Janssen Research & Development

Statistical Analysis Plan

A Randomized, Double-blind, Placebo-controlled Phase 2a Study to Evaluate the Safety and Immunogenicity of Seasonal Influenza Vaccine and Ad26.RSV.preF, with and without Co-administration, in Adults Aged 60 Years and Older in Stable Health

Protocol VAC18193RSV2003; Phase 2a

VAC18193 (JNJ-64400141)

Status: Approved
Date: 17 April 2018
Prepared by: Janssen Vaccines & Prevention B.V.
Document No.: EDMS-ERI-152844757

Compliance: The study described in this report was performed according to the principles of Good Clinical Practice (GCP).

TABLE OF CONTENTS

ABBREVIATIONS

Ad26	adenovirus serotype 26
ADaM	analysis data model
ANOVA	analysis of variance
AE	adverse event
DPS	data presentation specifications
CI	confidence interval
CRF	case report form
CTP	clinical trial protocol
DRC	data review committee
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
FA	full analysis set
FDA	Food and Drug Administration
GMT	geometric mean titer
HI	hemagglutination inhibition
HAI	hemagglutination inhibition assay
ICH	International Conference on Harmonization
ICS	intracellular cytokine staining
IFNγ	interferon gamma
IL	interleukin
IRB	Institutional Review Board
IEC	Independent Ethics Committee
LLOQ	lower limit of quantification
NSAIDs	non-steroidal anti-inflammatory drugs
PBMC	peripheral blood mononuclear cell
PI	principal investigator
PPII	Per-protocol Influenza Immunogenicity Set
PPRI	Per-protocol RSV Immunogenicity Set
RT-PCR	reverse transcriptase polymerase chain reaction
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SDTM	standard data tabulation model
SE	standard error
SRP	study responsible physician
RSV	respiratory syncytial virus
Th	T-helper (cell)
TLF	tables, listings and figures
TNFα	tumor necrosis factor alpha
ULOQ	upper limit of quantification
VNA	Viral Neutralization Assays
vp	viral particles
WHO	World Health Organization

1. INTRODUCTION

This statistical analysis plan (SAP) contains all information needed to perform a full safety and immunogenicity analysis of the VAC18193RSV2003 trial. It applies to both the primary and the final analysis:

- Primary analysis: 28 days post-second dose safety and immunogenicity analysis. This analysis will be performed based on unblinded data. The main goal of this analysis will be to evaluate the primary objectives. The blind will be maintained at the subject/site level. The date when all subjects have reached the Day 57 visit or discontinued earlier will be used as the database cut-off point. All available safety and immunogenicity data gathered so far will be included in the analysis.

- Final analysis: 6 months post-second dose safety analysis. This analysis will be performed based on unblinded data.

Individual tables, listings and figures (TLF) to be generated in each analysis will be described in a separate data presentation specifications (DPS) document.

This document is based on the clinical trial protocol (CTP) amendment 2 (EDMS-ERI-146966519).

1.1. Trial Objectives

See CTP, Section 2.1.

1.2. Trial Design

This is a single center, randomized, placebo-controlled, double-blind Phase 2a study, to be conducted in 180 adult male and female subjects aged ≥60 years of age in stable health randomized in parallel in a 1:1 ratio to one of two groups. Group 1 will receive $1x10^{11}$ vp Ad26.RSV.preF on Day 1 administered at the same time as a commercially available seasonal influenza vaccine, and placebo on Day 29. Group 2 will receive placebo on Day 1, administered at the same time as a commercially available seasonal influenza vaccine, and $1x10^{11}$ vp Ad26.RSV.preF on Day 29. All study vaccines will be given by the intramuscular route. An internal data review committee (DRC) will be commissioned for this study.

Group	N	Day 1	Day 29
1	90	Ad26.RSV.preF ($1x10^{11}$ vp) + Fluarix	Placebo
2	90	Placebo + Fluarix	Ad26.RSV.preF ($1x10^{11}$ vp)

N = number of subjects; vp = viral particles

See section 3.1 of the CTP for further details on the study design and evaluations.

1.3. Statistical Hypotheses for Trial Objectives

To demonstrate the non-inferiority of the concomitant administration of Ad26.RSV.preF and seasonal influenza vaccine versus the administration of seasonal influenza vaccine, 28 days after the administration of the seasonal influenza vaccine in terms of humoral immune response, the following hypothesis will be tested for each one of the four influenza vaccine strains:

Null Hypothesis:

- The GMT of HI antibody titers against one vaccine strain, 28 days after concomitant administration of Ad26.RSV.preF and seasonal influenza vaccine is inferior by at least 2 to the GMT 28 days after the administration of seasonal influenza vaccine and placebo

Alternative Hypothesis:

- The GMT of HI antibody titers against one vaccine strain, 28 days after concomitant administration of Ad26.RSV.preF and seasonal influenza vaccine is non-inferior to the GMT 28 days after the administration of seasonal influenza vaccine and placebo, using a non-inferiority margin of 2, for the ratio $GMT_{\text{control group}}/GMT_{\text{co-administration group}}$

No formal statistical testing of safety data is planned. All safety data will be analyzed descriptively by regimen.

1.4. Sample Size Justification

See CTP, Section 11.2.

1.5. Randomization and Blinding

See CTP, Section 5.

1.6. Changes to planned analyses

No changes from the planned analysis have been determined.

2. GENERAL ANALYSIS DEFINITIONS

2.1. Study phases

A baseline (or reference) value will be defined as the value of the last available assessment prior to the first vaccination on Day 1, except for the immunogenicity endpoints related to RSV in post-dose 2 of Group 2, where the baseline value will be the value of the last available assessment prior to the second vaccination (i.e. Day 1 for post-dose 2).

Study day or relative day is defined as follows:

Study Day = visit date – date of Day 1[*] + 1; if visit date > date of Day 1 (date of first vaccination).
Study Day = visit date – date of Day 1[*]; if visit date < date of Day 1 (date of first vaccination).

[*] For immunogenicity endpoints related to RSV in post-dose 2 of Group 2, the day of second vaccination will be considered as Day 1.

2.1.1. Phase definitions

The phases in the study will be constructed as follows:

Phase	Phase #	Period	Period #	Interval	
				From	To
Screening	1			Date and time of signing the informed consent form[a]	One minute prior to start of post-dose 1 period
Regimen	2	Post-dose 1	1	Date and time of first vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of interim c) One minute prior to post-dose 2. If second vaccination was not administered, then use maximum of (Day 28 after the first vaccination at 23:59, scheduled visit 28 days after first vaccination at 23:59).
Regimen	2	Post-dose 2	2	Date and time of second vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of interim c) Maximum (Day 28 after the second vaccination at 23:59, scheduled visit 28 days after second vaccination at 23:59)
Follow-up	3			One minute after the end of the last post-dose period.	Minimum of: a) 23:59 at the Date of last contact b) 23:59 at the date of database cut-off date in case of interim

[a] In case an earlier date is available (e.g. for vital signs), then use the very first date to include all data in the screening phase.
Note: If the second dose is not administered, the observations will end up in the follow-up phase.

2.2. Pooling Algorithm for Analysis Centers

This is a single center study, pooling is not applicable.

2.3. Analysis Sets

Vaccination assignment will follow the as-treated principle.

2.3.1. Full Analysis (FA) Set

The FA set includes all subjects who were randomized and received at least one dose of study vaccine, regardless of the occurrence of protocol deviations and vaccine type (seasonal influenza, Ad26.RSV.preF or placebo). All safety and subject information analyses will be based on the FA set.

2.3.2. Per-protocol Influenza Immunogenicity (PPII) Set

The PPII set will include all subjects who were randomized and received the first vaccination for whom immunogenicity data are available, excluding subjects with major protocol deviations expecting to impact the immunogenicity outcomes.

In addition, the following samples will not be included in the PPII set:

- For subjects who experience a seasonal influenza infection (based on reverse transcriptase polymerase chain reaction (RT-PCR), or other sources), samples taken after the natural infection will not be taken into account in the assessment of the immunogenicity of the seasonal influenza vaccine.

The analysis of primary immunogenicity endpoint will be based on the PPII set. The analysis of all secondary and exploratory immunogenicity endpoints related to influenza will also be based on the PPII set. Depending on the number of samples excluded, a post-hoc exploratory analysis might be performed, including the excluded samples. To visualize these excluded samples, subject profiles from several assays might be repeated, indicating the excluded samples.

2.3.3. Per-protocol RSV Immunogenicity (PPRI) Set

The PPRI set will include all randomized and fully vaccinated subjects (all three vaccinations, ie, seasonal influenza, Ad26.RSV.preF and placebo) for whom immunogenicity data are available, excluding subjects with major protocol deviations expecting to impact the immunogenicity outcomes.

In addition, the following samples will not be included in the PPRI set:

- For subjects who experience a natural RSV infection (based on reverse transcriptase polymerase chain reaction (RT-PCR), or other sources), samples taken after the natural infection will not be taken into account in the assessment of the immunogenicity of Ad26.RSV.preF.

The analysis of all secondary and exploratory immunogenicity endpoints related to RSV will also be based on the PPRI set. Depending on the number of samples excluded, a post-hoc exploratory analysis might be performed, including the excluded samples. To visualize these excluded samples, subject profiles from several assays might be repeated, indicating the excluded samples.

2.4. Definition of Subgroups

No subgroup analysis is planned for this study.

3. INTERIM ANALYSIS AND DATA MONITORING COMMITTEE REVIEW

The DRC will convene if an effect is seen on immune responses to the seasonal influenza vaccine with concomitant administration based on the immunogenicity analysis at 28 days after the first dose, and to discuss any significant or unexpected safety issues.

This SAP is applicable to all analyses the primary and final analysis. For further information related to the DRC analysis, refer to the CTP section 11.7, DRC charter and the corresponding DPS.

4. SUBJECT INFORMATION

Subject information will be shown for the FA set.

4.1. Demographics and Baseline Characteristics

Demographic characteristics and screening/baseline characteristics will be tabulated and summarized with descriptive statistics per randomization group and over all subjects.
The following demographic and baseline characteristics will be summarized.

- Sex (Female/Male)
- Age (years)
- Race
- Ethnicity
- Height (cm)
- Weight (kg)
- BMI (kg/m^2)

4.2. Disposition Information

The number and percentage provided for subjects:

- screened
- in the FA set
- in the PPII set
- in the PPRI set
- randomized, vaccinated
- not randomized, not vaccinated
- not randomized, vaccinated
- randomized, not vaccinated

Discontinued subjects (study discontinuation and vaccination discontinuation) with the reason of discontinuation will be tabulated per randomization group and overall.

The number of subjects and percentage per phase will also be tabulated.

4.3. Protocol Deviations

Major protocol deviations will be summarized.

4.4. Concomitant Medications

The analysis of concomitant therapies will be done using the WHO drug coded terms.

There will be special attention to analgesics/antipyretics such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin, administered during 8 days following each vaccination (00:00 of day of vaccination + 7 days).

Concomitant therapies will be reported in each applicable phase based on their start and stop date.

If a concomitant therapy record misses components of its start and/or stop dates (day and/or month and/or year):

- In case of partial start or stop dates, the concomitant therapy records will be allocated to periods using the available partial information, without imputations. If, for example, only month and year are available, these will be compared to the month and the year of the periods, and the concomitant therapy record will be allocated to the period(s) where these date parts match. This rule may lead to assignment to multiple periods.
- In case of a completely missing start date, the concomitant therapy will be considered as having started before the trial.
- In case of a completely missing end date, the concomitant therapy will be considered as ongoing at the end of the trial.

Concomitant therapies will be tabulated per period.

4.5. Medical History

Medical history data will be listed.

5. IMMUNOGENICITY ANALYSIS

The analysis of the immunogenicity of the Ad26.RSV.preF and seasonal influenza vaccine will use the PPRI set and PPII set respectively.

5.1. Handling of Missing and/or Unquantifiable Immune Response Data

Missing immune response data will not be imputed.

In the analyses of the immunogenicity of the seasonal influenza vaccine at Day 29 (post-dose 1), only samples taken within Day 22 and 35 (inclusive) will be used. In the analyses of the immunogenicity of Ad26.RSV.preF at Day 29 post-dose 1 and post-dose 2, only samples taken within Day 22 and 40 (inclusive) will be used. If two measurements fall within this interval, the one closest to the target day (Day 29) will be used in the analysis.

Values below the lower limit of quantification (LLOQ) will be treated differently according to the assay:

- Values will be imputed based on the type of analysis. For the calculation of the geometric mean titer, values below LLOQ will be imputed to LLOQ/2. While for the calculation of the geometric mean of the increase from baseline, values below LLOQ will be imputed to LLOQ. The LLOQ values per assay are available in the database.

- For ICS assays: no validated LLOQ is available. A provisional cut-off is put at 0.02% (only for total cytokine response). For the individual cytokine combinations of IFNγ, TNFα and IL2, negative values will be imputed with 0. For descriptive statistics or graphs on actual values, values below the cut-off will be imputed to a value of cut-off/2. For Th1 and Th2, a provisional cut-off of 0.001% is used. These values might change in the future, depending on progressing insight. For descriptive statistics or graphs on actual values, values below the cut-off will be imputed to a value of cut-off/2.

- For all assays: values above the upper limit of quantification (ULOQ) will be imputed with 2xULOQ.

5.2. Primary Immunogenicity Endpoint

5.2.1. Definitions

The primary immunogenicity endpoint is the HI titers against all four influenza vaccine strains included in the seasonal vaccination.

5.2.2. Primary Immunogenicity Analysis

The primary immunogenicity objective is to demonstrate the non-inferiority of the concomitant administration of Ad26.RSV.preF and seasonal influenza vaccine versus the administration of seasonal influenza vaccine alone in terms of humoral immune response expressed by the GMTs of HI antibody titers against all four influenza vaccine strains 28 days after the administration of influenza vaccine, using a non-inferiority margin of 2 for the GMT ratio (Control group/CoAd group).

The primary immunogenicity objective will be assessed by calculating the 95% one-sided upper confidence limit for the difference in log-transformed HI antibody titers for each of the four seasonal influenza vaccine strains between Control and CoAd groups, using an analysis of variance (ANOVA) model with the Day 28 titer as dependent variable and regimen as covariate. The confidence limit will be calculated using Welch's t-interval method, also referred to as the Satterthwaite method[3,4], to allow for the estimation of separate variances per regimen. The confidence limit will be back-transformed (by exponentiation) to a GMT ratio and compared to the non-inferiority limit of 2. The results will be presented in tables as well as graphs using forest plots.

Non-inferiority of co-administration versus the control group will be concluded only if the 95% one-sided upper confidence limit for the GMT ratio (Control group/CoAd group) of the HI antibody titers lies below 2 for each of the four vaccine strains. If one or more confidence limits for the GMT ratio exceed 2, non-inferiority cannot be concluded.

As a sensitivity analysis, the primary endpoint will also be evaluated adjusting for baseline HI levels in the model.

The primary immunogenicity endpoint will be based on the PPII set. The analysis set for the secondary and exploratory immunogenicity responses related to influenza will be the PPII set. As a sensitivity analysis, key tables may also be based on the FA set. Depending on their occurrence, the effect of natural infections might be further explored.

5.3. Secondary and Exploratory Immunogenicity Endpoint

5.3.1. Definitions

The following humoral and cellular immune responses will be measured.

Immunogenicity against the insert:

Humoral Immune Response

- RSV A2 neutralizing titers of the vaccine-induced immune response
- Antibodies binding to RSV F protein in post-fusion and pre-fusion form (RSV F-protein enzyme-linked immunosorbent assay [ELISA])

In addition, exploratory analyses may be performed to further investigate vaccine-elicited immune responses. These may include, but are not limited to, the following:

- RSV cross-neutralization of B and/or other A strain
- F-protein antibody specificity characterization
- Functional and molecular antibody characterization
- Influenza virus neutralization assay

Cell-mediated Immune Response to Ad26.RSV.preF and Seasonal Influenza Vaccine

- IFNγ ELISpot cytokine analysis
- Intracellular cytokine staining

Immunogenicity against the vector:

Adenovirus neutralization assay: this assay assesses neutralizing antibody responses against the Ad26 vectors. This is an exploratory endpoint and will only be analyzed, if available.

5.3.2. Secondary and Exploratory Immunogenicity Analysis

5.3.2.1. Humoral assays

No formal non-inferiority assessments for the secondary immunological parameters for seasonal influenza and RSV will be performed, but upper confidence limits for GMT ratios, as described for the primary endpoint, may be calculated for RSV A2 neutralizing titers, and assessed for relevance by comparison to the non-inferiority limit of 2 as used in the primary analysis.

Similarly, the difference in proportions (Control minus CoAD) of seroconverted and seroprotected subjects between Control and CoAd groups will be estimated together with the 95% one-sided confidence limit (calculated based on Wilson score method[1]). Seroconversion is defined as a post-vaccination titer ≥1:40 in subjects with a pre-vaccination titer of <1:10, or a ≥4-fold titer increase in subjects with a pre-vaccination titer of ≥1:10. Seroprotection is defined as the percentage of subjects with a post-vaccination titer ≥1:40. Note that the study is only powered for the four primary comparisons and does not have sufficient power to show non-inferiority of co-administration compared to control for all immunogenicity markers for seasonal influenza and RSV. Consequently, by chance the study may fail to show non-inferiority for one or more secondary endpoints even in the absence of interference between seasonal influenza and RSV vaccine administrations.

The proportions of seroconverted and seroprotected subjects will also be tabulated together with the exact mid-P two-sided 95% confidence interval (CI)[2]. For other categorical variables, frequency tables will be presented. Difference in proportions and corresponding CIs may be calculated where appropriate.

In addition, the following will be presented by vaccine regimen:

For **HAI, VNA** and **ELISA** assays, N, geometric mean[§] and corresponding 95% CI of the actual values will be calculated and fold increases from baseline will be tabulated and graphically presented. *[§]calculate the mean and corresponding 95%CI of the log_2 transformed values, back-transform this mean [i.e. 2^mean] and CI [i.e. 2^CI].*

Actual values and fold changes from baseline are tabulated and shown as dot plots with dots for subject values, and the corresponding geometric mean and 95% CI per time point for each assay. In addition, GMT plots over time, combining the regimens in one graph (without individual subject dots) will also be created.

Subject profiles of the actual values over time will be graphically presented.

Reverse distribution curves of the actual values are provided for per time point.

In the graphs, original values will be displayed on the log_2 scale.

A scatterplot with the VNA versus ELISA will be provided at Day 29. In these scatterplots, the actual values will be shown, even if they are below the LLOQ, but the LLOQ cut-off will be visualized in the graph per assay if some values are below LLOQ. Similar tables and figures of the ratios of secondary endpoints will be presented as described in the DPS. Other exploratory parameters may be analyzed at the discretion of the sponsor.

Note: for the immunogenicity endpoints related to RSV in post-dose 2 of Group 2, the baseline value will be the value of the last available assessment prior to the second vaccination (i.e. Day 1 for post-dose 2).

5.3.2.2. Cellular assays

For **ELISpot**, the following results will be calculated: N, median, quartiles and range of the actual values will be tabulated and graphically presented.

Subject profiles of the actual values over time will be graphically presented.

Actual values are shown as box plots with dots for subject values, and the corresponding median and interquartile range per time point for each assay. In addition, box plots over time, combining the regimens in one graph (without individual subject dots) will also be created. For the graphs, original values will be displayed on the $\log_{10}$ scale.

For **ICS** and **PBMC secreted cytokines** (if available) possible analyses may include:

Total Cytokine response: the % of subsets expressing at least IFNγ, TNFα or IL2 will be calculated for CD4 and CD8.

Tables with the corresponding descriptive statistics will be provided.

Subject profiles of the actual values over time will be graphically presented.

Actual values are shown as box plots with dots for subject values, and the corresponding median and interquartile range per time point for each assay.

In addition, box plots over time, combining the regimens in one graph (without individual subject dots) will also be created.

For all cytokine combinations (IFNγ and/or TNFα and/or IL2) piecharts reflecting the distribution of each of the cytokine combinations (the proportion of a specific cytokine combination of the CD4 or CD8 T-cells secreting at least one cytokine) and bar charts reflecting the median magnitude of each combination will be graphically presented. Tables with the corresponding descriptive statistics will be provided.

For the graphs, original values will be displayed on the $\log_{10}$ scale.

Th1 and Th2: Th1 is defined as all CD4+ IFNγ+ and Th2 as all CD4+ IL4+ cells. Subject profiles and graphs of the actual values over time (box-plot type) might be created. In addition, at time points of interest, scatterplots of Th1 vs Th2 might be created.

The technical details for the calculation of the ICS values to be used in the graphs will be outlined in the DPS.

5.3.2.3. Immunogenicity against the vector

For **Ad26 VNA** following statistics will be calculated: N, geometric mean[§(see above for the calculation)] and corresponding 95% CI of the actual values.

Subject profiles of the actual values over time will be graphically presented for each vector.

Actual values and fold changes (from baseline) are tabulated and shown as dot plots with dots for subject values, and the corresponding geometric mean and 95% CI per time point for each assay.

Subject profiles of the assays against the insert will be repeated, highlighting subjects with pre-existing immunity at baseline against the vectors. For immunogenicity endpoints related to RSV in post-dose 2 of Group 2, the baseline value will be the value of the last available assessment prior to the second vaccination (i.e. Day 1 for post-dose 2).

Scatterplots with the Adeno assays at baseline versus the assays against the inserts will be provided for the most important time points. In these scatterplots, the actual values will be shown, even if they are below the LLOQ.

6. SAFETY

Safety analyses will be performed on the FA set. Continuous variables will be summarized using the following statistics, as appropriate: number of observations, arithmetic mean (mean), 95% CI for the mean, standard deviation (SD), standard error (SE), median, quartiles (Q1 and Q3), minimum and maximum. Frequencies and percentages (one decimal place) will be generated for categorical variables. No formal comparisons between groups will be provided.

Safety data will be analyzed and presented by phase and group. Denominator for the percentages is the number of subjects in the considered population and period for a certain regimen (incidence per 100 subjects/phase).

6.1. Adverse Events (AE)

6.1.1. Definitions

Solicited AEs shown in the tables are extracted from the diary pages of the CRF. For unsolicited AEs, only the AEs starting within the 28-day period following each vaccination will be presented in the safety tables except for SAE, which will be captured and tabulated in the outputs covering the whole study period. Non-serious unsolicited events captured outside the 28-day periods will be presented through listings.

Solicited local AEs will be by definition considered as related to the study vaccine.

The severity of the AEs will be classified as grade 1 to 4. Solicited events of grade 0 are not considered as AE.

6.1.2. Analysis of Adverse Events

Number and percentage of subjects with at least one particular AE (unsolicited/solicited) will be tabulated. Unsolicited AEs will be summarized by System Organ Class and Preferred Term. Solicited AEs will be summarized by class (local, systemic) and preferred term.

For solicited AEs, the following tables will be provided: summary, by worst severity grade, grade 3, related (systemic only), time to onset (in days) and duration (in days) for most frequent events and body temperature. Note: for solicited AEs, duration is defined as number of days

from the start of the event until resolution of the event. The time to first onset is defined as (date of first onset – reference date + 1). The reference date is the start date of the regimen period.

For unsolicited AEs, the following tables will be provided: summary table (including SAE, fatal outcome, discontinuation), all events, most frequent, grade 3, permanent stop of vaccine, related, SAE.

Listings and/or subject narratives will be provided as appropriate, for those subjects who die, discontinue study vaccinations due to an AE, or experience a severe or serious AE.

6.1.3. Phase allocation of Unsolicited Adverse Events

Solicited events are always allocated to the respective post-dose period.

Solicited AEs from the diary will be added to the analysis data model (ADaM) for unsolicited AEs according to the same principles. This means the same event occurring on different days will be allocated to one row with the start date of the AE being the first date of the event and the end date for the event is the last subsequent day of the event. A change in grade will trigger a new row to be added. The same occurs in case of non-subsequent events (for example grade 1 nausea on day 1, 2 and 3 and also on day 6 and 7, which will be allocated to two rows; the duration of the event is 7 days). For further details related to transforming the on-site assessments and diaries of solicited AEs into analysis format please refer to Attachment 1.

Step 1: Allocation of events to the periods:

Adverse events in the standard data tabulation model (SDTM) database are allocated to periods based on their start date/time. If the start date/time of an event falls between (or on) the start and stop date/time of a period, the AE is attributed to that period (treatment-emergent principle).

- In case of partial start or stop dates (i.e. time and/or day and/or month and/or year missing), the events are allocated to the periods using the available partial information on start and end date; no imputation will be done. If, for instance, the AE start date only month and year are available, these data are compared to the month and year information of the periods. This rule may lead to multiplication of the event as a consequence of its assignment to multiple periods.

- In case of a completely missing start date, the event is allocated to the first active treatment phase (post dose 1 period), except if the end date of the AE falls before the start of the first active treatment phase (post dose 1 period).

- In case of a completely missing end date, the date is imputed by the cut-off date of the analysis for subjects still ongoing in the study, and by the end date of the last period for subjects who discontinued or completed the trial.

Step 2: Combination of events:

Overlapping/consecutive events are defined as events of the same subject with the same preferred term which have at least 1 day overlap or for which the start date of an event is 1 day

after the end date of the preceding event. Overlapping/consecutive events may be combined into one AE or not, according to the following rules:

1) In case a non-active period (e.g. Screening) is followed by an active period (post-dose 1, post-dose 2) and the overlapping/consecutive events start in both periods, they are allocated to their respective periods and are considered as separate events.

2) In case overlapping/consecutive events start within a single period, they are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM database but are assigned the same onset, period, and total duration.

3) In case overlapping/consecutive events start in both an active period followed by a non-active period, they are allocated to the active period only and are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM database but are assigned the same onset, treatment period, and total duration.

4) In case an active period is followed by another active period, and the overlapping/consecutive events start in both periods, they are allocated to their respective period and are considered as separate AEs. The same rule applies for 2 non-active periods.

Remarks:

1. Events can only be combined into one and the same AE if their start and stop dates are known.

2. In case the completely missing end date is imputed (for period allocation), this date is also considered as a complete date.

3. Time is not considered when determining overlap of events.

6.1.4. Missing Data

Missing data will not be imputed. Subjects who do not report an event will be considered as subjects without an event. The analysis of the solicited AEs will include only documented safety data.

6.1.5. Solicited Local (Injection Site) Reactions

The analysis of local solicited adverse events will include:

- Erythema
- Induration/swelling
- Pain/tenderness

6.1.6. Solicited Systemic Adverse Events

The analysis of systemic solicited adverse events will include:

- Fatigue

- Headache
- Myalgia
- Arthralgia
- Chills
- Nausea
- Fever (ie, body temperature ≥ 38 °C)

6.2. Vital Signs and Physical Examination Findings

For vital signs, only abnormalities emerging after vaccination will be tabulated by worst abnormality grade. The respective vital signs abnormalities are defined in the table below. Only the vital signs values will be used to derive abnormality grades, no clinical interpretations will be used. Therefore, grade 3 and 4 will be combined, as grade 4 always requires clinical interpretation.

Vital Signs	**Grade 1**	**Grade 2**	**Grade 3/4**
Tachycardia (pulse) – beats per minute	101 – 115	116 – 130	> 130
Bradycardia (pulse) – beats per minute	50 – 54	45 – 49	< 45
Hypertension (systolic) – mm Hg	141 – 150	151 – 160	> 160
Hypertension (diastolic) – mm Hg	91 – 95	96 – 100	> 100
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25

An abnormality (toxicity grade) will be considered as emerging in a particular period if it is worse than the baseline value. If the baseline is missing, the abnormality is always considered emerging.

Temperature (diary, on site assessments) will be allocated to predefined temperature intervals (from 37.5° C until 40°C, in steps of half degree increments; eg <37.5, 37.5-<38, 38-<38.5, … >40). Tables will show the maximum temperature for both diary and onsite assessments combined. A listing of subjects with fever according to the FDA grading table will also be provided.

Physical examination abnormalities will be reported by the investigator as AEs, hence will not be analyzed separately.

6.3. Electrocardiogram (ECG)

Any abnormalities in ECG parameters (at screening on Day 1) will be listed.

REFERENCES

1. Newcombe R. G. Interval estimation for the difference between independent proportions: comparison of eleven methods. Stat Med.; 1998;17:873-890
2. Newcombe, R. G. Two-sided confidence intervals for the single proportion: comparison of seven methods. Statistics in Medicine; 1998; 17, 857-872
3. Satterthwaite F. E. An Approximate Distribution of Estimates of Variance Components. Dec. 1946; Biometrics Bulletin, Vol. 2, No. 6, pp. 110-114
4. Welch B. L. The Generalization of `Student's´ Problem when Several Different Population Variances are Involved. Jan., 1947; Biometrika, Vol. 34, No. 1/2, pp. 28-35

ATTACHMENTS

ATTACHMENT 1: TRANSFORMING THE ON-SITE ASSESSMENTS AND DIARIES OF SOLICITED AES INTO ANALYSIS FORMAT

When creating the analysis dataset for solicited AEs, solicited AEs (recorded by day on the SR and FA domains) need to be converted into the same format as unsolicited AEs (recorded by event). For this purpose, the start date of the AE will be considered as the date of first occurrence of the solicited AE. If on subsequent day(s), the same grade is reported, the last reported date is used as the end date of the AE. A new record is created in case the grade of the event changes. If there is a time gap of at least one day between two (or more) occurrences of the same solicited AE, then the second (and/or next) occurrence will be considered as a new AE. In case no data is reported for a day, this is analyzed as no event reported. If the on-site assessment differs in grade or relatedness with the Day 1 diary data, the on-site assessment should be recorded as a separate record in the database.

The example below shows how the solicited AE should be converted into a format of unsolicited AEs:

Data from the Subject Diary
Subject: 0001
Solicited systemic AE: Headache

	On site assessment	DIARY DATA							
Solicited AE	Day 1 01Jan16	Day 1 01Jan16	Day 2 02Jan16	Day 3 03Jan16	Day 4 04Jan16	Day 5 05Jan16	Day 6 06Jan16	Day 7 07Jan16	Day 8 08Jan16
Grade	2	1	1	0	3	3	1	0	0
Relatedness	Doubtful	Probable							

The data should be converted and stored in the AE dataset as follows:

Subject No.	AE	Start Date (Char)	Stop Date (Char)	Severity	Relatedness	AEID
0001	Headache	01Jan16	01Jan16	2	Doubtful	1
0001	Headache	01Jan16	02Jan16	1	Probable	1
0001	Headache	04Jan16	05Jan16	3	Probable	1
0001	Headache	06Jan16	06Jan16	1	Probable	1

If a solicited AE ends after day 8:

- The last day that AE was reported and the maximum severity (or/and diameter for local AEs) after Day 8 are captured in the CRF. For this a separate record needs to be created, in case this severity deviates from the previous record.

For the **calculation of duration**, the first and last day is used, irrespective of whether interruptions occurred in between by missing reporting days or Grade 0 events. In the above example, the 4 records contribute to the same AE, therefore AEID (AE identification) is set to the same value and the duration of the AE is set to 6 for all records.

Notes:

- For solicited AEs time should not be taken into account to allocate an event to a phase, the event is per definition of solicited AEs collected post-dose and should therefore not be allocated to inactive phases.
- To complete the start and end-date based on diary data, the date will be calculated based on the day the AE is reported relative to vaccination and not on the reported date. For example, if the vaccination is on 1st JAN2016, and the AE starts on DAY 3, the start date will be set to the 3rd of January 2016 independent of the reported actual date.