Statistical Analysis Plan

Detailed Title:	A phase IIIB, open, long term extension study to evaluate the persistence of immune responses and the safety of GSK Biologicals' Herpes Zoster subunit (HZ/su) vaccine 1437173A, at Months 108 and 120 post-vaccination and the assessment of re-vaccination with two additional doses administered at 10 years after the initial vaccination in study ZOSTER-003 in healthy subjects aged 60 years of age and older
eTrack study number and Abbreviated Title	204926 (ZOSTER-060 EXT 003)
Scope:	All data pertaining to the above study.
Date of Statistical Analysis Plan	Amendment 1 Final: 14 May 2018
Co-ordinating author:	PPD
Adhoc reviewers:	PPD (SERM physician), PPD (Regulatory Affairs)
Approved by:	
Clinical Research Development Lead	PPD
Clinical and Epidemiology Project Lead	PPD
Project Statistician	PPD
Lead Statistician	PPD
Science Writer	PPD

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

TABLE OF CONTENTS

PAGE

LIST OF FIGURES

PAGE

LIST OF ABBREVIATIONS

Ab	Antibody
AE	Adverse event
AIC	Akaike's Information Criterion
AS01B	MPL, QS21, liposome based Adjuvant System [50µg MPL and 50µg QS21]
ATP	According-To-Protocol
CD40L	CD40 Ligand
CMI	Cell-Mediated Immunity
eCRF	electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
gE	Glycoprotein E
GMC	Geometric Mean Concentration
GSK	GlaxoSmithKline
HZ	Herpes Zoster
ICS	Intracellular Cytokine Staining
IFN-γ	Interferon gamma
IL-2	Interleukin- 2
LTFU	Long-Term Follow-up
mIU	Milli International Unit
MGI	Mean Geometric Increase
MPL	3-*O*-desacyl-4'-Monophosphoryl Lipid A
pIMDs	Potential Immune-Mediated diseases
QS21	*Quillaja saponaria* Molina, fraction 21 (Antigenics, New York, NY, USA)
SAE	Serious Adverse Event
SBIC	Schwarz' Bayesian Information Criterion
TNF-α	Tumor Necrosis Factor alpha
TVC	Total Vaccinated Cohort
US	United States
VRR	Vaccine response rate
VZV	Varicella-Zoster virus
YOA	Years of Age

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

1. DOCUMENT HISTORY

Date	Description	Protocol Version
19 August 2016	Version 1	Final version 1: 12 November 2015
14 May 2018	Amendment 1: amendment of the SAP to better match with the planned analyses described in the protocol and the approved TFL for the Y10 and re-vaccination part (dated 26MAR2018). None of those modifications are major changes to planned analysis	Final version 1: 12 November 2015

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
1	gE501B	50 μg gE/AS01$_B$	gE501B

The following subgroup names will be used for the statistical analyses by age stratum:

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
1	60-69 YOA	60-69 years old subjects at time of initial vaccination in study ZOSTER-003
2	≥70 YOA	Over 70 years old subjects at time of initial vaccination in study ZOSTER-003

3. OBJECTIVES

3.1. Primary objective

- To evaluate persistence of humoral and cell-mediated immune responses overall at Months 108 and 120 post first dose of initial vaccination course in study ZOSTER-003.

Refer to Section 4.1 for the definition of the primary endpoints.

3.2. Secondary objectives

For the persistence phase Months 108 and 120 post first dose of initial vaccination in study ZOSTER-003:

- To evaluate the persistence of humoral and cell-mediated immune responses within each age cohort (60-69 YOA and ≥70 YOA at the time of the initial vaccination) at Months 108 and 120 post first dose of initial vaccination course.

- To evaluate the safety of the study vaccine from Months 108 to Months 120 post first dose of initial vaccination course.

For the re-vaccination phase:

- To evaluate humoral and cell-mediated immune responses to a two dose re-vaccination course at one month after each dose (Months 121 and 123) and 12 months after last dose (Month 134) when administered 10 years after the initial vaccination course.

- To evaluate the reactogenicity and safety of the study vaccine after re-vaccination with two additional doses.

Refer to Section 4.2 for the definition of the secondary endpoints.

4. ENDPOINTS

4.1. Primary endpoints

- Antigen-specific antibody (Ab) concentrations at Months 108 and 120 post first dose of initial vaccination course in study ZOSTER-003.
  - Anti-gE Ab concentrations as determined by ELISA at Months 108 and 120.
- Cell-Mediated Immunity (CMI) in terms of frequencies of antigen-specific CD4+T-cells at Months 108 and 120 post first dose of initial vaccination course in study ZOSTER-003.
  - Frequencies of CD4+T cells with antigen-specific Interferon gamma (IFN-γ) and/or Interleukin-2 (IL-2) and/or Tumour Necrosis Factor alpha (TNF-α) and/or CD40 Ligand (CD40L) secretion/expression to gE as determined by Intracellular Cytokine Staining (ICS) at Months 108 and 120.

4.2. Secondary endpoints

For the follow-up of persistence phase Months 108 and 120 post first dose of initial vaccination course in study ZOSTER-003:

- Antigen-specific antibody (Ab) concentrations within each age cohort (60-69 YOA and ≥70 YOA at the time of initial vaccination) at persistence Months 108 and 120.
  - Anti-gE Ab concentrations as determined by ELISA at Months 108 and 120.
- Cell-Mediated Immunity (CMI) in terms of frequencies of antigen-specific CD4+T-cells within each age cohort (60-69 YOA and ≥70 YOA at the time of initial vaccination) at persistence Months 108 and 120 post first dose of initial vaccination course.
  - Frequencies of CD4+T cells with antigen-specific Interferon gamma (IFN-γ) and/or Interleukin-2 (IL-2) and/or Tumour Necrosis Factor alpha (TNF-α) and/or CD40 Ligand (CD40L) secretion/expression to gE as determined by Intracellular Cytokine Staining (ICS) at Months 108 and 120.
- Serious Adverse events:
  - Occurrence of all serious adverse events (SAEs) related to study participation or to a concurrent GSK medication/vaccine (including HZ/su administered during the ZOSTER-003 study) between Months 108 and 120.

For the re-vaccination phase:

- Antigen-specific antibody (Ab) concentrations post re-vaccination.
  - Anti-gE antibody concentrations as determined by ELISA in all subjects at one month after each vaccine dose (Months 121 and 123) and 12 months after last dose (Month 134).
- Cell-Mediated Immunity (CMI) in terms of frequencies of antigen-specific CD4+T-cells at one month after each vaccine dose (Months 121 and 123) and 12 months after last dose (Month 134).
  - Frequencies of CD4+T cells with antigen-specific Interferon gamma (IFN-γ) and/or Interleukin-2 (IL-2) and/or Tumour Necrosis Factor alpha (TNF-α) and/or CD40 Ligand (CD40L) secretion/expression to gE as determined by Intracellular Cytokine Staining (ICS) at Months 121, 123 and 134.
- Solicited local and general symptoms:
  - Occurrence and intensity of each solicited local symptom within 7 days (Days 0-6) after each vaccination in all subjects;
  - Occurrence, intensity and relationship to vaccination of each solicited general symptom within 7 days (Days 0-6) after each vaccination, in all subjects;
- Unsolicited adverse events (AEs)
  - Occurrence, intensity and relationship to vaccination of unsolicited AEs during 30 days (Days 0-29) after each vaccination, according to the Medical Dictionary for Regulatory Activities (MedDRA) classification in all subjects
- SAEs
  - Occurrence and relationship to vaccination of all SAEs from dose 1 of re-vaccination until study end.
  - Occurrence of any fatal SAEs from dose 1 of re-vaccination until study end.
- Potential immune-mediated diseases (pIMDs)
  - Occurrence and relationship to vaccination of any pIMDs from dose 1 of re-vaccination until study end in all subjects.

5. STUDY POPULATION

The TVC and ATP cohorts are described in section 10.4 of the protocol. 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 immunogenicity Y9	1040-2500	PR
ATP cohort for immunogenicity Y10	1040-2500	P1
Total Vaccinated cohort for re-vaccination phase	1030	MA
ATP cohort for analysis for immunogenicity after re-vaccination	1030-2500	MA
ATP cohort for immunogenicity Y11	1040-2500	FU

6. STATISTICAL METHODS

6.1. Analysis of demographics/baseline characteristics

The analysis of demography for the persistence phase at Month 108 and Month 120 post first dose of initial vaccination course will be performed on the Total enrolled cohort, the ATP cohort for immunogenicity Y9 and ATP cohort for immunogenicity Y10 respectively.

- Demographic characteristics (age at first initial vaccination dose, gender, geographic ancestry, race and ethnicity), cohort description and withdrawal status will be summarized overall.
- The mean age (plus range and standard deviation) as a whole and stratified by age category at dose 1 of initial vaccination in ZOSTER-003 will be calculated.
- The distribution of subjects enrolled among the study sites will be tabulated.
- Frequency tables will be generated for categorical variables such as gender.
- Mean, median and standard error will be provided for continuous data such as age.

The analysis of demography for the re-vaccination phase will be performed on the total vaccinated cohort (TVC) for re-vaccination phase, ATP cohort for immunogenicity after re-vaccination and ATP cohort for immunogenicity Y11.

- Demographic characteristics (age at first re-vaccination dose, gender, geographic ancestry, race and ethnicity), cohort description and withdrawal status will be summarized overall.
- The mean age at first re-vaccination dose (plus range and standard deviation) as a whole and stratified by age category at dose 1 of initial vaccination in ZOSTER-003 will be calculated.
- The distribution of subjects vaccinated among the study sites will be tabulated.
- Frequency tables will be generated for categorical variables such as gender.
- Mean, median and standard error will be provided for continuous data such as age.

6.2. Analysis of immunogenicity

6.2.1. Assessment of follow-up of persistence phase Months 108 and 120 after initial vaccination course.

The analysis for the persistence phase at Month 108 and Month 120 after initial vaccination course will be performed on the ATP cohort for immunogenicity at Y9 and ATP cohort for immunogenicity at Y10. If, the percentage of subjects with serological results excluded from the ATP cohort for analysis of persistence is 5% or more, a second analysis based on the total enrolled cohort will be performed to complement the ATP analysis. All analyses will be performed overall and by age category at dose 1 of initial vaccination in ZOSTER-003 if the number of subjects is sufficient in each stratum.

The exploratory analysis of modelling the persistence of immune response (humoral and cellular) after initial vaccination will be performed on subjects who received two doses of HZ/su vaccine in ZOSTER-003.

Humoral Immune response

- At each timepoint that a blood sample is available (at Months 0, 3, 12, 24, 36, 48, 60, 72, 108 and 120) post initial vaccination, the following parameters will be tabulated overall, and some by age category at dose 1 of initial vaccination in ZOSTER-003:
  - Geometric mean concentrations (GMCs) of anti-gE Ab with 95% confidence interval (CIs).
  - Anti-gE seropositivity rates with exact 95% confidence interval (CIs);
  - Descriptive statistics on Anti-gE antibody concentrations (mean, standard deviation, min, Q1, median, Q3, max)
  - Vaccine response rates for anti-gE antibody concentrations
  - Descriptive statistics of the fold increase over pre-vaccination in the initial study at months 3, 12, 24, 36, 48, 60, 72, 108 and 120 (mean, standard deviation, min, Q1, median, Q3, max).
  - Distribution of the fold over pre-vaccination in the initial study at months 3, 12, 24, 36, 48, 60, 72, 108 and 120
  - Mean Geometric Increase (MGI), ie geometric mean of the ratio Post-vaccination at months 3, 12, 24, 36, 48, 60, 72, 108 and 120 over pre-vaccination in the initial study, with 95% CI.
- The distribution of antibody titers at Months 48, 60, 72, 108 and 120 will be presented using reverse cumulative curves.

CMI response

- Descriptive statistics (N, mean, SD, min, Q1, median, Q3, max) for the following parameters will be tabulated at Months 0, 3, 12, 24, 36, 48, 60, 72, 108 and 120 post first dose of initial vaccination overall and some by age category at dose 1 of initial vaccination in ZOSTER-003 [60-69, ≥70 YOA]:
  - the frequency of gE specific CD4+T-cells secreting at least 2 activation markers (among IFN-γ, IL-2, TNF-α, CD40L)
  - the frequency of CD4+T-cells following induction with gE secreting at least two activation markers (among IFN-γ, IL-2, TNF-α, CD40L)
  - the fold increase over pre-vaccination in the frequency of gE-specific CD4(2+) T-cells
  - the fold increase over pre-vaccination in the frequency of CD4(2+) T-cells following induction with gE
- Vaccine response rates of frequency of gE-specific CD4(2+) T-cells up to Month 120 post-vaccination will be tabulated with 95% CI

Exploratory analysis

- A piece-wise linear mixed model, power law model and modified power law model [David , 2009] for repeated measurements (all data available from month 0, 3, 12, 24, 36, 48, 60, 72, 108 and 120 post first dose of initial vaccination) will be used to model over time
  - the frequency of gE specific CD4+T-cells secreting at least 2 activation markers (among IFN-γ, IL-2, TNF-α, CD40L)
  - the frequency of CD4+T-cells following induction with gE secreting at least 2 activation markers (among IFN-γ, IL-2, TNF-α, CD40L)
  - the gE antibody concentrations
- The three models based on data up to M120 will be presented graphically. Models based on data up to M108 and on data up to M120 will be presented side by side.

6.2.2. Assessment of re-vaccination phase

The analysis of re-vaccination phase will be based on the ATP cohort for analysis of immunogenicity after re-vaccination. If, the percentage of vaccinated subjects with serological results excluded from the ATP cohort for analysis of immunogenicity after re-vaccination is 5% or more, a second analysis based on the TVC will be performed to complement the ATP analysis.

All analyses will be performed overall and by age category at dose 1 of initial vaccination in ZOSTER-003 (60-69, ≥70 YOA) if the number of subjects is sufficient in each stratum.

Humoral Immune response

- At each timepoint that a blood sample is available (at Months 120, 121 and 123) post-re-vaccination, the following parameters will be tabulated overall and some by age category at dose 1 of initial vaccination in ZOSTER-003 (60-69, ≥70 YOA):
  - GMCs of anti-gE Ab with 95% confidence interval (CIs);
  - Anti-gE seropositivity rates with exact 95% confidence interval (CIs);
  - Vaccine response rates [post re-vaccination (M121 and M123) over pre-vaccination in the initial ZOSTER-003 study] with 95% confidence interval (CIs);
  - MGI (geometric mean of the ratio) Post Month 1 (Month 121) and post Month 3 (Month 123) post re-vaccination (current study) over Pre-re-vaccination (Month 120), with 95% CI;
  - Descriptive statistics of the fold increase over pre-re-vaccination at months 121 and 123 (mean, standard deviation, min, Q1, median, Q3, max).
  - Distribution of the fold increase over pre-re-vaccination at months 121 and 123
  - MGI (geometric mean of the ratio) Post Month 1 (Month 121) and post Month 3 (Month 123) post re-vaccination (current study) over pre-vaccination in the initial ZOSTER-003 study, with 95% CI;
- The distribution of antibody titers at Months 120, 121 and 123 will be presented using reverse cumulative curves.

CMI response

- Descriptive statistics (N, mean, SD, min, Q1, median, Q3, max) for the following parameters will be tabulated at Months 120, 121 and 123 overall and some by age category at dose 1 of initial vaccination in ZOSTER-003 [60-69,≥70 YOA]:
  - the frequency of gE specific CD4+T-cells secreting at least 2 activation markers (among IFN-γ, IL-2, TNF-α, CD40L)
  - the frequency of CD4+ T-cells following induction with gE secreting at least 2 activation markers (among IFN-γ, IL-2, TNF-α, CD40L)
  - the fold increase over pre-re-vaccination in the frequency of gE specific CD4[2+] T-cells at months 121 and 123
  - the fold increase over pre-re-vaccination in the frequency of CD4[2+] T-cells following induction with gE at months 121 and 123
  - the fold increase over pre-vaccination in the initial ZOSTER-003 study in the frequency of gE specific CD4[2+] T-cells at months 121 and 123
  - the fold increase over pre-vaccination in the initial ZOSTER-003 study in the frequency of CD4[2+] T-cells following induction with gE at months 121 and 123
- Vaccine response rates of frequency of gE-specific CD4(2+) T-cells at Months 121 and 123 over pre-vaccination in the initial ZOSTER-003 study will be tabulated with 95% CI.

6.2.3. Assessment of persistence after re-vaccination

Persistence data will be analyzed at Month 134 timepoint after re-vaccination (i.e. 12 months after last dose of re-vaccination).

The analysis of antibody persistence after re-vaccination at Month 134 will be based on the ATP cohort for analysis of immunogenicity at Y11. If the percentage of subjects who come back for this follow-up with serological results excluded from the ATP cohort is higher than 5%, a second analysis based on the TVC for revaccination course will be performed to complement the ATP analysis.

Humoral immune response:

- If a blood sample is available at Month 120, 121, 123 and 134, the following parameters (with 95% CIs) will be tabulated overall and some by age group (60-69, ≥70 YOA at time of initial vaccination in study ZOSTER-003):
  - GMCs of anti-gE Ab with 95% confidence interval (CIs);
  - Anti-gE seropositivity rates with exact 95% confidence interval (CIs);
  - Vaccine response rates [post re-vaccination over pre-vaccination in the initial ZOSTER-003 study] with 95% confidence interval (CIs);
  - MGI (geometric mean of the ratio) post re-vaccination (current study) over Pre-re-vaccination (Month 120), with 95% CI;
  - Descriptive statistics of the fold increase over pre-re-vaccination at months 121, 123 and 134 (mean, standard deviation, min, Q1, median, Q3, max).
  - Distribution of the fold increase over pre-re-vaccination at months 121, 123 and 134
  - MGI (geometric mean of the ratio) post re-vaccination (current study) over pre-vaccination in the initial ZOSTER-003 study, with 95% CI;
- The distribution of antibody titers at Months 121, 123 and 134 will be presented using reverse cumulative curves.

CMI response:

- Descriptive statistics (N, mean, SD, min, Q1, median, Q3, max) for the following parameters will be tabulated at Months 120, 121, 123 and 134 overall and some by age category at dose 1 of initial vaccination in ZOSTER-003 [60-69, ≥70 YOA]:
  - the frequency of gE specific CD4+T-cells secreting at least 2 activation markers (among IFN-γ, IL-2, TNF-α, CD40L)
  - the frequency of CD4+ T-cells following induction with gE secreting at least 2 activation markers (among IFN-γ, IL-2, TNF-α, CD40L)
  - the fold increase over pre-re-vaccination in the frequency of gE specific CD4[2+] T-cells at months 121, 123 and 134
  - the fold increase over pre-re-vaccination in the frequency of CD4[2+] T-cells following induction with gE at months 121, 123 and 134
  - the fold increase over pre-vaccination in the initial ZOSTER-003 study in the frequency of gE specific CD4[2+] T-cells at months 121, 123 and 134
  - the fold increase over pre-vaccination in the initial ZOSTER-003 study in the frequency of CD4[2+] T-cells following induction with gE at months 121, 123 and 134
- Vaccine response rates of frequency of gE-specific CD4(2+) T-cells at Months 121, 123 and 134 over pre-vaccination in the initial ZOSTER-003 study will be tabulated with 95% CI.

6.3. Analysis of safety and reactogenicity

6.3.1. Persistence phase

Analyses in the follow-up of persistence phase will be performed on the Total enrolled cohort.

Description of SAEs and HZ cases will be provided.

6.3.2. Re-vaccination phase

The primary analysis for safety will be based on the TVC for re-vaccination course.

Safety and reactogenicity analyses will be performed overall and by age category at dose 1 of initial vaccination in ZOSTER-003 [60-69, ≥70 YOA] if the number of subjects is sufficient in each stratum.

When appropriate, tabulations will be presented overall and by time of occurrence relative to last vaccination (e.g., using windows such as Days 0-6, Days 0-29 and more than 30 days post-vaccination).

The results for the analysis of safety will be tabulated as follows:

- The number and percentage of subjects with at least one local solicited AE, with at least one general solicited AE and with any solicited AE during the 7-day follow-up period with exact 95% CIs after each vaccine dose and overall will be provided; the same tabulation will be performed for grade 3 symptoms and for solicited and unsolicited symptoms.
- The percentage of subjects reporting each individual solicited local and general AE during the solicited 7-day follow-up period will be tabulated with exact 95% CI;
- For all solicited symptoms, the same tabulation will be performed for grade 3 solicited AEs and for solicited general AEs with relationship to vaccination;
- Summary of temperature value by half degree increment reported during the 7-day (Days 0-6) post-vaccination period following each dose and subjects
- Number of days with solicited symptoms will be tabulated
- The proportion of subjects/doses with at least one report of unsolicited AE classified by the MedDRA preferred terms and reported up to 30 days after each vaccination will be tabulated with exact 95% CI;
- The same tabulations will be performed for grade 3 unsolicited AEs (not including SAEs) and for unsolicited AEs with a relationship to vaccination. The proportion of AEs resulting in a medically attended visit will also be tabulated;
- Number and percentage of subjects with pIMDs classified by the MedDRA preferred terms will be tabulated and described in detail; the same for SAEs.
- SAEs and withdrawal due to AE(s) will be described in detail.
- Potential and confirmed HZ cases identified during the study will be listed.

7. STATISTICAL CALCULATIONS

7.1. Derived and transformed data

7.1.1. Demography

For a given subject and a given demographic variable, missing measurement will not be replaced except for age.

7.1.1.1. Age at vaccination

Age will be calculated as the number of years between the date of birth and the date of first vaccination (either at first initial vaccination dose or at the first re-vaccination dose as specified).

To ensure that the collection of date of birth will not jeopardize the privacy of Personally Identifiable Information (PII), only a partial date of birth (MMYYYY) will be collected.

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

In case the month is missing, the date will be replaced by the June 30th of the year.

7.1.2. Immunogenicity

7.1.2.1. CMI response

- The log-transformation of the frequency of CD4+T-cells producing at least 2 immunological activation markers (IFN-γ, IL-2, TNF-α and /or CD40L, termed "all doubles" or CD4[2+]) upon in vitro stimulation with the antigen (induction condition) is calculated by adding an offset of 0.5 to the number of activated CD4+T-cells producing at least 2 immunological activation markers ("all doubles", CD4[2+]) upon in vitro stimulation in medium only (background condition).

$$Freq_{Induction}^{CD4\ 2+} = \frac{n_{Induction}^{2+} + 0.5}{N_{Induction}^{CD4}} \qquad Log_e\left(Freq_{Induction}^{CD4\ 2+}\right) = Log_e\left(\frac{n_{Induction}^{2+} + 0.5}{N_{Induction}^{CD4}}\right)$$

$$Freq_{Background}^{CD4\ 2+} = \frac{n_{Background}^{2+} + 0.5}{N_{Background}^{CD4}} \qquad Log_e\left(Freq_{Background}^{CD4\ 2+}\right) = Log_e\left(\frac{n_{Background}^{2+} + 0.5}{N_{Background}^{CD4}}\right)$$

$n_{Induction}^{2+} =$ **Number of antigen – specific T-cells expressing at least 2 cytokines**

$n_{Background}^{2+} =$ **Number of CD4 T-cells expressing at least 2 cytokines in the medium only**

$N^{CD4} =$ **Total number of CD4 involved in the assay (induction or background)**

- The individual frequency of antigen –specific (gE) CD4+T-cells is calculated as the difference between the frequency of CD4+T-cells producing at least 2 immunological activation markers ("all doubles", CD4[2+]) upon in vitro stimulation

in medium only (background condition). When the log-transformation is applied to that variable prior to analysis, differences less or equal to zero (0) are imputed to 1 gE -specific cytokine secreting CD4+T-cells per 10^6 CD4+T-cells.

$$Freq_{Specific}^{CD4\ 2+} = \frac{n_{Induction}^{2+} + 0.5}{N_{Induction}^{CD4}} - \frac{n_{Background}^{2+} + 0.5}{N_{Background}^{CD4}}$$

$$Log_e\left(Freq_{Specific}^{CD4\ 2+}\right) = Log_e\left(\frac{n_{Induction}^{2+} + 0.5}{N_{Induction}^{CD4}} - \frac{n_{Background}^{2+} + 0.5}{N_{Background}^{CD4}}\right) \ if\ \frac{n_{Induction}^{CD4\ 2+}}{N_{Induction}^{CD4}} > \frac{n_{Background}^{CD4\ 2+}}{N_{Background}^{CD4}}$$

$$Log_e\left(Freq_{Specific}^{CD4\ 2+}\right) = Log_e\left(\frac{1}{10^6 cells}\right) \qquad if\ \frac{n_{Induction}^{CD4\ 2+}}{N_{Induction}^{CD4}} \leq \frac{n_{Background}^{CD4\ 2+}}{N_{Background}^{CD4}}$$

- The Geometric Mean (GM) calculations are performed by taking the anti-log of the mean of the log frequency transformations.
- The vaccine response after vaccination or after re-vaccination for the frequency of gE-specific CD4(2+) T-cells over pre-vaccination in the initial ZOSTER-003 study is defined as follows:
  - For initially subjects with pre-vaccination T cell frequencies below the threshold, at least a 2-fold increase as compared to the threshold (2x<320> Events/10E6 CD4+ T cells)
  - For initially subjects with pre-vaccination T cell frequencies above the threshold, at least a 2-fold increase as compared to pre-vaccination T cell frequencies

7.1.2.2. Humoral Immune response

- Cut-off for anti-gE Ab assay: 97 mIU/ml.
- A seronegative subject is a subject whose anti-gE Ab concentration is below the cut-off value.
- A seropositive subject is a subject whose anti-gE Ab concentration is greater than or equal to the cut-off value.
- The seropositivity rate is defined as the percentage of seropositive subjects.
- The vaccine response after vaccination or after re-vaccination for anti-gE over pre-vaccination in the initial ZOSTER-003 study is defined as the percentage of subjects who have at least:
  - For seronegative subjects at PRE (M0), antibody concentration at post-re-vaccination ≥ 4 fold the cut-off for anti-gE (4x97 mIU/ml)
  - For seropositive subjects at PRE (M0), antibody concentration at post-re-vaccination ≥ 4 fold the pre-vaccination antibody concentration
- The MGI of anti-gE antibody concentrations over pre-vaccination in the initial ZOSTER-003 study is defined as the geometric mean of the within -subject ratios of the post-vaccination reciprocal anti-gE concentration to the Day 0 reciprocal anti-gE concentration
- The MGI of anti-gE antibody concentrations (after re-vaccination) over pre-re-vaccination is defined as the geometric mean of the within -subject ratios of the post-re-vaccination reciprocal anti-gE concentration to the M120 reciprocal anti-gE concentration
- The GMCs calculations are performed by taking the anti-log of the mean of the log concentration transformations. Antibody concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMC calculation.

7.1.3. Safety and reactogenicity

7.1.3.1. Counting rule

The way the percentage of subjects will be derived will depend on the event analyzed (see the following table for details). As a result, the denominator (N) will differ from one table to another.

Event	N used for deriving %	Terminology used in the tables for N
Concomitant medication	All vaccinated subjects	Number of subjects with at least one administered dose
Solicited general symptom	All vaccinated subjects with at least one solicited general symptom documented as either present or absent	For each dose and overall/subject: N= number of subjects with at least one documented dose For overall/dose: N= number of documented doses
Solicited local symptom	All vaccinated subjects with at least one solicited local symptom documented as either present or absent	For each dose and overall/subject: N= number of subjects with at least one documented dose For overall/dose: N= number of documented doses
Unsolicited symptom from day 0 to day X	All vaccinated subjects	Number of subjects with at least one administered dose
SAE	All vaccinated subjects	Number of subjects with at least one administered dose

7.1.3.2. Grading rule

The maximum intensity of local injection site redness/swelling will be scored at GSK Biologicals using GSK Biologicals' standard grading scale based on the US Food and Drug Administration (FDA) guidelines for Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers enrolled in Preventive Vaccine Clinical Trials" (see protocol).

0 : <20 mm diameter

1 : ≥20 mm to ≤50 mm diameter

2 : >50 mm to ≤100 mm diameter

3 : >100 mm diameter

The preferred route to evaluate body temperature in this study is oral. When there is no other alternative, temperature may be recorded by other route (e.g., axillary, rectal or tympanic), If the temperature is not taken by oral route the route should be documented.

Grade 3 fever will be defined as temperature > 39.0°C (regardless the route used).

Note that Fever is defined as temperature ≥ 37.5°C / 99.5°F for oral, axillary or tympanic route, or ≥ 38.0°C / 100.4°F for rectal route.

7.1.3.3. Conversion of temperature to °C

The following conversion rule is used for the conversion of temperature to °C

Temperature in °Celsius = ((Temperature in °Fahrenheit -32) *5)/9

The result is rounded to 1 decimal digit.

7.2. Handling of missing data

7.2.1. Immunogenicity

For a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, analyses will exclude subjects with missing or non-evaluable measurements.

7.2.2. Reactogenicity and safety

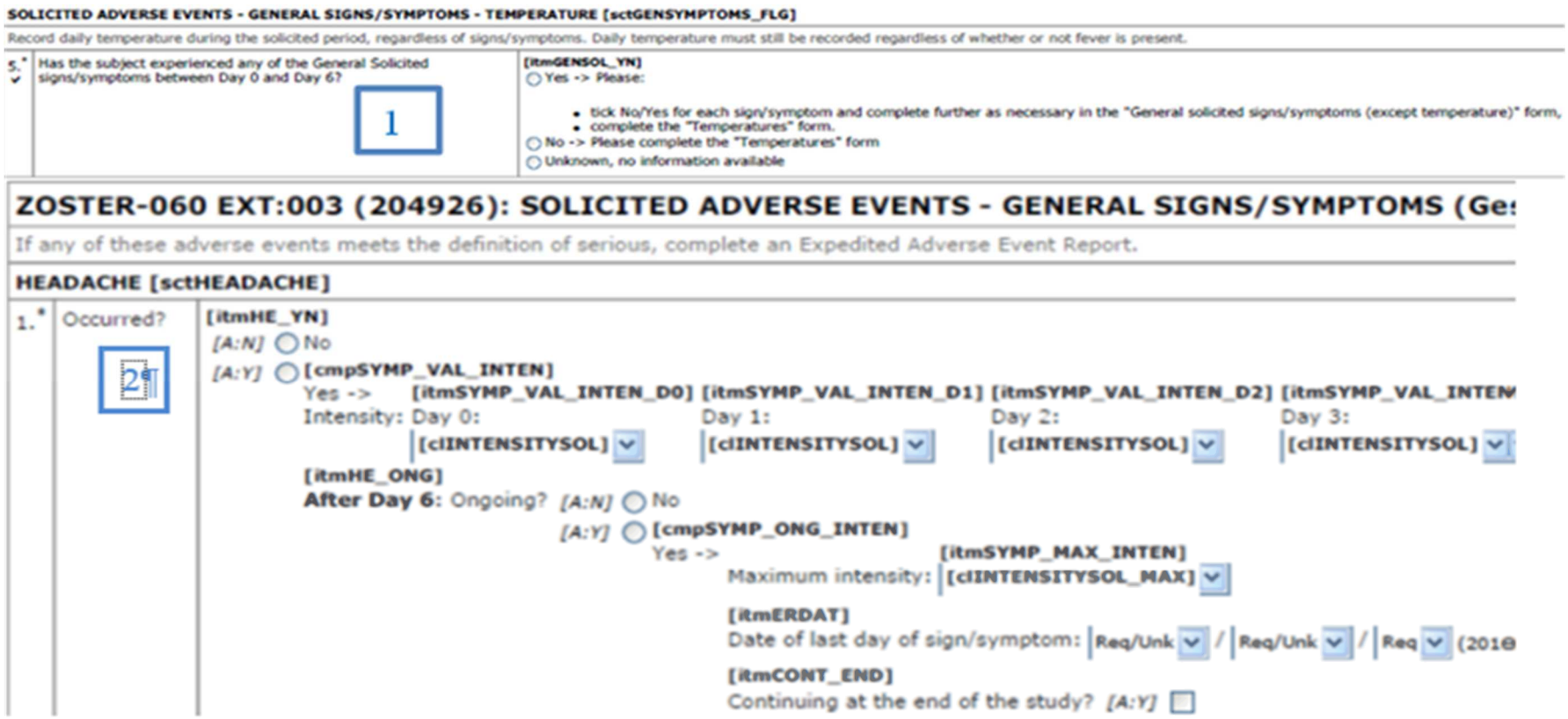
- For a given subject and a given measurement, missing or non-evaluable measurements will not be imputed. The missing endpoint and censoring are supposed to occur independently, and the pattern of the missing value(s) being either Missing Completely At Random (MCAR) or Missing At Random (MAR) only.
- For the analysis of solicited symptoms, missing or non-evaluable measurements will not be replaced. Therefore, the analysis of the solicited symptoms based on the TVC will include only subjects/doses with documented safety data (i.e., symptom screen/sheet completed).
- For the analysis of unsolicited symptoms/SAEs/pIMDs/concomitant medication, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.

7.2.2.1. Solicited general symptoms

The analysis of solicited general symptoms will include all subjects for whom the question (1) in Figure 1 about the presence of any solicited general symptom has been answered by ‘Yes’ or ‘No’ (see the GENSOL_YN item).

The next sections describe how each subject contributes to the analyses, depending on the endpoints.

Figure 1 Information captured in the clinical database for solicited general symptoms



For each solicited general symptom if the answer to question (2) in Figure 1 is "No", the subject will be considered as not having that symptom after that dose.

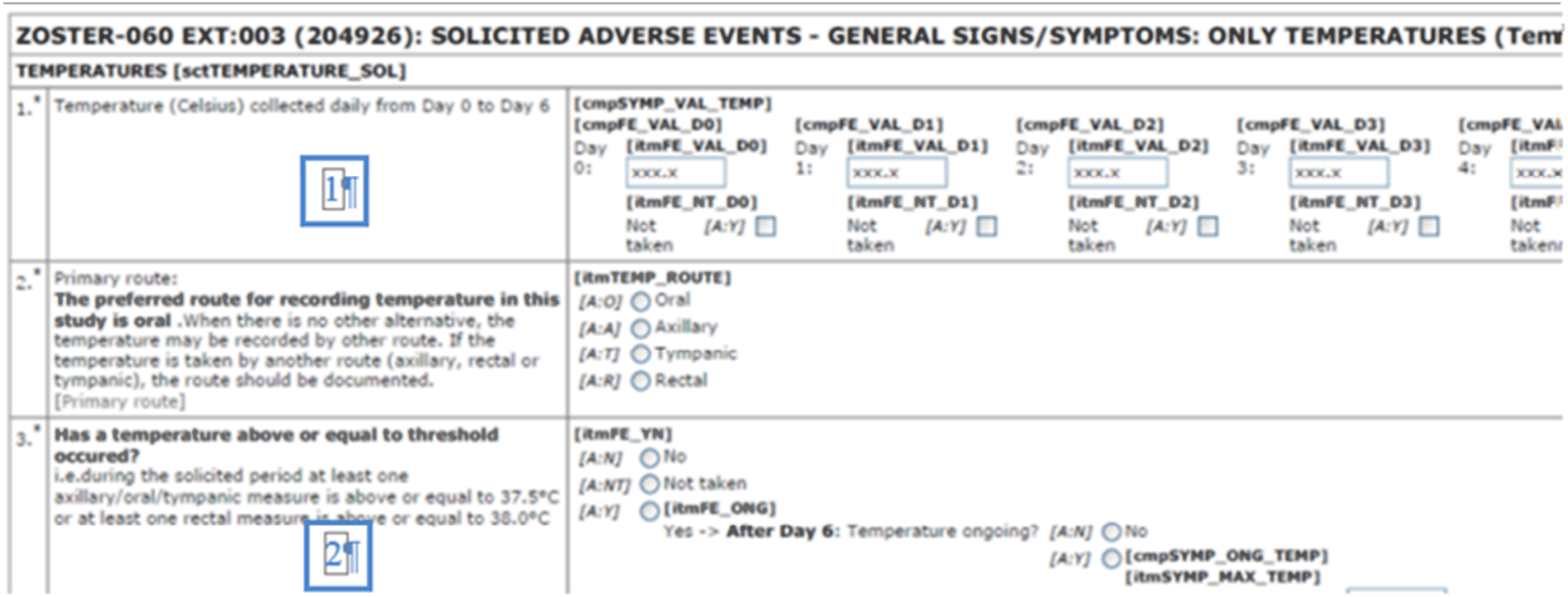
Subjects who documented the presence of a specific symptom i.e. if the answer to question (2) in Figure 1 is "Yes" (ex FA_YN=Y), the maximum intensity recording over the considered follow-up period is used for the analysis of the percentage of subjects with symptoms.

If the subject answered "Yes" to (2) in Figure 1 for a specific symptom BUT partially recorded daily measurement (e.g. intensity missing for Day 3) over the considered solicited period, she/he will be included in the summaries and classified according to their maximum observed daily recording over the solicited period.

If the subject answered "Yes" to (2) in Figure 1 for a specific symptom BUT no daily measurement is recorded, she/he will not be included and counted in the summary of subjects with symptoms above a specified threshold, however she/he will be part of the summary corresponding to the 'All' category.

7.2.2.2. Solicited general fever

Figure 2 Information captured in the clinical database for temperature record



ZOSTER-060 EXT:003 (204926): SOLICITED ADVERSE EVENTS - GENERAL SIGNS/SYMPTOMS: ONLY TEMPERATURES (Tem

TEMPERATURES [sctTEMPERATURE_SOL]

1.* Temperature (Celsius) collected daily from Day 0 to Day 6

[cmpSYMP_VAL_TEMP]

[cmpFE_VAL_D0]	**[cmpFE_VAL_D1]**	**[cmpFE_VAL_D2]**	**[cmpFE_VAL_D3]**	**[cmpFE_VAL**
Day 0: **[itmFE_VAL_D0]** xxx.x	Day 1: **[itmFE_VAL_D1]** xxx.x	Day 2: **[itmFE_VAL_D2]** xxx.x	Day 3: **[itmFE_VAL_D3]** xxx.x	Day 4: **[itmF** xxx.x
[itmFE_NT_D0] Not taken *[A:Y]* ☐	**[itmFE_NT_D1]** Not taken *[A:Y]* ☐	**[itmFE_NT_D2]** Not taken *[A:Y]* ☐	**[itmFE_NT_D3]** Not taken *[A:Y]* ☐	**[itmF** Not taken

2.* Primary route:
The preferred route for recording temperature in this study is oral .When there is no other alternative, the temperature may be recorded by other route. If the temperature is taken by another route (axillary, rectal or tympanic), the route should be documented.
[Primary route]

[itmTEMP_ROUTE]
[A:O] ◯ Oral
[A:A] ◯ Axillary
[A:T] ◯ Tympanic
[A:R] ◯ Rectal

3.* **Has a temperature above or equal to threshold occured?**
i.e.during the solicited period at least one axillary/oral/tympanic measure is above or equal to 37.5°C or at least one rectal measure is above or equal to 38.0°C

[itmFE_YN]
[A:N] ◯ No
[A:NT] ◯ Not taken
[A:Y] ◯ **[itmFE_ONG]**
Yes -> **After Day 6:** Temperature ongoing? *[A:N]* ◯ No
[A:Y] ◯ **[cmpSYMP_ONG_TEMP]**
[itmSYMP_MAX_TEMP]

For temperature

To allow the temperature recording, a separate screen needs to be completed whatever the answer to question (2) in Figure 2 is, also in case no temperature equal or above 37.5°C has been found in the diary card.

All subjects for whom question (2) in Figure 2 has been answered as "Yes" or "No", will be included in the summaries of temperature by half degree (°C) cumulative increments and classified according to their maximum temperature value observed daily recording over the solicited period. If no daily measurement is recorded for temperature, the subject will not be counted in the summary of subjects with temperature above a specific threshold. For the summary of temperature, the "all" category will not be computed. This table will be produced overall, by route with no route conversion.

For summary of fever

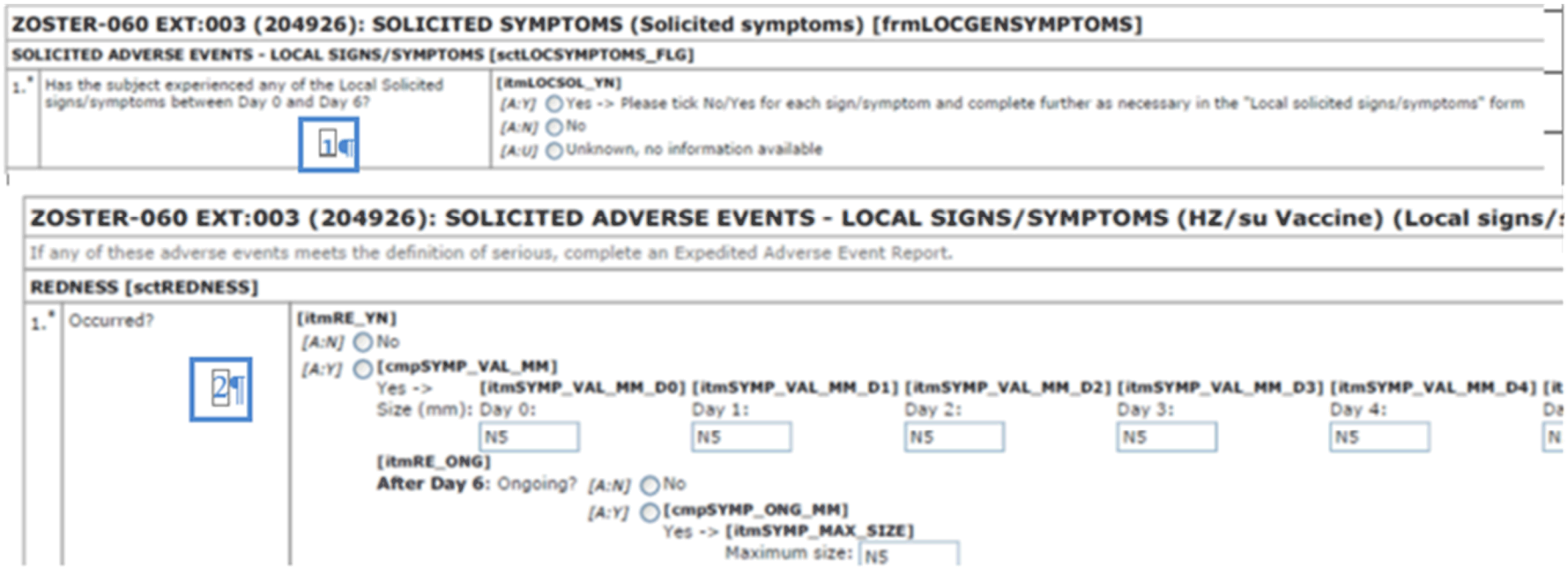
All subjects for whom question (2) in Figure 2 has been answered as "Yes" or "No", will be included in the summaries of fever and classified according to their maximum temperature value observed daily recording over the solicited period even if partial recording. If no daily measurement is recorded for temperature, the subject will not be counted in the summary of subjects with fever and the "all" category will count the number of subject with at least one temperature measurement ≥ 37.5°C / 99.5°F for oral, axillary or tympanic route, or ≥ 38.0°C / 100.4°F for rectal route.

7.2.2.3. Solicited local symptoms

The analysis of solicited local symptoms will include all subjects for whom the question (1) in Figure 3 about the presence of a solicited local symptoms has been answered as 'Yes' or 'No'(see LOCSOL_YN item).

The next sections describe how each subject contributes to the analyses, depending on the endpoints.

Figure 3 Information captured in the clinical database for solicited local symptoms



For each solicited local symptom, if the answer to question (2) in Figure 3 is "No", the subject will be considered as not having that local symptom at the injection site after that dose.

Subjects who documented the presence of a specific symptom i.e. if the answer to question (2) in Figure 3 is “Yes” (ex RE_YN=Y), the maximum intensity recording over the considered follow-up period is used for the analysis of the percentage of subjects with symptoms.

If the subject answered “Yes” to question (2) in Figure 3 for a specific symptom BUT partially recorded daily measurement (e.g., intensity missing for Day 3) over the considered solicited period, she/he will be included in the summaries and classified according to their maximum observed daily recording over the solicited period.

If the subject answered “Yes” to question (2) in Figure 3 for a specific symptom BUT no daily measurement is recorded, she/he will not be included and counted in the summary of subjects with symptoms above a specified threshold, however s/he will be part of the summary corresponding to the ‘All’ category.

7.2.2.4. Combined Solicited local and general symptoms

The analysis of the combined solicited general and local symptoms will include all vaccinated subjects for whom the question (1) in Figure 1 or Figure 3 about the presence of any solicited general or local symptoms has been answered by ‘Yes’ or ‘No’.

7.2.2.5. Unsolicited symptoms

All vaccinated subjects will be considered for the analysis of unsolicited symptoms.

The analysis of unsolicited adverse events, including SAEs, consists of evaluating the percentage of subjects with at least 1 report of an unsolicited adverse event classified by MedDRA.

Subjects who missed reporting unsolicited symptoms will be treated as subjects without unsolicited symptoms.

7.2.2.6. Concomitant medication

All vaccinated subjects will be considered for the analysis of concomitant medication use. Subjects who did not report the use of a concomitant medication will be considered as subjects without medication. Subjects will be counted in the summary who started a concomitant medication during the mentioned period and took at least one dose.

7.2.2.7. Compliance with respect to documenting safety

The number of doses injected, the number of doses not given according-to-protocol, and the number of symptom sheets (SS) transcribed for local and general symptoms, the compliance for local and general symptoms are tabulated for the TVC.

Compliance (%) is defined as the number of general (local) symptom sheets/screens completed divided by the number of doses administered for a specified vaccination (dose) and group.

The number of doses not given according to protocol, are the doses injected at the wrong site and/or side, or injected using the wrong route as defined in the study protocol for each study vaccine. This number is issued from the following question in the vaccine administration sheet of the CRF: "Has the study vaccine been administered according-to-protocol?" Study vaccine dose not administered according to protocol can lead to elimination from the ATP cohort, depending to the ATP cohort definition in the protocol.

7.3. Number of decimals:

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

Display Table	Parameters	Number of decimal digits
Demographic characteristics	Mean, median age	1
Demographic characteristics	SD (age)	2
Immunogenicity	Ratio of GMT/C	2
Reactogenicity	Mean, min, Q1, median, Q3, max for duration	1
All summaries	% of count, including LL & UL of CI	1
All summaries	% of difference, including LL & UL of CI	2

7.4. Methodology for computing CI

Unless otherwise mentioned, the confidence intervals will be 2sided 95% CI and calculated according to the following methods:

7.4.1. Binomial Data

The exact 95% CIs for a proportion within a group will be calculated according to [Clopper , 1934].

7.4.2. Continuous Data

The 95% CI for geometric mean titers/concentrations (GMTs/GMCs) analyses will be obtained. The 95% CI for the mean of log-transformed titer/concentration will be first obtained assuming that log-transformed values were normally distributed with unknown variance. The 95% CI for the GMTs/GMCs will be then obtained by exponential-transformation of the 95% CI for the mean of log-transformed titer/concentration.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

Description	Analysis ID	Disclosure Purpose	Reference for TFL
Final Analysis including persistence 1 year post revaccination	E1_01	CSR/CTRS	TFL E1_01
Persistence Year 9	E1_02	Internal	TFL E1_02
Main analysis: persistence Year 10 and revaccination	E1_03	CSR/CTRS	TFL E1_03

8.2. Statistical considerations for interim analyses

All analyses before the end of study will be conducted on final data as clean as possible and therefore no statistical adjustment for interim analyses is required. The analyses are purely descriptive, therefore no adjustment on type I error is foreseen.

9. CHANGES FROM PLANNED ANALYSES

NA

10. REFERENCES

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

David MP, Van Herck K, Hardt K, Tibaldi F, Dubin G, Descamps D et al. Long-term persistence of anti-HPV-16 and -18 antibodies induced by vaccination with the AS04-adjuvanted cervical cancer vaccine: modeling of sustained antibody responses. Gynecol Oncol 2009; 115 (Suppl 3):S1-6.

Statistical Analysis Plan

Study alias & e-track number(s): ZOSTER-060 EXT 003 (204926)

Detailed Title:	A phase IIIB, open, long term extension study to evaluate the persistence of immune responses and the safety of GSK Biologicals' Herpes Zoster subunit (HZ/su) vaccine 1437173A, at Months 108 and 120 post-vaccination and the assessment of re-vaccination with two additional doses administered at 10 years after the initial vaccination in study ZOSTER-003 in healthy subjects aged 60 years of age and older
SAP version	Version 1
SAP date	19-AUG-2016
Scope:	All data pertaining to the above study.
Co-ordinating author:	PPD
Other author(s):	
Adhoc reviewers:	PPD (Sr Mgr, Clinical Safety & PV), PPD (Sr Mgr, Global Reg Affairs)
Approved by:	
Clinical Research Development Lead	PPD
Project Statistician	PPD
Lead Statistician	PPD
Science Writer	PPD

TABLE OF CONTENTS

PAGE

LIST OF FIGURES

PAGE

LIST OF ABBREVIATIONS

Ab	Antibody
AE	Adverse event
AIC	Akaike's Information Criterion
$AS01_B$	MPL, QS21, liposome based Adjuvant System [50µg MPL and 50µg QS21]
ATP	According-To-Protocol
CD40L	CD40 Ligand
CMI	Cell-Mediated Immunity
eCRF	electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
gE	Glycoprotein E
GMC	Geometric Mean Concentration
GSK	GlaxoSmithKline
HZ	Herpes Zoster
ICS	Intracellular Cytokine Staining
IFN-γ	Interferon gamma
IL-2	Interleukin- 2
LTFU	Long-Term Follow-up
mIU	Milli International Unit
MGI	Mean Geometric Increase
MPL	3-*O*-desacyl-4'-Monophosphoryl Lipid A
pIMDs	Potential Immune-Mediated diseases
QS21	*Quillaja saponaria* Molina, fraction 21 (Antigenics, New York, NY, USA)
SAE	Serious Adverse Event
SBIC	Schwarz' Bayesian Information Criterion
TNF-α	Tumor Necrosis Factor alpha
TVC	Total Vaccinated Cohort
US	United States

VRR	Vaccine response rate
VZV	Varicella-Zoster virus
YOA	Years of Age

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.

1. DOCUMENT HISTORY

Date	Description	Protocol Version
19-AUG-2016	Version 1	Final Version 1: 12 November 2015

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
1	gE501B	50 μg gE/AS01$_B$	gE501B

The following subgroup names will be used for the statistical analyses by age stratum:

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
1	60-69 YOA	60-69 years old subjects at time of initial vaccination in study ZOSTER-003
2	≥70 YOA	Over 70 years old subjects at time of initial vaccination in study ZOSTER-003

3. OBJECTIVES

3.1. Primary objective

- To evaluate persistence of humoral and cell mediated immune responses overall at Months 108 and 120 post first dose of initial vaccination course in study ZOSTER-003.

Refer to Section 4.1 for the definition of the primary endpoints.

3.2. Secondary objectives

For the persistence phase Months 108 and 120 post first dose of initial vaccination in study ZOSTER-003:

- To evaluate the persistence of humoral and cell mediated immune responses within each age cohort (60-69 YOA and ≥70 YOA at the time of the initial vaccination) at Months 108 and 120 post first dose of initial vaccination course.

- To evaluate the safety of the study vaccine from Months 108 to Months 120 post first dose of initial vaccination course.

For the re-vaccination phase:

- To evaluate humoral and cell mediated immune responses to a two dose re-vaccination course at one month after each dose (Months 121 and 123) and 12 months after last dose (Month 134) when administered 10 years after the initial vaccination course.

- To evaluate the reactogenicity and safety of the study vaccine after re-vaccination with two additional doses.

Refer to Section 4.2 for the definition of the secondary endpoints.

4. ENDPOINTS

4.1. Primary endpoints

- Antigen-specific antibody (Ab) concentrations at Months 108 and 120 post first dose of initial vaccination course in study ZOSTER-003.
  - Anti-gE Ab concentrations as determined by ELISA at Months 108 and 120.

- Cell-Mediated Immunity (CMI) in terms of frequencies of antigen-specific CD4 T-cells at Months 108 and 120 post first dose of initial vaccination course in study ZOSTER-003.
  - Frequencies of CD4+ T cells with antigen-specific Interferon gamma (IFN-γ) and/or Interleukin-2 (IL-2) and/or Tumour Necrosis Factor alpha (TNF-α) and/or CD40 Ligand (CD40L) secretion/expression to gE as determined by Intracellular Cytokine Staining (ICS) at Months 108 and 120.

4.2. Secondary endpoints

For the follow-up of persistence phase Months 108 and 120 post first dose of initial vaccination course in study ZOSTER-003:

- Antigen-specific antibody (Ab) concentrations within each age cohort (60-69 YOA and ≥70 YOA at the time of initial vaccination) at persistence Months 108 and 120.
  - Anti-gE Ab concentrations as determined by ELISA at Months 108 and 120.
- Cell-Mediated Immunity (CMI) in terms of frequencies of antigen-specific CD4 T-cells within each age cohort (60-69 YOA and ≥70 YOA at the time of initial vaccination) at persistence Months 108 and 120 post first dose of initial vaccination course.
  - Frequencies of CD4+ T cells with antigen-specific Interferon gamma (IFN-γ) and/or Interleukin-2 (IL-2) and/or Tumour Necrosis Factor alpha (TNF-α) and/or CD40 Ligand (CD40L) secretion/expression to gE as determined by Intracellular Cytokine Staining (ICS) at Months 108 and 120.
- Serious Adverse events:
  - Occurrence of all serious adverse events (SAEs) related to study participation or to a concurrent GSK medication/vaccine (including HZ/su administered during the ZOSTER-003 study) between Months 108 and 120.

For the re-vaccination phase:

- Antigen-specific antibody (Ab) concentrations post re-vaccination.
  - Anti-gE antibody concentrations as determined by ELISA in all subjects at one month after each vaccine dose (Months 121 and 123) and 12 months after last dose (Month 134).
- Cell-Mediated Immunity (CMI) in terms of frequencies of antigen-specific CD4 T-cells at one month after each vaccine dose (Months 121 and 123) and 12 months after last dose (Month 134).

  - Frequencies of CD4+ T cells with antigen-specific Interferon gamma (IFN-γ) and/or Interleukin-2 (IL-2) and/or Tumour Necrosis Factor alpha (TNF-α) and/or CD40 Ligand (CD40L) secretion/expression to gE as determined by Intracellular Cytokine Staining (ICS) at Months 121, 123 and 134.

- Solicited local and general symptoms:
  - Occurrence and intensity of each solicited local symptom within 7 days (Days 0-6) after each vaccination in all subjects;
  - Occurrence, intensity and relationship to vaccination of each solicited general symptom within 7 days (Days 0-6) after each vaccination, in all subjects;
- Unsolicited adverse events (AEs)
  - Occurrence, intensity and relationship to vaccination of unsolicited AEs during 30 days (Days 0-29) after each vaccination, according to the Medical Dictionary for Regulatory Activities (MedDRA) classification in all subjects
- SAEs
  - Occurrence and relationship to vaccination of all SAEs from dose 1 of re-vaccination until study end.
  - Occurrence of any fatal SAEs from dose 1 of re-vaccination until study end.
- Potential immune-mediated diseases (pIMDs)
  - Occurrence and relationship to vaccination of any pIMDs from dose 1 of re-vaccination until study end in all subjects.

5. STUDY POPULATION

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 for immunogenicity Y9	1040-2500	PR
ATP cohort for analysis for immunogenicity Y10	1040-2500	P1
Total Vaccinated cohort for re-vaccination phase	1030	MA
ATP cohort for analysis for immunogenicity	1030-2500	MA
ATP cohort for analysis for immunogenicity Y11	1040-2500	FU

6. STATISTICAL METHODS

6.1. Analysis of demographics/baseline characteristics

Demographic characteristics (age, gender, geographic ancestry, race and ethnicity) will be tabulated.

- The mean age (plus range and standard deviation) of the enrolled subjects, as a whole, and stratified by age group will be calculated.
- The distribution of subjects enrolled among the study sites will be tabulated as a whole.
- No inferential analyses of demographic data or baseline characteristics are planned.

6.2. Analysis of immunogenicity

The primary analysis will be based on the ATP cohorts for analysis of immunogenicity (Y9, Y10, post re-vaccination, Y11). If the percentage of enrolled subjects excluded from this ATP cohort is more than 5%, a second analysis based on the TVC will be performed to complement the ATP analyses.

Immunogenicity descriptive analyses will be performed overall and by age stratum if the number of subjects is sufficient in each stratum.

CMI endpoint

Descriptive statistics of the gE- and VZV- specific frequency (induction-background frequency) of CD4 T-cells producing at least 2 immunological activation markers following stimulation with gE or VZV-antigen will be presented for all subjects participating in Zoster-024. For the purpose of inferential analysis, all pre-vaccination data and following last vaccination data from ZOSTER-003, ZOSTER-011, ZOSTER-012, ZOSTER-013, ZOSTER-024 and ZOSTER-060 (50 μg/gE $AS01_B$ group only) will be included in the analysis set. The frequency of CD4 T-cells producing at least 2-immunological activation markers following stimulation with gE or with VZV-antigen will be analysed (i.e. induction CD4 frequency without subtraction of CD4 frequency due to medium only [i.e. background frequency]). Least square geometric means will be adjusted for the background (medium only) CD4 frequency and pre-vaccination induction response using analysis of covariance methodology. The dependant variable will be log-transformed prior to analysis and least square means will be back-transformed on the original axis.

CMI persistence will be assessed by analysing the frequencies of CD4 T-cells producing at least 2 immunological activation markers following stimulation with gE or VZV.

A mixed effect model for repeated measurements will be used to model over time the frequency of CD4 T-cells producing at least 2 immunological activation markers following stimulation with gE or with VZV [Ledent, 2009]

The covariates will include log-transformed background CD4 frequency, log-transformed pre-vaccination response and the log-transformed of the time elapsed (measured in months) following the last vaccination. Exploratory analyses may include additional effects included in the fixed effect model. A similar "power-law decay" model is described by Fraser [Fraser, 2007] in Appendix A2. The model allows for random individual deviations from the overall mean response (random intercept) and, if necessary according to Schwarz' Bayesian Information Criterion (SBIC) and Akaike's information Criterion (AIC) goodness-of-fit statistics, for a random individual deviation from the overall CD4-frequency decay over time (random coefficient [slope]). Other approaches to modelize the antibody decay may be applied.

Humoral Immune response endpoint

All gE-ELISA samples for the 50 µg/AS01$_B$ ZOSTER-003, ZOSTER-011, ZOSTER-012, ZOSTER-013 have been reanalyzed using the GSK gE ELISA. When the GSK gE ELISA result for a subject cannot be determined or is missing, the predicted value using the Deming regression performed in ZOSTER-024 will be used instead:

$$\text{Log}_{10}\,(\text{GSK gE ELISA}) = 0.6291 + 0.9778 * \text{Log}_{10}(gE\ \text{Henogen})$$

For the persistence analysis, a similar approach as to that described for the CMI endpoint will be followed. The covariates will include pre-vaccination response and time OR the log-transformed of the time elapsed following the last vaccination (measured in months) according to the goodness of fit statistics (AIC or SBIC). Other approaches to modelize the antibody decay may be applied.

The following parameters will be tabulated by vaccine group at each time point when anti-gE Ab concentration result is available:

- Seropositivity rate with exact 95% CI
- GMC with 95% CI
- VRR with exact 95% CI
- Mean Geometric Increase (MGI) with exact 95% CI
- Descriptive statistics (N, mean, SD, min, Q1, median, Q3, max) of Mean Geometric Increase (MGI)

- Distribution of the fold increase i.e Percentage of subjects with a more than X-fold (e.g. >2, >4, >6,.-fold) increase will be tabulated with 95%CI.
- Anti-gE Ab concentrations will be displayed using reverse cumulative curves.

6.3. Analysis of safety and reactogenicity

The primary analysis for safety will be based on the TVC.

Safety and reactogenicity analyses will be performed overall and by age stratum if the number of subjects is sufficient in each stratum.

When appropriate, tabulations will be presented overall and by time of occurrence relative to last vaccination (e.g., using windows such as Days 0-6, Days 0-29 and more than 30 days post-vaccination).

The results for the analysis of safety will be tabulated as follows:

- The number and percentage of subjects with at least one local solicited AE with at least one general solicited AE and with any solicited AE during the 7-day follow-up period with exact 95% CIs after each vaccine dose and overall by vaccination group will be provided;
- The percentage of subjects reporting each individual solicited local and general AE during the solicited 7-day follow-up period will be tabulated with exact 95% CI;
- For all solicited symptoms, the same tabulation will be performed for grade 3 solicited AEs and for solicited general AEs with relationship to vaccination;
- The proportion of subjects with at least one report of unsolicited AE classified by the MedDRA Preferred Terms and reported up to 30 days after each vaccination will be tabulated with exact 95% CI;
- The same tabulation will be performed for grade 3 unsolicited AEs and for unsolicited AEs with a relationship to vaccination. The proportion of AEs resulting in a medically attended visit will also be tabulated;
- Total number/percentages of doses (per dose and overall) followed by AEs will be tabulated;
- Number of subjects with pIMDs will be tabulated and described in detail;
- SAEs and withdrawal due to AE(s) will be described in detail.
- Potential and confirmed HZ cases identified during the study will be listed. Medical data reviewer will provide the listing of all the Preferred Terms associated to an HZ case based on the MedDRA dictionary. This list will be reviewed by Clinical R&D Lead and stored with the clinical database.

- Summary of temperature value by half degree increment reported during the 7-day (Days 0-6) post-vaccination period following each dose and subjects with no conversion rule.
- Summary of temperature value by half degree (°C) increment by route temperature taken reported during the 7-day (Days 0-6) post-vaccination period following each dose with no conversion rule.

The following tables will be done for CTRS posting

- The number of occurrence of the 5% most frequent non-serious unsolicited AE classified by the MedDRA Preferred Terms and reported up to 30 days after each vaccination will be tabulated.
- The number of occurrence of SAEs classified by the MedDRA Preferred Terms and reported during the entire study period will be tabulated.

7. STATISTICAL CALCULATIONS

7.1. Derived and transformed data

7.1.1. Demography

For a given subject and a given demographic variable, missing measurement will *not be replac*ed except for age.

7.1.1.1. Age at vaccination

Age will be calculated as the number of years between the date of birth and the date of first vaccination.

To ensure that the collection of date of birth will not jeopardise the privacy of Personally Identifiable Information (PII), only a partial date of birth (MMYYYY) will be collected.

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

In case the month is missing, the date will be replaced by the June 30th of the year.

7.1.2. Immunogenicity

7.1.2.1. CMI response

- The log-transformation of the frequency of CD4 T-cells producing at least 2 immunological activation markers (IFN-γ, IL-2, TNF-α and /or CD40L, termed "all doubles" or CD4[2+]) upon in vitro stimulation with the antigen (induction condition) is calculated by adding an offset of 0.5 to the number of activated CD4 T-cells producing at least 2 immunological activation markers ("all doubles", CD4[2+]) upon in vitro stimulation in medium only (background condition).

$$Freq_{Induction}^{CD4\ 2+} = \frac{n_{Induction}^{2+} + 0.5}{N_{Induction}^{CD4}} \qquad Log_e\left(Freq_{Induction}^{CD4\ 2+}\right) = Log_e\left(\frac{n_{Induction}^{2+} + 0.5}{N_{Induction}^{CD4}}\right)$$

$$Freq_{Background}^{CD4\ 2+} = \frac{n_{Background}^{2+} + 0.5}{N_{Background}^{CD4}} \qquad Log_e\left(Freq_{Background}^{CD4\ 2+}\right) = Log_e\left(\frac{n_{Background}^{2+} + 0.5}{N_{Background}^{CD4}}\right)$$

$n_{Induction}^{2+}$ = Number of antigen – specific T-cells expressing at least 2 cytokines

$n_{Background}^{2+}$ = Number of CD4 T-cells expressing at least 2 cytokines in the medium only

N^{CD4} = Total number of CD4 involved in the assay (induction or background)

- The individual frequency of antigen –specific (gE or VZV) CD4 T-cells is calculated as the difference between the frequency of CD4 T-cells producing at least 2 immunological activation markers ("all doubles", CD4[2+]) upon in vitro stimulation in medium only (background condition). When the log-transformation is applied to that variable prior to analysis, differences less or equal to zero (0) are imputed to 1 gE or VZV-specific cytokine secreting CD4 T-cells per 10^6 CD4 T-cells.

$$Freq_{Specific}^{CD4\ 2+} = \frac{n_{Induction}^{2+} + 0.5}{N_{Induction}^{CD4}} - \frac{n_{Background}^{2+} + 0.5}{N_{Background}^{CD4}}$$

$$Log_e\left(Freq_{Specific}^{CD4\ 2+}\right) = Log_e\left(\frac{n_{Induction}^{2+} + 0.5}{N_{Induction}^{CD4}} - \frac{n_{Background}^{2+} + 0.5}{N_{Background}^{CD4}}\right) \ if\ \frac{n_{Induction}^{CD4\ 2+}}{N_{Induction}^{CD4}} > \frac{n_{Background}^{CD4\ 2+}}{N_{Background}^{CD4}}$$

$$Log_e\left(Freq_{Specific}^{CD4\ 2+}\right) = Log_e\left(\frac{1}{10^6 cells}\right) \qquad if\ \frac{n_{Induction}^{CD4\ 2+}}{N_{Induction}^{CD4}} \leq \frac{n_{Background}^{CD4\ 2+}}{N_{Background}^{CD4}}$$

- The Geometric Mean (GM) calculations are performed by taking the anti-log of the mean of the log frequency transformations.
- Since almost all subjects are VZV-positive, the cut-off value for CMI response is defined as the 95% percentile of the pre-vaccination distribution for the variable considered, calculated using a non-parametric method. For the frequency of gE-specific CD4[2+], that threshold was equal to $587/10^6$ CD4 T-cells in the previous dose-ranging study.
- A responder is a subject with a CMI response greater than or equal to the cut-off value.

7.1.2.2. Humoral Immune response

- Cut-off for anti-gE Ab assay: 97 mIU/ml.
- A seronegative subject is a subject whose anti-gE Ab concentration is below the cut-off value.
- A seropositive subject is a subject whose anti-gE Ab concentration is greater than or equal to the cut-off value.
- The seropositivity rate is defined as the percentage of seropositive subjects.
- The VRR for anti-gE is defined as the percentage of subjects who have at least:
  - a 4-fold increase in the post-dose 2 anti-gE antibody concentration as compared to the pre-revaccination anti-gE antibody concentration, for subjects who are seropositive at baseline (year 10), or,
  - a 4-fold increase in the post dose 2 anti-gE antibody concentrations as compared to the anti-gE antibodies cut-off value for seropositivity, for subjects who are seronegative at baseline (year 10).
- The MGI is defined as the geometric mean of the within subject ratios of the post-vaccination titre to the Day 0 titre.
- The Geometric Mean Concentrations (GMCs) calculations are performed by taking the anti-log of the mean of the log concentration transformations. Antibody concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMC calculation.

7.1.3. Safety and reactogenicity

7.1.3.1. Counting rule

The way the percentage of subjects will be derived will depend on the event analysed (see the following table for details). As a result, the denominator (N) will differ from one table to another.

Event	N used for deriving %	Terminology used in the tables for N
Concomitant medication	All vaccinated subjects	Number of subjects with at least one administered dose
Solicited general symptom	All vaccinated subjects with at least one solicited general symptom documented as either present or absent	For each dose and overall/subject: N= number of subjects with at least one documented dose For overall/dose: N= number of documented doses
Solicited local symptom	All vaccinated subjects with at least one solicited local symptom documented as either present or absent	For each dose and overall/subject: N= number of subjects with at least one documented dose For overall/dose: N= number of documented doses
Unsolicited symptom from day 0 to day X	All vaccinated subjects	Number of subjects with at least one administered dose
SAE	All vaccinated subjects	Number of subjects with at least one administered dose

7.1.3.2. Grading rule

The maximum intensity of local injection site redness/swelling will be scored at GSK Biologicals using GSK Biologicals' standard grading scale based on the US Food and Drug Administration (FDA) guidelines for Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers enrolled in Preventive Vaccine Clinical Trials" (see protocol).

0 : < 20 mm diameter

1 : ≥ 20 mm to ≤ 50 mm diameter

2 : > 50 mm to ≤ 100 mm diameter

3 : > 100 mm diameter

The preferred route to evaluate body temperature in this study is oral. When there is no other alternative, temperature may be recorded by other route (e.g., axillary, rectal or tympanic), If the temperature is not taken by oral route the route should be documented. Temperature (measured by oral, axillary or tympanic route) will be scored at GSK Biologicals as follows:

0 : < 37.5°C

1 : 37.5°C to 38.0°C

2 : 38.1°C to 39.0°C

3 : > 39.0°C

Note that Fever is defined as temperature ≥ 37.5°C / 99.5°F for oral, axillary or tympanic route, or ≥ 38.0°C / 100.4°F for rectal route.

7.1.3.3. Conversion of temperature to °C

The following conversion rule is used for the conversion of temperature to °C

Temperature in °Celsius = ((Temperature in °Fahrenheit -32) *5)/9

The result is rounded to 1 decimal digit.

7.2. Handling of missing data

7.2.1. Immunogenicity

For a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, analyses will exclude subjects with missing or non-evaluable measurements.

7.2.2. Reactogenicity and safety

- For a given subject and a given measurement, missing or non-evaluable measurements will not be imputed. The missing endpoint and censoring are supposed to occur independently, and the pattern of the missing value(s) being either Missing Completely At Random (MCAR) or Missing At Random (MAR) only.
- For the analysis of solicited symptoms, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited symptoms based on the TVC will include only subjects/doses with documented safety data (i.e., symptom screen/sheet completed).
- For the analysis of unsolicited symptoms/SAEs/pIMDs/concomitant medication, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.

7.2.2.1. Solicited general symptoms

The analysis of solicited general symptoms will include all subjects for whom the question (1) in Figure 1 about the presence of any solicited general symptom has been answered by 'Yes' or 'No' (see the GENSOL_YN item).

The next sections describe how each subject contributes to the analyses, depending on the endpoints.

Figure 1 Information captured in the clinical database for solicited general symptoms

For each solicited general symptom if the answer to question (2) in Figure 1 is "No", the subject will be considered as not having that symptom after that dose.

Subjects who documented the presence of a specific symptom i.e., if the answer to question (2) in Figure 1 is “Yes” (ex FA_YN=Y), the maximum intensity recording over the considered follow-up period is used for the analysis of the percentage of subjects with symptoms.

If the subject answered “Yes” to (2) in Figure 1 for a specific symptom BUT partially recorded daily measurement (e.g. intensity missing for Day 3) over the considered solicited period, she/he will be included in the summaries and classified according to their maximum observed daily recording over the solicited period.

If the subject answered “Yes” to (2) in Figure 1 for a specific symptom BUT no daily measurement is recorded, she/he will not be included and counted in the summary of subjects with symptoms above a specified threshold, however she/he will be part of the summary corresponding to the ‘All’ category.

7.2.2.2. Solicited general fever

Figure 2 Information captured in the clinical database for temperature record

ZOSTER-060 EXT:003 (204926): SOLICITED ADVERSE EVENTS - GENERAL SIGNS/SYMPTOMS: ONLY TEMPERATURES (Tem

TEMPERATURES [sctTEMPERATURE_SOL]

1.* Temperature (Celsius) collected daily from Day 0 to Day 6

[cmpSYMP_VAL_TEMP]

[cmpFE_VAL_D0]	**[cmpFE_VAL_D1]**	**[cmpFE_VAL_D2]**	**[cmpFE_VAL_D3]**	**[cmpFE_VAL**
Day 0: **[itmFE_VAL_D0]** XXX.X	Day 1: **[itmFE_VAL_D1]** XXX.X	Day 2: **[itmFE_VAL_D2]** XXX.X	Day 3: **[itmFE_VAL_D3]** XXX.X	Day 4: **[itmF** XXX.X
[itmFE_NT_D0] Not taken [A:Y] ☐	**[itmFE_NT_D1]** Not taken [A:Y] ☐	**[itmFE_NT_D2]** Not taken [A:Y] ☐	**[itmFE_NT_D3]** Not taken [A:Y] ☐	**[itmF** Not taken

2.* Primary route:
The preferred route for recording temperature in this study is oral .When there is no other alternative, the temperature may be recorded by other route. If the temperature is taken by another route (axillary, rectal or tympanic), the route should be documented.
[Primary route]

[itmTEMP_ROUTE]
[A:O] ◯ Oral
[A:A] ◯ Axillary
[A:T] ◯ Tympanic
[A:R] ◯ Rectal

3.* **Has a temperature above or equal to threshold occured?**
i.e.during the solicited period at least one axillary/oral/tympanic measure is above or equal to 37.5°C or at least one rectal measure is above or equal to 38.0°C

[itmFE_YN]
[A:N] ◯ No
[A:NT] ◯ Not taken
[A:Y] ◯ **[itmFE_ONG]**
Yes -> **After Day 6:** Temperature ongoing? [A:N] ◯ No
[A:Y] ◯ **[cmpSYMP_ONG_TEMP]**
[itmSYMP_MAX_TEMP]

For temperature

To allow the temperature recording, a separate screen needs to be completed whatever the answer to question (2) in Figure 2 is, also in case no temperature equal or above 37.5°C has been found in the diary card.

All subjects for whom question (2) in Figure 2 has been answered as "Yes" or "No", will be included in the summaries of temperature by half degree (°C) cumulative increments and classified according to their maximum temperature value observed daily recording over the solicited period. If no daily measurement is recorded for temperature, the subject will not be counted in the summary of subjects with temperature above a specific threshold. For the summary of temperature, the "all" category will not be computed. This table will be produced overall, by route with no route conversion.

For summary of fever

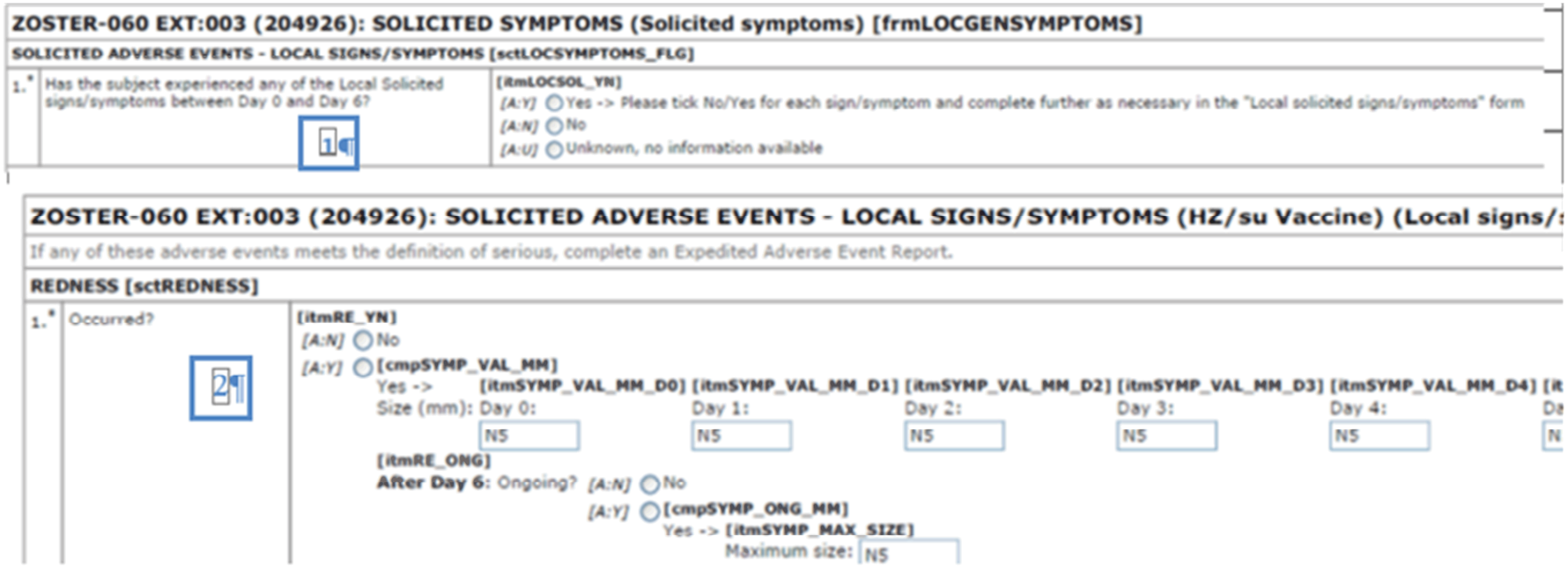
All subjects for whom question (2) in Figure 2 has been answered as "Yes" or "No", will be included in the summaries of fever and classified according to their maximum temperature value observed daily recording over the solicited period even if partial recording. If no daily measurement is recorded for temperature, the subject will not be counted in the summary of subjects with fever and the "all" category will count the number of subject with at least one temperature measurement ≥ 37.5°C / 99.5°F for oral, axillary or tympanic route, or ≥ 38.0°C / 100.4°F for rectal route.

7.2.2.3. Solicited local symptoms

The analysis of solicited local symptoms will include all subjects for whom the question (1) in Figure 3 about the presence of a solicited local symptoms has been answered as 'Yes' or 'No'(see LOCSOL_YN item).

The next sections describe how each subject contributes to the analyses, depending on the endpoints.

Figure 3 Information captured in the clinical database for solicited local symptoms



For each solicited local symptom, if the answer to question (2) in Figure 3 is "No", the subject will be considered as not having that local symptom at the injection site after that dose.

Subjects who documented the presence of a specific symptom i.e. if the answer to question (2) in Figure 3 is "Yes" (ex RE_YN=Y), the maximum intensity recording over the considered follow-up period is used for the analysis of the percentage of subjects with symptoms.

If the subject answered "Yes" to question (2) in Figure 3 for a specific symptom BUT partially recorded daily measurement (e.g., intensity missing for Day 3) over the considered solicited period, she/he will be included in the summaries and classified according to their maximum observed daily recording over the solicited period.

If the subject answered "Yes" to question (2) in Figure 3 for a specific symptom BUT no daily measurement is recorded, she/he will not be included and counted in the summary of subjects with symptoms above a specified threshold, however s/he will be part of the summary corresponding to the 'All' category.

7.2.2.4. Combined Solicited local and general symptoms

The analysis of the combined solicited general and local symptoms will include all vaccinated subjects for whom the question (1) in Figure 1 or Figure 3 about the presence of any solicited general or local symptoms has been answered by 'Yes' or 'No'.

7.2.2.5. Unsolicited symptoms

All vaccinated subjects will be considered for the analysis of unsolicited symptoms.

The analysis of unsolicited adverse events, including serious adverse events, consists of evaluating the percentage of subjects with at least 1 report of an unsolicited adverse event classified by the Medical Dictionary for Regulatory Activities (MedDRA).

Subjects who missed reporting unsolicited symptoms will be treated as subjects without unsolicited symptoms.

7.2.2.6. Concomitant medication

All vaccinated subjects will be considered for the analysis of concomitant medication use. Subjects who did not report the use of a concomitant medication will be considered as subjects without medication. Subjects will be counted in the summary who started a concomitant medication during the mentioned period and took at least one dose.

7.2.2.7. Compliance with respect to documenting safety

The number of doses injected, the number of doses not given according-to-protocol, and the number of symptom sheets (SS) transcribed for local and general symptoms, the compliance for local and general symptoms are tabulated for the Total Vaccinated Cohort.

Compliance (%) is defined as the number of general (local) symptom sheets/screens completed divided by the number of doses administered for a specified vaccination (dose) and group.

The number of doses not given according-to-protocol, are the doses injected at the wrong site and/or side, or injected using the wrong route as defined in the study protocol for each study vaccine. This number is issued from the following question in the vaccine administration sheet of the CRF: "Has the study vaccine been administered according-to-protocol?" Study vaccine dose not administered according to protocol can lead to elimination from the ATP cohort, depending to the ATP cohort definition in the protocol.

7.3. Number of decimals:

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

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

7.4. Methodology for computing CI

Unless otherwise mentioned, the confidence intervals will be 2 sided 95% CI and calculated according to the following methods:

7.4.1. Binomial Data

The exact 95% CIs for a proportion within a group will be calculated according to Clopper & al. (1934).

7.4.2. Continuous Data

The 95% CI for geometric mean titres/concentrations (GMTs/GMCs) analyses will be obtained. The 95% CI for the mean of log-transformed titre/concentration will be first obtained assuming that log-transformed values were normally distributed with unknown variance. The 95% CI for the GMTs/GMCs will be then obtained by exponential-transformation of the 95% CI for the mean of log-transformed titre/concentration.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

Description	Analysis ID	Disclosure Purpose	Reference for TFL
Final Analysis including persistence 1 year post revaccination	E1_01	CSR/CTRS	TFL E1_01
Persistence Year 9	E1_02	Internal	TFL E1_02
Main analysis: persistence Year 10 and revaccination	E1_03	CSR/CTRS	TFL E1_03

8.2. Statistical considerations for interim analyses

All analyses will be conducted on final data and therefore no statistical adjustment for interim analyses is required. The analysis is purely descriptive, therefore no adjustment on type I error is foreseen.

9. CHANGES FROM PLANNED ANALYSES

NA

10. REFERENCES

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