Janssen Research & Development

Statistical Analysis Plan for the Primary and the Final Analyses Amendment 2

A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate Safety and Immunogenicity of the ExPEC4V (JNJ-63871860) Clinical Trial Material After a Single Intramuscular Dose and a Second Dose 6 Months Later in Healthy Subjects Aged 18 Years and Older

Protocol 63871860BAC2003; Phase 2

JNJ-63871860 (ExPEC4V)

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

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

AMENDMENT HISTORY

SAP Version	Issue Date
Original SAP	25 June 2018
Amendment 1	23 October 2018
Amendment 2	15 May 2019

Amendments below are listed beginning with the most recent amendment.

Amendment 1 (23 October 2018)
The overall reason for the amendment: (1) to change that the sample not the subject is excluded from per protocol immunogenicity analysis if the second vaccination or the blood is drawn outside the allowed protocol time window, (2) to change the age group on which safety and immunogenicity analysis will be performed and (3) to change the definition of smoking history.

Applicable Sections
Rationale: The sample not the subject is excluded from per protocol immunogenicity analysis if the second vaccination or the blood is drawn outside the allowed protocol time window.
Section 2.3.2
Rationale: Safety and immunogenicity will be performed by age group, "≥ 18 to < 50 years" and "≥ 50 years" rather than the age group "≥ 18 to < 60 years" and "≥ 60 years".
Section 2.4
Rationale: Add the age group "≥ 18 to < 50 years" and "≥ 50 years" to the list of variables for demographic and baseline information and add the use of e-cigarettes to the definition of smoking.
Section 4.1

Amendment 2 (15 May 2019)
The reason for the amendment: the algorithm described in section 5.1.5 about how solicited adverse events from the ediary are added to the ADaM for unsolicited adverse events is being implemented and built into the system at the SDTM level. Hence, this algorithm is no longer relevant to be included in this SAP because it is already described in the completion guideline.

ABBREVIATIONS

ADaM	Analysis Data Model
AE	Adverse Event
CI	Confidence Interval
CSR	Clinical Study Report
CTM	Clinical Trial Material
CTP	Clinical Trial Protocol
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
EPA	exoProtein A
ExPEC	Extraintestinal Pathogenic *Escherichia Coli*
FAS	Full Analysis Set
GMR	Geometric Mean ratio
GMT	Geometric Mean of Titer
ICF	Informed Consent Form
IWRS	Interactive Web Response System
LLOQ	Lower Limit of Quantification
OPKA	Opsonophagocytic Killing Assay
PPI	Per-Protocol Immunogenicity Analysis Set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDTM	Study Data Tabulation Model

1. INTRODUCTION

This statistical analysis plan (SAP) is based on the revised protocol (v2) approved on 19 December 2017. It describes both the primary analysis and the final (end of study) analysis. The primary analysis is performed when all subjects have completed the Day 30 visit or discontinued earlier and the final analysis when all subjects have completed the Day 360 visit (telephone contact) or discontinued earlier.

Unless otherwise specified in the Data Presentation Specifications, tables, figures and listings created during the primary analysis will be generated in the final analysis. Sponsor personnel who were involved in the primary analysis will be unblinded at the time of the primary analysis.

1.1. Trial Objectives

The primary objectives of the trial are

1. to evaluate the safety/reactogenicity of the ExPEC4V CTM (defined in 1.2) after the first vaccination,
2. to evaluate the immunogenicity of the ExPEC4V CTM, as measured by the enzyme-linked immunosorbent assay (ELISA), 14 days after the first vaccination (on Day 15).

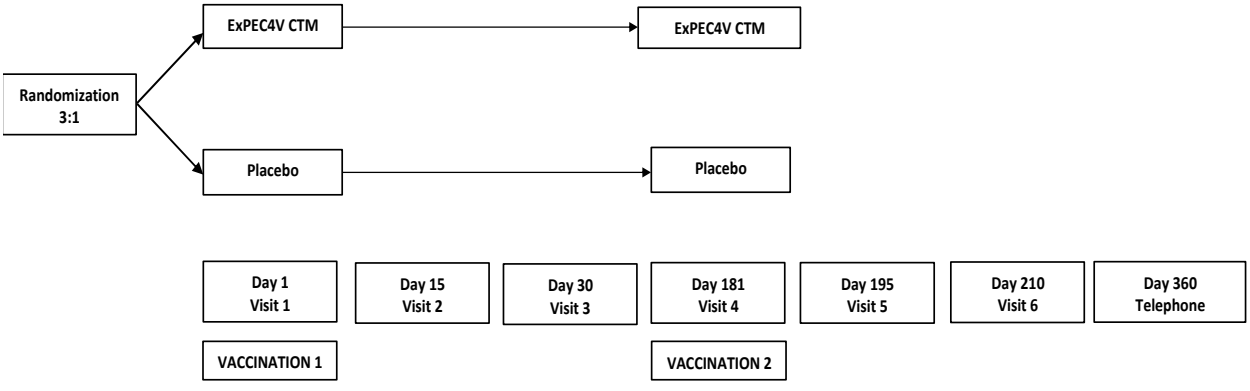
The secondary objectives of the trial are

1. to evaluate the immunogenicity of the ExPEC4V CTM, as measured by the opsonophagocytic killing assay (OPKA) assay, 14 days after the first vaccination (on Day 15),
2. to evaluate the safety/reactogenicity of the ExPEC4V CTM after the second vaccination,
3. to evaluate the immunogenicity of the ExPEC4V CTM, as measured by the ELISA, 14 days after the second vaccination (on Day 195),
4. to evaluate the immunogenicity of the ExPEC4V CTM, as measured by the OPKA, 14 days after the second vaccination (on Day 195).

1.2. Trial Design

Refer to section 3.1 of the clinical trial protocol (CTP).

Figure 1: Schematic Overview of the Study



1.3. Statistical Hypotheses for Trial Objectives

No statistical hypothesis is pre-specified. All analyses are descriptive.

1.4. Sample Size Justification

Refer to section 11.3 of the CTP.

1.5. Randomization and Blinding

Refer to section 5 of the CTP.

1.6. Changes to Planned Analyses

There are no changes to the planned analysis.

2. GENERAL ANALYSIS DEFINITIONS

The study data will be analyzed as follows.

- Categorical variables will be summarized by frequency tabulation, presenting counts and percentages (to 1 decimal place, as appropriate).
- Continuous variables will be summarized using the following statistics, as appropriate: number of observations, arithmetic mean, geometric mean, 95% confidence interval, standard deviation, median, minimum and maximum.

2.1. Analysis Visit Windows and Study Phases

2.1.1. Analysis Visit Windows

A baseline value will be defined as the value of the last available assessment prior to the first vaccination on Day 1. The safety analysis will present results by phase. Immunogenicity results will be presented per scheduled time point as appropriate. 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.

Study Day = visit date – date of "Day 1"; if visit date < date of Day 1.

There is no Day 0.

Protocol visits are scheduled at screening/baseline (Day 1), and on Days 15, 30, 181, 195, 210 and 360. Day 360 is a telephone contact. Each protocol visit time window is specified in the Table 1 below (Section 9.1.2 of the CTP).

Table 1: Visit Windows

VISIT	Visit Day	Window	Primary Purpose
Visit 1	Day 1 (VACC 1)	-	First vaccination
Visit 2	Day 15 (VACC 1 + 14 days)	± 2 days	14 days post-first vaccination safety and immunogenicity visit
Visit 3	Day 30 (VACC 1 + 29 days)	± 3 days	29 days post-first vaccination safety only visit
Visit 4	Day 181 (VACC 2)	± 7 days	Second vaccination (6 months post-first vaccination)
Visit 5	Day 195 (VACC 2 + 14 days*)	± 2 days	14 days post-second vaccination safety and immunogenicity visit
Visit 6	Day 210 (VACC 2 + 29 days*)	± 3 days	29 days post-second vaccination safety only visits
Visit 7	Day 360 (VACC 2 + 179 days*)	± 7 days	6 months post-second vaccination safety only visit (Telephone contact)

VACC 1: First dose of ExPEC4V or placebo on Day 1; **VACC 2**: Second dose of ExPEC4V or placebo on Day 181.
*The timings of visits after the second vaccination will be determined relative to the actual day of that vaccination.

2.1.2. Study Phase definitions

The phases in the study will be constructed as follows. There is one regimen and two post-dose periods and follow-ups. A subject who does not receive a second vaccination will not have a Post-dose 2 period.

Table 2: Analysis Phase and Period

Phase	Phase #	Period	Period #	Interval	
				From	To
Screening	1			Date and time of signing the informed consent form (ICF)	One minute prior to start of Post-dose 1 period
Regimen	2.1	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 on 29 days after the first vaccination (23:59 of day of vaccination + 29 days)
Follow-up 1	3			One minute after Post-dose 1 period end	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) One minute prior to Post-dose 2
Regimen	2.2	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 on Day 29 after the second vaccination (23:59 of day of vaccination + 29 days)
Follow-up 2	4			One minute after Post-dose 2 period end	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of last phone contact

The phases and periods will be used primarily for safety and concomitant medication allocation. For immunogenicity analysis, no phases will be constructed. The post-dose periods and the regimen phase are considered active phases. Screening and the follow-up phases are considered non-active phases. For descriptive statistics over time, assessments (regardless of the investigated parameter) will be allocated to an analysis visit based on the visit number as captured in the database.

2.2. Pooling Algorithm for Analysis Centers

This study is conducted in 2 centers in the US. The data from these two centers will be pooled.

2.3. Analysis Sets

Vaccination assignment will follow the "as-treated" principle.

2.3.1. Full Analysis Set (FAS)

The full analysis set will include all randomized subjects with at least one study vaccine administration documented regardless of the occurrence of protocol deviations.

2.3.2. Per-Protocol Immunogenicity Analysis Set (PPI)

The per-protocol immunogenicity analysis set will consist of all subjects from the FAS excluding those with major protocol deviations expecting to impact the immunogenicity outcomes. A list of these major protocol deviations is given in Attachment 2. Prior to interim or final database lock, the clinician will review the list of major deviations and determine which of these deviations is expecting to impact the immunogenicity outcomes.

If a subject misses the second dose but continues the planned visit schedule, data generated by samples taken after the missed dose will not be included in the per-protocol statistical analysis. Samples from subjects having a second vaccination or blood draw outside the protocol visit will be removed from the per-protocol analysis. If a subject's blood sample drawn from either pre- or post-first vaccination is lost, then this subject will not be included in the PPI.

2.4. Definition of Subgroups

Summary statistics will be presented for the following subgroups. More details are given in the immunogenicity analysis section.

1. Antigen baseline titer: low and high. For each antigen, pool the actual values of baseline across placebo and ExPEC4V CTM and determine the median. Use the median, or the lower limit of quantification (LLOQ) if the median is less than LLOQ, to split the values of baseline titers into low and high groups.
2. Onset of solicited AE: no AE, early and late. Onset is early if the first solicited AE started on or before 5 days post-vaccination and late if the AE started after 5 days post-vaccination.
3. Age group ($\geq$ 18 years to < 50 years, $\geq$ 50 years)

3. PLANNED ANALYSES

The primary analysis will be performed when all subjects have completed Day 30 visit or discontinued earlier, upon resolution of relevant queries and database lock. The primary analysis will include safety data through Day 30 and immunogenicity data through Day 15. Sponsor personnel involved in the analysis of the data will be unblinded at the time of this primary analysis. The primary analysis data will be used by the sponsor for planning of future studies and will not be communicated to the investigator(s) or site staff.

The final analysis will be performed for safety/reactogenicity and immunogenicity, when all subjects have completed the Day 360 visit or discontinued earlier.

4. SUBJECT INFORMATION

Subject information will be analyzed based on the FAS. Data will be presented by treatment group: ExPEC4V CTM, placebo and overall.

4.1. Demographics and Baseline Characteristics

The following demographic characteristics and screening/baseline characteristics will be tabulated, if appropriate or summarized with descriptive statistics over all subjects.

1. Sex (Female/Male)
2. Age (years)
3. Age group ($\geq$ 18 years to < 50 years, $\geq$ 50 years)
4. Age group ($\geq$ 18 years to < 60 years, $\geq$ 60 years)
5. Race
6. Ethnicity
7. Center
8. Height (cm)
9. Weight (kg)
10. BMI (kg/m^2)
11. History of smoking in the past 6 months (positive, negative)

Smoking includes the use of either tobacco, cigarettes, cigars, pipes or e-cigarettes. Subjects who have positive history of smoking in the past 6 months will include current or former smokers who quit smoking less than 6 months prior to screening. Those with a negative history of smoking in the past 6 months will include subjects who never smoked or former smokers who quit smoking at least 6 months prior to screening.

4.2. Disposition Information

The number and percentage of subjects with the following events and milestones will be tabulated.

1. randomized
2. randomized and received first vaccination
3. not randomized and received first vaccination
4. randomized and not vaccinated
5. in the FAS
6. in the PPI
7. completed Day 15 visit
8. completed Day 30 visit
9. did not receive 2nd vaccination
10. reason of not receiving 2nd vaccination
11. study discontinuation
12. reason of study discontinuation

Subjects assignment to study vaccine regimen will be provided in a data listing including the assigned regimen and the actual regimen received.

4.3. Treatment Compliance

The number of subjects with a missed second vaccination will be provided.

4.4. Protocol Deviations

Subjects with major protocol deviations will be identified prior to interim lock for the primary analysis and database lock for the final analysis. The major deviations will be summarized by deviations category. A subject may be counted in more than one deviation category. A listing of the major protocol deviations will be generated. The deviations that will have the potential to influence immune response will be flagged in the listing.

4.5. Medical History

Medical history abnormalities will be presented in a listing.

4.6. Pre-study and Concomitant Medications

Pre-study and concomitant medications will be tabulated. Except for vaccines, the analysis of pre-study and concomitant therapies will be based on the World Health Organization drug coded term as provided in the clinical database. Vaccines will not be coded. If the coded term for a medication is missing then the reported term will be used and noted in the table. The pre-study specific therapies will include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, antihistaminic, and vaccinations administered up to 30 days before signing the ICF.

Concomitant therapies which will include NSAIDs, corticosteroids, antihistaminic, and non-study vaccinations must be recorded for all subjects until 29 days after the first vaccination (from Day 1 to Day 30) and from the second vaccination through the following 29 days (from Day 181 to Day 210), and additionally outside of these periods when associated with a serious adverse event (SAE). Information on concomitant use of herbal supplements or vitamins will not be collected. A medication that is started prior to the date of administration of the first dose of study vaccine, but is continued to be taken after the first dose will be considered a concomitant medication.

Based on their start and stop dates, concomitant therapies will be reported in the phase during which they are applied. Time information, if available may also be used to allocate concomitant therapies to periods. For missing or partial start/stop dates the following allocation rules will apply.

1. In case the start date or the end date of the medication is partially missing, the concomitant therapy will be allocated to periods using the available partial information, without imputation. 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.
2. In case of a completely missing start date, the medication is listed as having started before the trial.
3. In case of a completely missing end date, the medication is listed as ongoing at the end of the trial.

5. SAFETY

The safety and reactogenicity endpoints are

1. Solicited local and systemic AEs (reactogenicity), collected daily from day of vaccination until 14 days post-vaccination
2. Unsolicited AEs collected through 29 days after each vaccination
3. SAEs collected after the first vaccination until the end of the study

Primary endpoints will include solicited AEs observed after first vaccination from Day 1 to Day 15, unsolicited AE from Day 1 to Day 30 and SAEs until Day 180. Secondary endpoints will include solicited AEs observed 14 days after second vaccination, unsolicited AEs for 29 days after vaccination and SAEs through until the end of the study.

AEs whether serious or non-serious related to the study procedures or non-investigational (concomitant) Janssen products are collected from the time a signed and dated ICF is obtained onwards. All other AEs, whether serious or non-serious are collected starting with the administration of first dose of the study vaccine on the AE electronic case report form (eCRF) page. Clinically relevant medical events, occurring between signing of ICF and time of first vaccination, are collected on the medical history eCRF page as pre-existing conditions.

5.1. Adverse Events

5.1.1. Definitions

Solicited AEs are precisely defined events (local and systemic) that subjects are specifically asked about and which are noted by subjects in the diary.

Unsolicited AEs are all AEs that a subject experienced but are not specifically asked about.

5.1.1.1. Solicited Local (Injection Site) Reactions

The analysis of solicited local AEs or injection-site reactions will include the following.

1. Injection-Site Pain/Tenderness
2. Injection-Site Erythema
3. Injection-Site Swelling/Induration

5.1.1.2. Solicited Systemic Adverse Events

The analysis of solicited systemic AEs will include the following.

1. Fatigue
2. Headache
3. Nausea
4. Myalgia
5. Fever

Fever is defined as endogenous elevation of body temperature ≥38°C (100.4°F), as recorded in at least one measurement if more than one measurement is made on any given day.

5.1.1.3. Unsolicited and Serious Adverse Events

All other AEs besides those in 5.1.1.1 and 5.1.1.2 are considered unsolicited.

Condition(s) on which a solicited or an unsolicited AE is considered a SAE is described in the CTP. For unsolicited AEs, only the AEs within the 29-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 period from first vaccination until the end of the study. All other collected unsolicited AEs will be presented through listings.

5.1.2. Causality

Solicited local AEs are always considered related to the use of study vaccine. Causality of the solicited systemic and unsolicited AEs will be considered either related or not related to the study vaccine. If a subject has experienced the same event multiple times in the same period, any of the event related to the study vaccine will be used.

5.1.3. Severity Criteria

The severity of the AEs will be classified by the investigator as either one of grades 1 to 4. Events that are less than grade 1, are not considered as AE. All AEs data will be coded for severity using the toxicity tables in Attachment 1. For AEs not identified in these toxicity tables, a guideline to determine severity grade is given in the CTP. If a subject has experienced the same event multiple times in the same period, the event with the worst severity is used.

5.1.4. Analysis of Adverse Events

The safety and reactogenicity will be summarized based on the FAS. The solicited and unsolicited AEs including SAE (through 29 days after vaccination) will be presented by study vaccine regimen (Post-dose 1 and Post-dose 2) and by treatment group. A separate table for SAEs will be presented by phases and by treatment group. The solicited AEs will be summarized by class (local, systemic) and terms as reported in the eCRF. The unsolicited AEs will be coded using Medical Dictionary for Regulatory Activities (MedDRA) and summarized by system organ class and preferred term. At each level of summarization, a subject is counted once if the subject reported one or more events.

If the second vaccination is not administered to a subject, this subject will not be included in the analysis of solicited AEs and unsolicited AEs in Post-dose 2 period. The denominator for the percentages is the number of subjects vaccinated at that phase or period.

A table indicating the number of subjects will be created for each of the following events.

1. Solicited local AE by worst severity grade
2. At least Grade 3 solicited local AE
3. Solicited systemic AE by worst severity grade
4. At least Grade 3 solicited systemic AE
5. Solicited systemic AE related to study vaccine
6. At least Grade 3 solicited systemic AE related to study vaccine
7. Unsolicited AE
8. At least Grade 3 unsolicited AE
9. Unsolicited AE related to study vaccine
10. At least Grade 3 unsolicited AE related to study vaccine
11. SAE
12. SAE related to study vaccine
13. AE leading to study discontinuation
14. AE leading to death
15. AE leading to vaccination discontinuation

For the table containing the unsolicited AEs only, the tabulation of AEs will be sorted by descending incidence in ExPEC4V CTM.

For the following, only the overall incidence will be calculated and presented in the summary table. The individual AEs will not be tabulated.

1. Any AE, including both solicited and unsolicited
2. Any AE related to study vaccine
3. Any AE with severity of at least grade 3
4. Any AE with severity of at least grade 3 related to study vaccine
5. Any solicited AE
6. Any solicited AE related to study vaccine
7. Any solicited AE with severity of at least grade 3
8. Any solicited AE with severity of at least grade 3 related to study vaccine

In the summary tables, a 95% CI (Clopper-Pearson) for incidence proportion (percentage) will be provided, as appropriate. Bar plots of the incidence proportion will be generated from selected tables.

For solicited AEs, duration (day) of AE and time to first onset (day) of AE will also be summarized. Duration is defined as the number of days from the start of the event until the resolution of the event. If a subject experiences more than one event of the same AE, the maximum duration of the events will be used. 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 phase (Post-dose 1 or Post-dose 2).

5.1.5. Period allocation of Adverse Events

Solicited events are always allocated to the respective period.

Step 1: Allocation of events to the periods:

AEs in the Study Data Tabulation Model (SDTM) database are allocated to periods based on their start date/time.

1. 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).
2. 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 doubling the event as consequence of its assignment to two periods.
3. In case of a completely missing end date (for the calculation of duration) and the AE is flagged as ongoing, the date is imputed by the cutoff 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 study. In case the AE is not flagged as ongoing, the end date is considered as unknown and the date will remain missing.

4. 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).

Remarks:

1. In addition to the date information, time information is used to allocate AEs to periods, if available.
2. The imputation of missing end dates of ongoing AEs will only be used to derive the duration of the events (i.e., to give an indication of the minimum duration). The imputed end dates will not be shown in the data listings.

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. If overlapping/consecutive events start in one of the following periods - screening or post-dose extension (i.e. non-active periods) - followed by an AE in - post-dose period (active period) - 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 Analysis Data Model (ADaM) database but are assigned the same onset, period, and total duration. All related attributes to the AE/phase/period should also be consistent with the new event.
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. All related attributes to the AE/phase/period should also be consistent with the new event.
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 derivation of duration), this date is also considered as a complete date.
3. Time is not considered when determining overlap of events.

5.1.6. Missing Data

Missing data will not be imputed including missing causality and missing severity. Subjects who do not report an event will be considered as subjects without an event.

An AE with a missing causality or severity will be considered as an AE reported, but will be considered as not reported for causality or severity. For example, an AE with missing severity will be considered as an AE reported for the analysis of any grade but will be considered as not reported for the analysis or tabulation of at least grade 3 severity.

5.2. Vital Signs and Physical Examination Findings

The vital signs are measured before vaccination and at the end of the 30-minute observation period after both doses of study vaccine on Day 1 and Day 181 (body temperature, pulse/heart rate, systolic and diastolic blood pressure). The worst toxicity grade of vital sign abnormalities emerging after vaccination will be tabulated separately by period and by treatment group.

A full physical examination is only performed at screening by the investigator or a designated medically trained clinical physician/associate. A targeted physical examination may be performed at subsequent visits (except Day 360, which will be a telephone contact) based on the complaints/symptomatology and health status of the subject. Therefore, only a listing of subjects with the worst (abnormal) physical examination findings following vaccination will be provided by period and treatment group.

6. IMMUNOGENICITY

Immunogenicity of the ExPEC4V vaccine response will be assessed by ELISA and OPKA specific for the ExPEC4V serotypes, measuring total IgG antibodies and functional antibodies, respectively. The immune response will be available for each of the following antigens: O1A, O2, O6A, O25B and EPA (ELISA only). Each immune response will be assessed in serum from blood samples taken at the following timepoints: Days 1 (pre-vaccination), 15, 181 (pre-vaccination) and 195.

The immunogenicity endpoints are

1. Total IgG antibody titers determined by ELISA
2. Functional antibody titers determined by OPKA

The primary endpoints are ELISA antibody titers on Days 1 and 15. Secondary endpoints will include ELISA antibody titers (Days 181 and 195) and OPKA antibody titers (Days 1, 15, 181 and 195).

The fold change in antibody titers based on both the ELISA and OPKA will also be calculated on Day 15 (from Day 1 pre-vaccination) and on Day 195 (from Days 1 and 181 pre-vaccination).

6.1. Parameters

The following summary measures will be evaluated.

1. Geometric mean titer (GMT) on Day 1 (pre-vaccination), Day 15, Day 181 (pre-second vaccination) and Day 195.
2. Proportion of subjects with a ≥2-fold and ≥4-fold increase in serum antibody titers (Day 15 compared to Day 1 and Day 195 compared to Day 181).
3. Geometric mean ratio (GMR): fold change from baseline, calculated from the Day 15/Day 1 values and fold change from pre-second dose, calculated from Day 195/Day 181 values

There will be no formal statistical comparisons. Graphical representations of immunological parameters will be made as applicable.

The titer (fold change) will be log10 transformed to allow a normal distribution to be assumed in the calculation of the 95% CI. The 95% CI will be based on the *t* distribution. The GMT (GMR) is obtained by taking the anti-log10 of the mean of the log10 concentration (fold change) transformations. The CI of the GMT (GMR) is calculated as the back-transformed CI of the mean of the log10 transformed titer (fold change). The CI is used to describe the precision of the GMT and GMR.

6.2. Limit of Quantification and Limit of Detection

Both ELISA and OPKA were fully qualified on precision, trueness, linearity, accuracy/total error, specificity and sample stability. For GMT analysis, titer values below LLOQ will be replaced by ½ of the LLOQ. For GMR analysis, titer values below LLOQ will be replaced by LLOQ. Antibody concentrations at or above the LLOQ are considered accurate and their quantitated values will be reported.

ExoProtein A (EPA) is the carrier protein antibody.

Table 3: Lower Limit of Quantification

Antigen	ELISA (EC50)	OPKA (Opsonic Indices)
O1A	210	180
O2	514	129
O6A	234	36
O25B	253	42
EPA	317	Not Applicable

Missing immune response data will not be imputed.

6.3. Analysis

The immunogenicity analysis will be presented by treatment group (ExPEC4V CTM and placebo) and by timepoints.

A summary of immunogenicity measures and their corresponding 95% CI on ELISA and OPKA data by 4 serotypes (O1A, O2, O6A and O25B) and antigen ExoProtein A (EPA, ELISA only)

will be performed on both FAS and PPI analysis sets. PPI is the primary analysis set for immunogenicity. Each measure will be analyzed descriptively over time.

The proportion (or percentage) of subjects reaching at least two- and four-fold increase from pre-vaccination level at Day 15 and at Day 195 will be presented. A two-sided 95% Clopper-Pearson CI will be provided for each proportion (or percentage).

The association between onset of solicited AE and antigen titer level will be investigated on the FAS. The GMT of an antigen titer at baseline and 15 days post-vaccination will be numerically described for subjects with no solicited (local and systemic) AE, early (1 to 5 days post-vaccination) versus late (after 5 days post-vaccination) first onset of solicited (local and systemic) AE. The prevalence of late onset of solicited (local and systemic) AE, stratified by high vs low antigen level at baseline will be provided. Both analyses will be stratified by age group ($\geq$ 18 years to $<$ 50 years and $\geq$ 50 years). For the definition of these subgroups, see Section 2.4.

All immunogenicity plots will be based on the PPI. In each plot, the original values will be displayed on the log10 scale, as appropriate.

1. GMT plots over time will be created.
2. Reverse cumulative distribution curves of the actual values will be plotted for each treatment group and time point using step functions starting with 100% of subjects going down to 0% of subjects on the vertical (y-) axis and immunoglobulin titers on the horizontal (x-) axis.
3. Assess the relation between age and Days 1 and 181 titers
4. Assess the relation between age and fold changes from pre-vaccination level at Day 15 and at Day 195
5. Assess the relation between Day 1 pre-vaccination titers on increase in ELISA/OPK fold change from pre-vaccination observed at Day 15
6. Investigate the correlation between ELISA and OPK measurements at Days 1, 15, 181 and 195.

ATTACHMENTS

Attachment 1: Toxicity Tables

Adapted from the FDA Guidance document "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (September 2007)

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to Touch	Discomfort with Movement	Significant discomfort at rest	ER visit or Hospitalization
Erythema/redness*	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/swelling**	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Vital Signs *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Fever** (°C) Fever** (°F)	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	>40 >104
Tachycardia - beats per minute	101 – 115	116 – 130	>130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) - mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate - breaths per minute	17 – 20	21 – 25	>25	Intubation

* Subject should be at rest for all vital sign measurements.
** Oral temperature; no recent hot or cold beverages or smoking.
*** When resting heart rate is between 60 - 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Nausea/vomiting	No interference with activity or 1 - 2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Systemic Illness	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life-threatening (Grade 4)**
Illness or clinical AE (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

Attachment 2: Major Protocol Deviations Influencing Immunogenicity
INCLUSION CRITERIA
Inclusion 2 Subject is medically stable as confirmed by documented medical history, physical examination and vital signs. Subject may have underlying illnesses such as hypertension, diabetes, or ischemic heart disease, as long as their symptoms/signs are medically controlled. If he/she is on medication for a condition, the medication dose must have been stable for at least 12 weeks preceding vaccination.
EXCLUSION CRITERIA
Exclusion 2 Subject with an acute illness (this does not include minor illnesses such as diarrhea or mild upper respiratory tract infection) or temperature ≥38.0ºC (100.4ºF) within 24 hours prior to administration of study vaccine; enrollment at a later date is permitted.
Exclusion 3 Subject with known allergies, hypersensitivity, or intolerance to ExPEC4V or its excipients (refer to Investigator's Brochure).
Exclusion 4 Subject with abnormal function of the immune system resulting from: • clinical conditions (e.g., autoimmune disease or immunodeficiency). • chronic or recurrent use of systemic corticosteroids. *Note: Ocular, topical or inhaled steroids are allowed.* • administration of antineoplastic and immunomodulating agents or radiotherapy.
Exclusion 5 Subject has a history of neoplastic disease (excluding non-melanoma skin cancer or carcinoma *in situ* of the cervix that was successfully treated) within the past 1 year or a history of any hematological malignancy.
Exclusion 7 Subject who has received or plans to receive: • licensed live attenuated vaccines – within 28 days before or after each study vaccination. • other licensed (not live) vaccines - within 14 days before or after each study vaccination.
Exclusion 8 Subject who has received an investigational drug (including investigational vaccines) or used an invasive investigational medical device within 90 days before vaccination in the study, is currently enrolled in an investigational study, or plans to enroll in an investigational study during this study.
Exclusion 10 Subject has received treatment with immunoglobulins in the 2 months or blood products in the 4 months before vaccination in the study or any plans to receive such treatment during the study.
DOSAGE & ADMINISTRATION
Subject randomized but not treated with study medication
Subject not randomized but treated with study medication
Subject received Investigational Product is not stored properly
Subject received incorrect dose
Subjects received second dose outside the protocol time window (See Table 1 or CTP, section 9.1.2).
OTHER – STUDY PROCEDURES
Blood sampling for immunogenicity evaluation not done per-protocol schedule (See Table 1 or CTP, Section 9.1.2).
The collection, handling, storage, and shipment of blood samples was not done as indicated in the Laboratory Manual
Received concomitant immunosuppressive therapy after randomization and within 15 days after vaccination