AvGM_{XX}))$$

$$D_{CMIXX_g} = 1 / (1 + \mathrm{Exp}((AvGM_{CMIXX} - GM_{CMIXX_g})/AvGM_{CMIXX}))$$

The following groups will be evaluated at Post-dose 2:

- 10-10-10-AS summary score Post-dose 2

- 10-10-3-AS summary score Post-dose 2

The average GMCs used to calculate the summary scores will be an average of all vaccinated subjects from an active group at post dose 2.

The summary score for group g will be computed by taking the weighted geometric mean of the several D measures as follows:

$$D_g = \sqrt[15]{D_{PDg}^4 \times D_{PEg}^4 \times D_{PilAg}^4 \times D_{CMIPDg}^1 \times D_{CMIPEg}^1 \times D_{CMIPilAg}^1}$$

A group with a D score of ½ corresponds exactly at the average level of all subjects in the active groups. The highest the value of the summary score for a group, the better the group is performing in terms of immunogenicity and CMI compared to the others.

Differences of less than 0.02 in terms of summary measures will not be considered sufficient to establish a clear hierarchy between 2 groups.

6.2. Analysis up to Visit 8 (Day 420)

The final analyses will be descriptive and will be presented by study group, with pooled data from the two steps for the placebo groups.

6.2.1. Analysis of demographics

Withdrawal status will be summarised by group using descriptive statistics:

The number of subjects enrolled into the study as well as the number of subjects excluded from ATP analyses will be tabulated.

The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal.

6.2.2. Analysis of safety

The primary analysis will be performed on the TVC and, if in any vaccine group and at any time point the percentage of vaccinated subjects excluded from the ATP cohort is at least 10%, a second analysis will be performed on the ATP cohort to complement the TVC.

The percentage of subjects with at least one **local AE** (solicited and unsolicited), with at least one **general AE** (solicited and unsolicited) and with any AE during the 7-day or 30-day follow-up period will be tabulated after Dose 1 and Dose 2 and overall with exact

95% CI. The same computations will be done for Grade 3 AEs, any AEs causally related to vaccination and any Grade 3 AEs causally related to vaccination.

The percentage of subjects/doses reporting each individual solicited local (any grade, Grade 3) and general (any grade, Grade 3, any causally related to vaccination and any Grade 3 causally related to vaccination) AE during the 7-days (Day 0 to Day 6) follow-up period will be tabulated for each group as follows:

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

The exact 95% CIs will be calculated assuming independence between doses. For fever, additional analyses will be performed by 0.5°C increments.

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

For each group and for each **haematology and biochemistry parameter**:

- The percentage of subjects having haematology and biochemistry results below or above the normal laboratory ranges will be tabulated by time point.
- The maximum grading from Screening up to Visit 8 (Day 420) will be tabulated (grades will be based on local laboratory normal ranges and derived from FDA Guidance to Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". Those laboratory parameters not included in the FDA grading scale will not be graded).

The number of subjects who experienced at least one **SAE** or any **pIMDs** from first vaccination up to study conclusion will be reported.

The percentage of subjects/dose using **concomitant medication/ product** (any medication/ product, any antipyretic and any antipyretic taken prophylactically, respectively) during the 30-day follow-up period (Day 0 – Day 29) will be summarised per group for each dose and overall per dose.

The number of subjects who experienced any **AE leading to study withdrawal**, or any **SAE related to study participation or concurrent GSK medication/ vaccination,** from first vaccination up to study conclusion will be reported.

Pregnancy reports from first vaccination up to study conclusion and pregnancy outcomes will be described in detail.

6.2.3. Analysis of immunogenicity

The primary analysis will be performed on the ATP cohort for immunogenicity and if, in any study group, the percentage of vaccinated subjects with serological results excluded from the ATP cohort is at least 10%, a second analysis will be performed on the TVC.

6.2.3.1. Humoral immune response

Within group evaluation

For each group, at each time point during which blood samples are collected for humoral immune response and for each assay:

- Seropositivity rate and their exact 95% CI will be tabulated.
- GMCs and their 95% CI will be calculated.
- Antibody concentrations distribution will be investigated using Reverse Cumulative Curves.

These analyses will also be performed for each level of the following minimisation factors: age (50-59 years vs. 60-70 years), smoking status (current vs. former smoker), FEV_1/FCV ratio ($\geq$0.7 vs. <0.7).

Between groups evaluation (only for Step 2)

Comparative analyses will be exploratory with the aim to characterise the difference between the 10-10-10-AS and 10-10-3-AS groups in humoral immune response.

The difference in terms of GMCs will be evaluated, one month post-Dose 2, by computing the 95% CIs of the GMC ratio between groups for Step 2 by using a one-way ANCOVA model on the logarithm10 transformation of the concentrations/ titres. The ANCOVA model will include the group category, the age category, the smoking status and FEV_1/FVC ($\geq$ 0.7 or < 0.7) and the pre-Dose 1 concentration (as co-variable) as fixed effects. The groups will be considered significantly different if the 95% CI for the GMC ratio between the two groups does not contain the value 1.

However, these differences should be interpreted with caution considering that there will be no adjustment for multiplicity of endpoints.

6.2.3.2. CMI response

The frequency of specific $CD4^+$/ $CD8^+$ T-cells will be summarised (mean, SD, minimum, Q1, median, Q3, and maximum) by group, at each time point during which blood samples are collected for CMI (descriptive statistics).

Each data will be obtained by subtracting the summary of the background value to each summary value. Values less than or equal to zero will be set to 1 for the purpose of geometric mean calculation and graphical representation.

6.2.3.3. Summary score to support dose selection

The calculation of a summary score for both active groups of Step 2 (i.e. 10-10-10-AS and 10-10-3-AS) will be proposed in order to help the formulation selection. The group(s) with the highest summary score(s) should potentially be the one(s) considered for selection provided it (they) has (ve) an acceptable safety and CMI profile. However note that a difference of less than 0.02 between 2 summary scores will not be sufficient to consider that a formulation should be ranked higher than/preferred to the other. Unacceptable safety profile encompasses cases such as high occurrence of grade 3 events, occurrence of specific serious adverse event. Unacceptable CMI profile encompasses cases with a pronounced Th_2 response in which there is an isolated elevated IL-13 response, in particular without a response in terms of IFN-γ. The discarding of a group from the selection because of specific safety or CMI reason will be substantiated in the CSR.

If the group(s) ultimately selected for use in upcoming studies for COPD patients is(/are) not the one with the highest summary scores at post dose 2 and/or post dose 3, the disqualification of the group(s) with higher summary scores should be adequately justified in the CSR.

The summary scores will be calculated based on the subjects from the ATP cohort.

For each group *g*, the summary score will summarize how well the group is performing compared to the average active group in terms of the following immunogenicity and CMI results. Before defining the summary score several quantities are introduced (for *XX* being PD, PE, or PilA):

- GM_{XX_g} : the 30 days post last vaccination GMC of Anti-*XX* of group g
- $AvGM_{XX}$: the 30 days post last vaccination GMC of Anti-*XX* of all subjects in any active group
- GM_{CMIXX_g} : the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of group g

- AvGM$_{CMIXX}$ the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of all subjects in any active group

These measures will be transformed in order to be contained between 0 and 1 via the following formulae:

$$D_{XXg} = 1 / (1 + \text{Exp}((\text{AvGM}_{XX} - \text{GM}_{XXg})/\text{AvGM}_{XX}))$$

$$D_{CMIXXg} = 1 / (1 + \text{Exp}((\text{AvGM}_{CMIXX} - \text{GM}_{CMIXXg})/\text{AvGM}_{CMIXX}))$$

The following groups will be evaluated at Post-dose 2:

- 10-10-10-AS summary score Post-dose 2
- 10-10-3-AS summary score Post-dose 2

The average GMCs used to calculate the summary scores will be an average of all vaccinated subject from an active group at post dose 2.

The summary score for group g will be computed by taking the weighted geometric mean of the several D measures as follows:

$$D_g = \sqrt[15]{D_{PDg}^4 \times D_{PEg}^4 \times D_{PilAg}^4 \times D_{CMIPDg}^1 \times D_{CMIPEg}^1 \times D_{CMIPilAg}^1}$$

A group with a D score of ½ corresponds exactly at the average level of all subjects in the active groups. The highest the value of the summary score for a group, the better the group is performing in terms of immunogenicity and CMI compared to the others.

Differences of less than 0.02 in terms of summary measures will not be considered sufficient to establish a clear hierarchy between 2 groups. Therefore several groups may be considered to be classified as the best ones.

Although these comparisons are exploratory and should be interpreted with caution considering that there is no adjustment for multiplicity, a statistical significance on at least one GMC of the selected antigen is expected to confirm the potential use of the selected active group in future studies.

7. STATISTICAL CALCULATIONS

The study groups will be defined by treatment actually administered at Dose 1.

Demography

- For a given subject and a given demographic variable, missing measurement will not be replaced.
- Age: Age at the reference activity, computed as the number of units between the date of birth and the reference activity In case of partial dates of any of these 2 dates:
  - 15th of month, If only the day is missing
  - 30th of June, if day and months are missing

Immunogenicity

- For a given subject and the analysis of a given immunogenicity measurement, missing or un-evaluable measurements will not be replaced.
- A seronegative subject is defined as a subject whose antibody concentration is below the assay cut-off value.
- A seropositive subject is defined as a subject whose antibody concentration is greater than or equal to the assay cut-off value.
- Antibody concentrations below the assay cut-off will be given an arbitrary value of half the assay cut-off for the purpose of geometric mean concentration (GMC) calculation.
- Calculation of the GMCs will be performed by taking the anti-logarithm in base 10 (anti-log10) of the mean of the log10 concentration/titre transformations.
- All confidence intervals (CIs) computed will be two-sided 95% CIs.
- The assay cut-off is the value under which there is no quantifiable result available. For an assay with a specific 'cut_off' , numerical immuno result is derived from a character field (rawres):
  - If rawres is 'NEG' or '-' or '(-)', numeric result= cutt_off/2,
  - if rawres is 'POS' or '+' or '(+)', numeric result = cut_off,
  - if rawres is '< value' and value<=cut_off, numeric result =cut_off/2,
  - if rawres is '< value' and value>cut_off, numeric result =value,
  - if rawres is '> value' and value<cut_off, numeric result =cut_off/2,
  - if rawres is '> value' and value>=cut_off, numeric result =value,
  - if rawres is '<= value' or '>= value' and value<cut_off, numeric result =cut_off/2,
  - if rawres is '<= value' or '>= value' and value>=cut_off, numeric result =value,
  - if rawres is a value < cut_off, numeric result = cut_off/2,

- if rawres is a value >= cut_off, numeric result = rawres,
- else numeric result is left blank.

Safety/reactogenicity

- For **solicited symptoms**, the analysis will exclude subjects with missing or un-evaluable measurements (e.g. total analysis of solicited symptoms will include all vaccinated subjects with documented solicited symptom sheets).
- For the **unsolicited symptoms** and concomitant medications/ products/ vaccinations, subjects who did not report unsolicited symptoms/concomitant medications/ products/ vaccinations will be treated as subjects without unsolicited symptoms or concomitant medications/ products/ vaccinations, respectively.
- Note that for all tables described in this section, the way the percentage of subjects will be derived will depend on the event analysed (see table below for details). As a result, the N value will differ from one table to another.

Event	N used for deriving % per subject
Concomitant vaccination	All subjects with study vaccine administered
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	All subjects with study vaccine administered
Concomitant medication	All subjects with study vaccine administered

7.1. Data presentation description

The following decimal description will be used for the demography and safety/ reactogenicity.

Display Table	Parameters	Number of decimal digits
All summaries	% of count, including LL & UL of CI	1
All summaries	% of difference, including LL & UL of CI	2
Demographic characteristics	Mean, median age	1
Demographic characteristics	SD (age)	2
Immunogenicity	GMT ratio	2

The following data presentation description will be used for the immunogenicity analyses.

Component	GMC/T	Assay		Assay cut-off	Number of decimal digits		Tick marks on RCC's	
					GMC/T	GMC/T ratio		
		method	Unit				Low value	High value
Anti-PD	GMC	ELISA	EL.U/mL	153	1	2	1	10000
Anti-PE	GMC	ELISA	EL.U/mL	8	1	2	1	10000
Anti-PilA	GMC	ELISA	EL.U/mL	7	1	2	1	10000
Anti-UspA2	GMC	ELISA	EL.U/mL	18	1	2	1	10000

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

- The analysis of data up to Visit 6 (Day 90, end of Epoch 001) will be performed in a first step. This analysis will include:
  - The final analysis of all immunogenicity results (including CMI), solicited AEs post-Dose 1 and post-Dose 2,
  - The assessment of unsolicited AEs up to 30 days post-Dose 1 and post-Dose 2, and of SAEs and pIMDs up to 30 days post-Dose 2 on as cleaned as possible data. No individual listings will be written at this stage.

  This analysis will be documented in a statistical report. At this point, the GSK statistician will be unblinded (i.e. will have access to the individual subject treatment assignments).
- The analysis of Epoch 002 (from Visit 7 [Day 210] up to Visit 8 [Day 420]) will be performed in a second step, once those data will be available and cleaned. This analysis will include:
  - The final analysis of all immunogenicity results (including CMI) up to Visit 8 (Day 420).
  - SAEs and pIMDs up to Visit 8 (Day 420) on cleaned data.

  In addition, all previous analyses will be re-produced based on cleaned data at this point.

  Individual listings will only be provided at this stage.

Description	Analysis ID	Disclosure Purpose
Analysis up to Visit 6 (Day 90)	E1_02	Internal
Final analysis (up to day 450)	E1_01	CTRS & Clinical Study Report

8.2. Statistical considerations for interim analyses

Not applicable.

9. CHANGES FROM PLANNED ANALYSES

None.

10. REFERENCES

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].

Statistical Analysis Plan

Study alias & e-track number(s): NTHI MCAT-001 (201281)

Detailed Title:	A Phase I, randomised, observer-blind, placebo-controlled, multi-centre study to evaluate the safety, reactogenicity and immunogenicity of GSK Biologicals' GSK3277511A investigational vaccine when administered intramuscularly according to a 0, 2 months schedule in adults
SAP version	Amendment 2
SAP date	06DEC2016
Scope:	All data pertaining to the above study.
Co-ordinating author:	PPD
Other author(s):	
Adhoc reviewers:	PPD, Global Regulatory Lead (RA) PPD, PPD, Safety Representatives CTRS team
Approved by:	PPD, Clinical Research & Development Lead PPD, Lead Statistician PPD, Statistician

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The complete statistical analysis plan 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 SR.

LIST OF ABBREVIATIONS

AE	Adverse event
AECOPD	Acute exacerbation of COPD
ALT	Alanine aminotransferase
ATP	According-To-Protocol
ANCOVA	Analysis of covariance
CI	Confidence Interval
CMI	Cell-mediated immunity
COPD	Chronic obstructive pulmonary disease
CRF	Case Report Form
CTRS	Clinical Trial Registry
EL.U/ml	ELISA unit per milliliter
ELISA	Enzyme-linked immunosorbent assay
Eli Type	Internal GSK database code for type of elimination code
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
FU	Internal GSK database code for Follow-up analysis (elimination codes) link to FORM-BIO-CLIN-9004-05 Criteria for eliminating subjects from the analyses
GMC	Geometric mean antibody concentration
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GSK	GlaxoSmithKline
H. influenzae	Haemophilus influenzae
ICF	Informed consent form
ICS	Intracellular cytokine staining
IU/ml	International units per milliliter
LL	Lower Limit of the confidence interval

MA	Internal GSK database code for Main analysis (elimination codes) link to FORM-BIO-CLIN-9004-05 Criteria for eliminating subjects from the analyses
MATEX	MATerial EXcellence
M. catarrhalis	Moraxella catarrhalis
MedDRA	Medical Dictionary for Regulatory Activities
N.A.	Not Applicable
NTHI	Non-Typeable *Haemophilus influenzae*
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PD	Protein D
PE	Protein E
PI	Prescribing information
PilA	Type IV pili subunit of non-typeable Haemophilus influenzae
pIMD	Potential immune-mediated disease
PT	Preferred term
SAE	Serious adverse event
S. aureus	Staphylococcus aureus
SAP	Statistical Analysis Plan
SBIR	GSK Biological's Internet Randomization System
SD	Standard Deviation
SR	Study Report
STGG	Skim milk, tryptone, glucose, and glycerin transport medium
SYN	Synopsis
TFL	Tables Figures and Listing template annexed to SAP
TVC	Total vaccinated cohort
UL	Upper Limit of the confidence interval
UspA2	Ubiquitous surface protein A2 of Moraxella catarrhalis
VE	Vaccine efficacy

1. DOCUMENT HISTORY

Date	Description	Protocol Version
11-MAR-2016	Version 1	Amendment 1 – 30JUL2015
27-JUN-2016	Amendment 1 – Anti-PD cut-off was updated from 100 EL.U/mL to 153 EL.U/mL following the development of the new anti-PD test.	Amendment 1 – 30JUL2015
6-DEC-2016	Amendment 2 – Update the desirability function in order to take into account the Mcat Protein.	Amendment 1 – 30JUL2015

2. RATIONALE FOR THE AMENDMENT

Summary score's formulas have been updated, taking into account MCat protein according to the rules described in section 7 Statistical Methods.

3. STUDY DESIGN

The following group names will be used for the statistical analyses:

Group order in tables	Group label in tables	Group definition for footnote	Pooled Groups label in tables	Pooled definition for footnote
1	10-10-10	2 doses of non-adjuvanted NTHI/Mcat vaccine containing 10 mcg of PD, PE-PilA and UspA2		
2	10-10-10-AS	2 doses of AS01E-adjuvanted NTHI/Mcat vaccine containing 10 mcg of PD, PE-PilA and UspA2		
3	10-10-3-AS	2 doses of AS01E-adjuvanted NTHI/Mcat vaccine containing 10 mcg of PD, PE-PilA and 3 mcg of UspA2		
4	PLACE1	2 doses of saline solution – Step 1	Placebo	2 doses of saline solution
5	PLACE2	2 doses of saline solution – Step 2	Placebo	

4. OBJECTIVES

4.1. Primary objective

- To evaluate the safety and reactogenicity profile of the NTHI-Mcat investigational vaccines.

4.2. Secondary objective

- To evaluate the humoral and cellular immune response of the NTHI-Mcat investigational vaccines.

4.3. Tertiary objective

- To collect blood samples for assay development/validation and/or for evaluation/characterisation of the humoral and cellular immune responses to components of either the NTHI-Mcat investigational vaccines and/or of other respiratory pathogens.

5. ENDPOINTS

5.1. Primary endpoints

- Occurrence of each solicited local and general AE, during a 7-day follow-up period (*i.e.* day of vaccination and 6 subsequent days) post-Dose 1 and post-Dose 2, in all subjects, in all vaccine groups.
- Occurrence of any unsolicited AEs, during a 30-day follow-up period (i.e. day of vaccination and 29 subsequent days) post-Dose 1 and post-Dose 2, in all subjects, in all vaccine groups.
- Occurrence of haematological and biochemical laboratory abnormalities, after vaccination, in all subjects, in all vaccine groups:
  - Any haematological (RBC, WBC and differential count, platelets count and haemoglobin level) or biochemical (ALT, AST and creatinine) laboratory abnormality on Day 7, Day 60, Day 67, Day 210 and Day 420.
- Occurrence of any SAE, occurring from first vaccination (Day 0) to study conclusion (Day 420) in all subjects, in all vaccine groups.
- Occurrence of any pIMD occurring from first vaccination (Day 0) to study conclusion (Day 420) in all subjects, in all vaccine groups.

5.2. Secondary endpoints

- Humoral immune response to the components of the NTHI-Mcat vaccine formulations, on Day 0, Day 30, Day 60, Day 90, Day 210 and Day 420, in all subjects, in all vaccine groups:
  - Anti-PD, anti-PE, anti-PilA and anti-UspA2 antibody concentrations.

- Cell-mediated immune response to components of the NTHI-Mcat vaccine formulations, on Day 0, Day 60, Day 90, Day 210 and Day 420, in a sub-cohort of subjects, in all vaccine groups:
  - Frequency of specific $CD4^+/CD8^+$ T-cells measured on cryopreserved peripheral blood mononuclear cells (PBMCs) and identified by flow cytometry intracellular cytokine staining (ICS) expressing two or more markers (such as IL-2, IL-13, IL-17, IFN-γ, TNF-α and CD40L).

6. STUDY POPULATION

The following subject cohorts will be evaluated.

6.1.1. Total vaccinated cohort

The total vaccinated cohort (TVC) will include all subjects with at least one vaccine administration documented:

- 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 analysis will be performed per treatment actually administered at Dose 1.

6.1.2. According-to-protocol cohort

The ATP cohorts will be defined by epoch and will consist of all subjects from the TVC who will comply with eligibility criteria, study procedures up to the end of the epoch and had immunogenicity results in the epoch.

6.1.2.1. According-to-protocol cohort for analysis of immunogenicity (Epoch 001)

The ATP cohort for immunogenicity will include all subjects in the TVC:

- Who met all eligibility criteria.
- For whom the administration route and site of the vaccines was according to protocol.
- Who complied with the vaccination schedule.
- Who did not receive a concomitant medication/ product/vaccine leading to the elimination from the ATP analysis up the one month post-Dose 2 visit (Visit 6, Day 90).

- Who did not present with an intercurrent medical condition leading to elimination from the ATP analysis up to the one month post-Dose 2 visit (Visit 6, Day 90).
- Who complied with the immunogenicity blood sample timings at 1 month post-Dose 2 visit (Visit 6, Day 90).
- For whom post-Dose 2 immunogenicity results are available for at least one assay.

6.1.2.2. According-to-protocol cohort for analysis of persistence of immunogenicity (Epoch 002)

The ATP cohort for persistence of immunogenicity will include all evaluable subjects, i.e., those who were included in the ATP cohort for immunogenicity, or were excluded from this cohort solely because they had no blood samples taken or because of non-compliance with blood sample timings up to the one month post-Dose 2 visit (Visit 6, Day 90), and:

- Who did not receive a concomitant medication/ product/vaccine leading to elimination from the ATP analysis for immunogenicity.
- Who did not present with an intercurrent medical condition leading to elimination from the ATP analysis for immunogenicity.
- Who complied with at least one of the blood sample timings after the one month post-last vaccination visit (Visit 6, Day 90).
- For whom persistence immunogenicity results are available for at least one assay in at least one of the two persistence time points (Visit 7, Day 210 and Visit 8, Day 420).

The list of applicable elimination codes for each cohort can be found in the study specific form FORM-BIO-CLIN-9004-05 Criteria for eliminating subjects from the analyses.

Cohort	Elimination codes	Eli Type
ATP cohort for analysis of immunogenicity	1010-2500	MA
ATP cohort for analysis of persistence of immunogenicity	1010-2500	FU

7. STATISTICAL METHODS

7.1. Analysis up to Visit 6 (Day 90)

The final analyses will be descriptive and will be presented by study group, with pooled data from the two steps for the placebo groups.

7.1.1. Analysis of demographics

Demographic characteristics (age at the first dose in years, gender and race), cohort description and other characteristics such as smoking/exposure history status, pulmonary function test baseline results, height, weight and previous vaccination with a pneumococcal vaccine or an influenza vaccine will be summarized by group using descriptive statistics:

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

- The distribution of subjects enrolled among the study sites will be tabulated as a whole and per group.
- Withdrawal status will be summarised by group using descriptive statistics:
  - The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal.
- The number of subjects enrolled into the study as well as the number of subjects excluded from ATP analyses will be tabulated.

7.1.2. Analysis of safety

The primary analysis will be performed on the TVC and, if in any vaccine group and at any time point the percentage of vaccinated subjects excluded from the ATP cohort is at least 10%, a second analysis will be performed on the ATP cohort to complement the TVC.

The percentage of subjects with at least one **local AE** (solicited and unsolicited), with at least one **general AE** (solicited and unsolicited) and with any AE during the 7-day or 30-day follow-up period will be tabulated after Dose 1 and Dose 2 and overall with exact 95% CI. The same computations will be done for Grade 3 AEs, any AEs causally related to vaccination and any Grade 3 AEs causally related to vaccination.

The percentage of subjects/doses reporting each individual solicited local (any grade, Grade 3) and general (any grade, Grade 3, any causally related to vaccination and any Grade 3 causally related to vaccination) AE during the 7-days (Day 0 to Day 6) follow-up period will be tabulated for each group as follows:

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

The exact 95% CIs will be calculated assuming independence between doses. For fever, additional analyses will be performed by 0.5°C increments.

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

For each group and for each **haematology and biochemistry parameter**:

- The percentage of subjects having haematology and biochemistry results below or above the normal laboratory ranges will be tabulated by time point.
- The maximum grading from Screening up to Visit 6 (Day 90) will be tabulated (grades will be based on local laboratory normal ranges and derived from FDA Guidance to Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". Those laboratory parameters not included in the FDA grading scale will not be graded).

The number of subjects who experienced at least one **SAE** or any **pIMDs** from first vaccination up to 30 days post-Dose 2 will be reported.

The percentage of subjects/dose using **concomitant medication/ product** (any medication/ product, any antipyretic and any antipyretic taken prophylactically, respectively) during the 30-day follow-up period (Day 0 – Day 29) will be summarised per group for each dose and overall per dose.

The number of subjects who experienced any **AE leading to study withdrawal**, or any **SAE related to study participation or concurrent GSK medication/ vaccination,** from first vaccination up to 30 days post-Dose 2 will be reported.

Pregnancy reports from first vaccination up to 30 days post-Dose 2 and pregnancy outcomes will be described in detail.

7.1.3. Analysis of immunogenicity

The primary analysis will be performed on the ATP cohort for immunogenicity and if, in any study group, the percentage of vaccinated subjects with serological results excluded from the ATP cohort is at least 10%, a second analysis will be performed on the TVC.

7.1.3.1. Humoral immune response

Within group evaluation

For each group, at each time point during which blood samples are collected for humoral immune response and for each assay:

- Seropositivity rate and their exact 95% CI will be tabulated.
- GMCs and their 95% CI will be calculated.
- Antibody concentrations distribution will be investigated using Reverse Cumulative Curves.

These analyses will also be performed for each level of the following minimisation factors: age (50-59 years vs. 60-70 years), smoking status (current vs. former smoker), FEV_1/FCV ratio ($\geq$0.7 vs. <0.7).

Between groups evaluation (only for Step 2)

Comparative analyses will be exploratory with the aim to characterise the difference between the 10-10-10-AS and 10-10-3-AS groups in humoral immune response.

The difference in terms of GMCs will be evaluated, one month post-Dose 2, by computing the 95% CIs of the GMC ratio between groups for Step 2 by using a one-way ANCOVA model on the logarithm10 transformation of the concentrations/ titres. The ANCOVA model will include the group category, the age category, the smoking status and FEV_1/FVC ($\geq$ 0.7 or < 0.7) and the pre-Dose 1 concentration (as covariate) as fixed effects. The groups will be considered significantly different if the 95% CI for the GMC ratio between the two groups does not contain the value 1.

However, these differences should be interpreted with caution considering that there will be no adjustment for multiplicity of endpoints.

7.1.3.2. CMI response

The frequency of specific $CD4^+$/ $CD8^+$ T-cells will be summarised (mean, SD, minimum, Q1, median, Q3, and maximum) by group, at each time point during which blood samples are collected for CMI (descriptive statistics).

Each data will be obtained by subtracting the summary of the background value to each summary value. Values less than or equal to zero will be set to 1 for the purpose of geometric mean calculation and graphical representation.

7.1.3.3. Summary score to support dose selection

The calculation of a summary score for both active groups of Step 2 (i.e. 10-10-10-AS and 10-10-3-AS) will be proposed in order to help the formulation selection. The group with the highest summary score should potentially be the one considered for selection provided it has an acceptable safety and CMI profile. However note that a difference of less than 0.02 between 2 summary scores will not be sufficient to consider that a formulation should be ranked higher than/preferred to the other. Unacceptable safety profile encompasses cases such as high occurrence of grade 3 events, occurrence of specific serious adverse event. Unacceptable CMI profile encompasses cases with a pronounced Th_2 response in which there is an isolated elevated IL-13 response, in particular without a response in terms of IFN-γ. The discarding of a group from the selection because of specific safety or CMI reason will be substantiated in the CSR.

If the group(s) ultimately selected for use in upcoming studies for COPD patients is(/are) not the one with the highest summary scores at post dose 2 and/or post dose 3, the disqualification of the group(s) with higher summary scores should be adequately justified in the CSR.

The summary scores will be calculated based on the subjects from the ATP cohort.

For each group *g*, the summary score will summarize how well the group is performing compared to the average active group in terms of the following immunogenicity and CMI results. Before defining the summary score several quantities are introduced (for *XX* being PD, PE, PilA, or ***UspA2***):

- GM_{XX_g} : the 30 days post last vaccination GMC of Anti-*XX* of group g
- $AvGM_{XX}$: the 30 days post last vaccination GMC of Anti-*XX* of all subjects in any active group
- GM_{CMIXX_g} : the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of group g
- $AvGM_{CMIXX}$ the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of all subjects in any active group

These measures will be transformed in order to be contained between 0 and 1 via the following formulae:

$$D_{XX_g} = 1 / (1 + \mathrm{Exp}((AvGM_{XX} - GM_{XX_g})/AvGM_{XX}))$$

$$D_{CMIXX_g} = 1 / (1 + \mathrm{Exp}((AvGM_{CMIXX} - GM_{CMIXX_g})/AvGM_{CMIXX}))$$

The following groups will be evaluated at Post-dose 2:

- 10-10-10-AS summary score Post-dose 2
- 10-10-3-AS summary score Post-dose 2

The average GMCs used to calculate the summary scores will be an average of all vaccinated subjects from an active group at post dose 2.

The summary score for group g will be computed by taking the weighted geometric mean of the several D measures as follows:

$$D_g = \sqrt[20]{D_{PDg}^4 \times D_{PEg}^4 \times D_{PilAg}^4 \times D_{UspA2g}^4 \times D_{CMIPDg}^1 \times D_{CMIPEg}^1 \times D_{CMIPilAg}^1 \times D_{CMIUspA2g}^1}$$

A group with a D score of ½ corresponds exactly at the average level of all subjects in the active groups. The highest the value of the summary score for a group, the better the group is performing in terms of immunogenicity and CMI compared to the others.

Differences of less than 0.02 in terms of summary measures will not be considered sufficient to establish a clear hierarchy between 2 groups.

7.2. Analysis up to Visit 8 (Day 420)

The final analyses will be descriptive and will be presented by study group, with pooled data from the two steps for the placebo groups.

7.2.1. Analysis of demographics

Withdrawal status will be summarised by group using descriptive statistics:

The number of subjects enrolled into the study as well as the number of subjects excluded from ATP analyses will be tabulated.

The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal.

7.2.2. Analysis of safety

The primary analysis will be performed on the TVC and, if in any vaccine group and at any time point the percentage of vaccinated subjects excluded from the ATP cohort is at least 10%, a second analysis will be performed on the ATP cohort to complement the TVC.

The percentage of subjects with at least one **local AE** (solicited and unsolicited), with at least one **general AE** (solicited and unsolicited) and with any AE during the 7-day or 30-day follow-up period will be tabulated after Dose 1 and Dose 2 and overall with exact 95% CI. The same computations will be done for Grade 3 AEs, any AEs causally related to vaccination and any Grade 3 AEs causally related to vaccination.

The percentage of subjects/doses reporting each individual solicited local (any grade, Grade 3) and general (any grade, Grade 3, any causally related to vaccination and any Grade 3 causally related to vaccination) AE during the 7-days (Day 0 to Day 6) follow-up period will be tabulated for each group as follows:

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

The exact 95% CIs will be calculated assuming independence between doses. For fever, additional analyses will be performed by 0.5°C increments.

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

For each group and for each **haematology and biochemistry parameter**:

- The percentage of subjects having haematology and biochemistry results below or above the normal laboratory ranges will be tabulated by time point.
- The maximum grading from Screening up to Visit 8 (Day 420) will be tabulated (grades will be based on local laboratory normal ranges and derived from FDA Guidance to Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". Those laboratory parameters not included in the FDA grading scale will not be graded).

The number of subjects who experienced at least one **SAE** or any **pIMDs** from first vaccination up to study conclusion will be reported.

The percentage of subjects/dose using **concomitant medication/ product** (any medication/ product, any antipyretic and any antipyretic taken prophylactically,

respectively) during the 30-day follow-up period (Day 0 – Day 29) will be summarised per group for each dose and overall per dose.

The number of subjects who experienced any **AE leading to study withdrawal**, or any **SAE related to study participation or concurrent GSK medication/ vaccination,** from first vaccination up to study conclusion will be reported.

Pregnancy reports from first vaccination up to study conclusion and pregnancy outcomes will be described in detail.

7.2.3. Analysis of immunogenicity

The primary analysis will be performed on the ATP cohort for immunogenicity and if, in any study group, the percentage of vaccinated subjects with serological results excluded from the ATP cohort is at least 10%, a second analysis will be performed on the TVC.

7.2.3.1. Humoral immune response

Within group evaluation

For each group, at each time point during which blood samples are collected for humoral immune response and for each assay:

- Seropositivity rate and their exact 95% CI will be tabulated.
- GMCs and their 95% CI will be calculated.
- Antibody concentrations distribution will be investigated using Reverse Cumulative Curves.

These analyses will also be performed for each level of the following minimisation factors: age (50-59 years vs. 60-70 years), smoking status (current vs. former smoker), FEV_1/FCV ratio ($\geq$0.7 vs. <0.7).

Between groups evaluation (only for Step 2)

Comparative analyses will be exploratory with the aim to characterise the difference between the 10-10-10-AS and 10-10-3-AS groups in humoral immune response.

The difference in terms of GMCs will be evaluated, one month post-Dose 2, by computing the 95% CIs of the GMC ratio between groups for Step 2 by using a one-way ANCOVA model on the logarithm10 transformation of the concentrations/ titres. The ANCOVA model will include the group category, the age category, the smoking status and FEV_1/FVC ($\geq$ 0.7 or < 0.7) and the pre-Dose 1 concentration (as co-variable) as fixed effects. The groups will be considered significantly different if the 95% CI for the GMC ratio between the two groups does not contain the value 1.

However, these differences should be interpreted with caution considering that there will be no adjustment for multiplicity of endpoints.

7.2.3.2. CMI response

The frequency of specific $CD4^+$/ $CD8^+$ T-cells will be summarised (mean, SD, minimum, Q1, median, Q3, and maximum) by group, at each time point during which blood samples are collected for CMI (descriptive statistics).

Each data will be obtained by subtracting the summary of the background value to each summary value. Values less than or equal to zero will be set to 1 for the purpose of geometric mean calculation and graphical representation.

7.2.3.3. Summary score to support dose selection

The calculation of a summary score for both active groups of Step 2 (i.e. 10-10-10-AS and 10-10-3-AS) will be proposed in order to help the formulation selection. The group(s) with the highest summary score(s) should potentially be the one(s) considered for selection provided it (they) has (ve) an acceptable safety and CMI profile. However note that a difference of less than 0.02 between 2 summary scores will not be sufficient to consider that a formulation should be ranked higher than/preferred to the other. Unacceptable safety profile encompasses cases such as high occurrence of grade 3 events, occurrence of specific serious adverse event. Unacceptable CMI profile encompasses cases with a pronounced Th_2 response in which there is an isolated elevated IL-13 response, in particular without a response in terms of IFN-γ. The discarding of a group from the selection because of specific safety or CMI reason will be substantiated in the CSR.

If the group(s) ultimately selected for use in upcoming studies for COPD patients is(/are) not the one with the highest summary scores at post dose 2 and/or post dose 3, the disqualification of the group(s) with higher summary scores should be adequately justified in the CSR.

The summary scores will be calculated based on the subjects from the ATP cohort.

For each group *g*, the summary score will summarize how well the group is performing compared to the average active group in terms of the following immunogenicity and CMI results. Before defining the summary score several quantities are introduced (for *XX* being PD, PE, or PilA):

- GM_{XX_g} : the 30 days post last vaccination GMC of Anti-*XX* of group g
- $AvGM_{XX}$: the 30 days post last vaccination GMC of Anti-*XX* of all subjects in any active group

- GM_{CMIXXg} : the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of group g
- $AvGM_{CMIXX}$ the 30 days post last vaccination GMC of specific Anti-*XX* CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-Alpha, IFN-Gamma, IL-13 and IL-17 per million cells of all subjects in any active group

These measures will be transformed in order to be contained between 0 and 1 via the following formulae:

$$D_{XXg} = 1 / (1 + \text{Exp}((AvGM_{XX} - GM_{XXg})/AvGM_{XX}))$$

$$D_{CMIXXg} = 1 / (1 + \text{Exp}((AvGM_{CMIXX} - GM_{CMIXXg})/AvGM_{CMIXX}))$$

The following groups will be evaluated at Post-dose 2:

- 10-10-10-AS summary score Post-dose 2
- 10-10-3-AS summary score Post-dose 2

The average GMCs used to calculate the summary scores will be an average of all vaccinated subject from an active group at post dose 2.

The summary score for group g will be computed by taking the weighted geometric mean of the several D measures as follows:

$$D_g = \sqrt[15]{D_{PDg}^4 \times D_{PEg}^4 \times D_{PilAg}^4 \times D_{CMIPDg}^1 \times D_{CMIPEg}^1 \times D_{CMIPilAg}^1}$$

A group with a D score of ½ corresponds exactly at the average level of all subjects in the active groups. The highest the value of the summary score for a group, the better the group is performing in terms of immunogenicity and CMI compared to the others.

Differences of less than 0.02 in terms of summary measures will not be considered sufficient to establish a clear hierarchy between 2 groups. Therefore several groups may be considered to be classified as the best ones.

Although these comparisons are exploratory and should be interpreted with caution considering that there is no adjustment for multiplicity, a statistical significance on at least one GMC of the selected antigen is expected to confirm the potential use of the selected active group in future studies.

8. STATISTICAL CALCULATIONS

The study groups will be defined by treatment actually administered at Dose 1.

Demography

- For a given subject and a given demographic variable, missing measurement will not be replaced.
- Age: Age at the reference activity, computed as the number of units between the date of birth and the reference activity In case of partial dates of any of these 2 dates:
  - 15th of month, If only the day is missing
  - 30th of June, if day and months are missing

Immunogenicity

- For a given subject and the analysis of a given immunogenicity measurement, missing or un-evaluable measurements will not be replaced.
- A seronegative subject is defined as a subject whose antibody concentration is below the assay cut-off value.
- A seropositive subject is defined as a subject whose antibody concentration is greater than or equal to the assay cut-off value.
- Antibody concentrations below the assay cut-off will be given an arbitrary value of half the assay cut-off for the purpose of geometric mean concentration (GMC) calculation.
- Calculation of the GMCs will be performed by taking the anti-logarithm in base 10 (anti-log10) of the mean of the log10 concentration/titre transformations.
- All confidence intervals (CIs) computed will be two-sided 95% CIs.
- The assay cut-off is the value under which there is no quantifiable result available. For an assay with a specific 'cut_off' , numerical immuno result is derived from a character field (rawres):
  - If rawres is 'NEG' or '-' or '(-)', numeric result= cutt_off/2,
  - if rawres is 'POS' or '+' or '(+)', numeric result = cut_off,
  - if rawres is '< value' and value<=cut_off, numeric result =cut_off/2,
  - if rawres is '< value' and value>cut_off, numeric result =value,
  - if rawres is '> value' and value<cut_off, numeric result =cut_off/2,
  - if rawres is '> value' and value>=cut_off, numeric result =value,
  - if rawres is '<= value' or '>= value' and value<cut_off, numeric result =cut_off/2,
  - if rawres is '<= value' or '>= value' and value>=cut_off, numeric result =value,
  - if rawres is a value < cut_off, numeric result = cut_off/2,

- if rawres is a value >= cut_off, numeric result = rawres,
- else numeric result is left blank.

Safety/reactogenicity

- For **solicited symptoms**, the analysis will exclude subjects with missing or un-evaluable measurements (e.g. total analysis of solicited symptoms will include all vaccinated subjects with documented solicited symptom sheets).
- For the **unsolicited symptoms** and concomitant medications/ products/ vaccinations, subjects who did not report unsolicited symptoms/concomitant medications/ products/ vaccinations will be treated as subjects without unsolicited symptoms or concomitant medications/ products/ vaccinations, respectively.
- Note that for all tables described in this section, the way the percentage of subjects will be derived will depend on the event analysed (see table below for details). As a result, the N value will differ from one table to another.

Event	N used for deriving % per subject
Concomitant vaccination	All subjects with study vaccine administered
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	All subjects with study vaccine administered
Concomitant medication	All subjects with study vaccine administered

8.1. Data presentation description

The following decimal description will be used for the demography and safety/ reactogenicity.

Display Table	Parameters	Number of decimal digits
All summaries	% of count, including LL & UL of CI	1
All summaries	% of difference, including LL & UL of CI	2
Demographic characteristics	Mean, median age	1
Demographic characteristics	SD (age)	2
Immunogenicity	GMT ratio	2

The following data presentation description will be used for the immunogenicity analyses.

Component	GMC/T	Assay		Assay cut-off	Number of decimal digits		Tick marks on RCC's	
					GMC/T	GMC/T ratio		
		method	Unit				Low value	High value
Anti-PD	GMC	ELISA	EL.U/mL	153	1	2	1	10000
Anti-PE	GMC	ELISA	EL.U/mL	8	1	2	1	10000
Anti-PilA	GMC	ELISA	EL.U/mL	7	1	2	1	10000
Anti-UspA2	GMC	ELISA	EL.U/mL	18	1	2	1	10000

9. CONDUCT OF ANALYSES

9.1. Sequence of analyses

- The analysis of data up to Visit 6 (Day 90, end of Epoch 001) will be performed in a first step. This analysis will include:
  - The final analysis of all immunogenicity results (including CMI), solicited AEs post-Dose 1 and post-Dose 2,
  - The assessment of unsolicited AEs up to 30 days post-Dose 1 and post-Dose 2, and of SAEs and pIMDs up to 30 days post-Dose 2 on as cleaned as possible data. No individual listings will be written at this stage.

  This analysis will be documented in a statistical report. At this point, the GSK statistician will be unblinded (i.e. will have access to the individual subject treatment assignments).
- The analysis of Epoch 002 (from Visit 7 [Day 210] up to Visit 8 [Day 420]) will be performed in a second step, once those data will be available and cleaned. This analysis will include:
  - The final analysis of all immunogenicity results (including CMI) up to Visit 8 (Day 420).
  - SAEs and pIMDs up to Visit 8 (Day 420) on cleaned data.

  In addition, all previous analyses will be re-produced based on cleaned data at this point.

  Individual listings will only be provided at this stage.

Description	Analysis ID	Disclosure Purpose
Analysis up to Visit 6 (Day 90)	E1_02	Internal
Final analysis (up to day 450)	E1_01	CTRS & Clinical Study Report

9.2. Statistical considerations for interim analyses

Not applicable.

10. CHANGES FROM PLANNED ANALYSES

None.

11. REFERENCES

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].