

Janssen Research & Development**Statistical Analysis Plan
AMENDMENT 7**

Randomized, Double-Blind, Placebo-controlled, Multicenter Phase 3 Study to Assess the Efficacy, Safety And Immunogenicity of Vaccination With ExPEC9V in the Prevention of Invasive Extraintestinal Pathogenic Escherichia coli Disease in Adults Aged 60 Years And Older with a History of Urinary Tract Infection in the Past 2 Years

Protocol VAC52416BAC3001; Phase 3; E.mbrace**VAC52416 (JNJ-78901563; ExPEC9V)**

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

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VERSION HISTORY**Table 1: SAP Version History Summary**

SAP Version	Approval Date	Change	Rationale
1.0	24 June 2021	Not Applicable	Initial release
2.0	17 March 2022	Description of the details and type of data to be reviewed by the Independent Data Monitoring Committee (IDMC) has been updated. The safety data to be assessed by the IDMC once the first 500 randomized participants have completed the Day 30 visit (Visit 3), or discontinued earlier, will include serious adverse events (SAEs) and unsolicited adverse events (AEs) from all participants and solicited AEs from participants in the Safety Subset. Added text to describe that in case of positive efficacy results of the first primary endpoint, the study will not be stopped, but follow-up of participants will be continued for efficacy (interim and final analyses of the second primary endpoint and secondary endpoints), safety, and immunogenicity. Rephrased text to indicate that cases with multiple pathogens in the urine culture results will not be considered for the primary and secondary endpoints if the systemic infection cannot be attributed only to <i>Escherichia coli</i> (<i>E. coli</i>), as per Independent Endpoint Adjudication Committee judgment. Cases with mixed pathogens and the presence of <i>E. coli</i> in urine cultures will be included in sensitivity analyses. All cases with multiple pathogens including <i>E. coli</i> in the culture results from blood or any other sterile site will be included in the efficacy analysis for the primary and secondary endpoints. Added text to indicate that in case of serious safety concerns during the IDMC safety review once 500 randomized participants have completed the Day 30 visit (Visit 3), the IDMC may recommend the sponsor to stop the study. Added text to indicate that participants who receive other <i>E. coli</i> vaccines will be excluded from the per protocol efficacy population and the per protocol immunogenicity population. Added text to indicate that participants will be excluded from the per-protocol immunogenicity or per-protocol efficacy population from the date of the major protocol deviation impacting immunogenicity or efficacy onwards.	Alignment with Protocol Amendment 3 (20 September 2021)

Table 1: SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
		Rephrased text to indicate that the presence of at least one risk factor for complicated urinary tract infection (cUTI) (instead of risk factors for cUTIs) at baseline will be used to stratify the randomization. Clarified that the significance level of 0.0122 that will be used for the secondary endpoints represents a Pocock boundary with 3 interim looks at information fractions 0.5, 0.7 and 1. Clarification to the IED Clinical Case Definition regarding sterile sites' definition and UTI signs & symptoms Definition of onset of IED, UTI and ABP added Added interaction test between the subgroup categories and the VE Adapted the Frailty Index calculation based on Curran et al. 2019 Added Section 5.7.7: Diagnosis of UTI/ABP and Clinical Data Collection, Appendix 9: Sterile Sites' Definition and Appendix 10: Primary Endpoint based on IEAC Adjudication Minor changes to make the SAP consistent throughout. Minor grammatical, formatting, or spelling changes were made.	To clarify how the significance level of 0.0122 was obtained To clarify the IED Clinical Case Definition Onset was not yet defined in the SAP Interaction test was not yet in SAP The calculation of the Frailty Index did not account for missing values in the questionnaires To clarify the categories for UTI and ABP, similar to IED. To clarify the sterile sites' definition and how primary endpoints will be calculated based on the IEAC adjudication Minor errors and inconsistencies were noted.
3.0	27 March 2023	The second primary endpoint was modified to clarify that invasive extraintestinal pathogenic <i>Escherichia coli</i> disease (IED) cases with microbiological confirmation from urine only are excluded. An exploratory objective/endpoint was added to include efficacy evaluation of ExPEC9V in the	Alignment with Protocol Amendment 4 (2 June 2022)

Table 1: SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
		prevention of the first acute bacterial prostatitis (ABP) event caused by any ExPEC O-serotype. An exploratory objective/endpoint was modified to include efficacy evaluation of ExPEC9V in the prevention of the first <i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>) ABP event. Included sequential organ failure assessment (SOFA) criteria as an alternative to systemic inflammatory response syndrome (SIRS) criteria and updated the footnote for urinary tract infection (UTI) signs and symptoms for urosepsis cases. During Independent Endpoint Adjudication Committee (IEAC) review, the IEAC reviewers need to decide if the collected data are pertinent to be included in the case definition evaluation for the same IED event. Added a schematic diagram for visual representation of the IED clinical case definition. Added that in case multiple SOFA scores are available before the onset of systemic infection, the acute change will be counted from the baseline score defined as the lowest score over the last 72 hours preceding the diagnosis of systemic infection. The cut-off for the urine sediment microscopy results was added to the definition of pyuria, ie, ≥ 5 white blood cells per high-power field (WBC/HPF) (400x enlargement). Subserotypes A or B were removed from O serotypes O1A, O6A, O18A, O25B that need to be available for the primary and secondary endpoints, and the ABP endpoint. Addition of the utilization of tokenization and matching procedures to obtain medical data 5 years prior to enrollment of the participant until 5 years after the participant completed the study from consenting participants in the United States. Added plazomicin to the antibiotic panel.	
		The study design has been updated to allow for continuous enrollment instead of staggered enrollment. A note has been added to indicate that over-enrollment may occur due to local requirements.	Alignment with Protocol Amendment 5 (2 December 2022)

Table 1: SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
		The signs and symptoms for complicated UTIs and pyelonephritis were updated to clarify that fever alone qualifies as symptom. The Charlson Comorbidity Index score will be calculated in addition to the Frailty Index. Text regarding the O-serotype testing has been updated with additional testing using the O-agglutination assay per FDA advice. The description of estimands was improved Minor changes to make the SAP consistent throughout. Minor grammatical, formatting, or spelling changes were made.	To align with the description of estimands in vaccine studies Minor errors and inconsistencies were noted.
4.0	20 February 2024	‘Any ExPEC O-serotype’ is reworded to ‘<i>E. coli</i>’ (Section 1.1, 2.1, 5.4.1.1, 5.4.1.2.1, 0, 5.5.2.1). For the MRU objective, ICU hospitalization and ICU length of stay for IED and UTI/ABP events are added (Section 1.1). Participants taking prophylactic antibiotics are allowed to enter the study since PA6. Therefore, blood samples of subjects who were on prophylactic antibiotics at the time of the sampling will be excluded from the MOPA analysis. (Section 4). For the secondary endpoints related to <i>E. coli</i>, it is clarified that local lab confirmation of <i>E. coli</i> is sufficient to fulfill the definition (Section 5.4.1.1). In Table 10 (Criteria of Interim and Final Analysis for the First Primary Endpoint) in Section 5.8.3, the footnote on blinding is removed. The IED Clinical Case Definition (CCD) is corrected for the criteria on WBC ($WBC \geq 10\%$) and to include $PaCO_2 < 32$ mm Hg in the definition (Section 6.2.1). In the ABP CCD, wording is added to describe that a temperature $\geq 38.0^\circ\text{C}$ is considered a fever (Section 6.2.4.).	Alignment with Protocol Amendment 6 (11 July 2023)

Table 1: SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
		Pyelonephritis and nephrolithiasis are added in Table 15 as risk factors for complicated UTI (Section 6.4). COVID-19 specific language has been removed as the pandemic has ended (Appendix 6: Prior and Concomitant Medications).	
		<i>E. coli</i> bacteremia is added as a risk factor for complicated UTI (Section 6.4). The decision tree for isolate characterization (Section 6.9) is updated. “Hospitalization in the past 2 years”, the combination (hospitalization in the past 2 years or history of urosepsis/<i>E. coli</i> bacteremia or risk factors for complicated UTI (any toxicity grade)) and use of antimicrobial prophylaxis at baseline are added to the list of baseline characteristics (Section 6.4) as well as to the subgroups of interest (Section 5.7.2).	Alignment with Protocol Amendment 7 (17 November 2023)
		The wording of the results of the adjudication is updated. (Section 6.10)	Alignment with IEAC Charter Amendment 3 (9 October 2023)
		QTL parameters and thresholds are defined (Section 1.2). Wording is added to clarify that participants who receive other <i>E. coli</i> vaccines will be excluded from the PPE as well as from the PPI population from the date of the vaccination onwards. The list of <i>E. coli</i> vaccines is provided (Section 4). Rephrasing of the text to clarify how cases with multiple pathogens in urine will be analyzed. For UTI/ABP related endpoints, it is clearly stated which events will be included in the analysis (Section 5.1). The database cut-off is added to the phase definitions (Section 5.1.2), Wording is added to emphasize that all isolates from blood, sterile site or urine will be serotyped - independently in which site <i>E. coli</i> was detected- and considered in the definition of the primary endpoint (Section 5.3.1).	Updates and clarifications

Table 1: SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
		Isolates confirmed by the central lab as <i>E. coli sensu stricto</i>, as well as species comprising or previously comprising <i>Escherichia</i> clades I through V will be sent for serotyping and if confirmed to be ExPEC9V, will be included in the primary statistical analysis (Section 5.3.1). IED episodes cannot overlap in time, and pyelonephritis episodes cannot overlap in time. Clarification is added (Sections 5.3.3 and 5.4.1.3). An additional sensitivity analysis including all IED in blood/sterile sites and IED in urine only with antibiotic treatment initiated before blood sample collection is added (Section 5.3.3) An additional sensitivity analysis including only IED with <i>E. coli sensu stricto</i> is added (Section 5.3.3). The definition of IED related deaths is updated to adjudicated deaths (Section 5.4.2). In the future, ongoing solicited AEs (beyond the 15-day period for ExPEC), will not be mapped anymore to the AE domain and therefore the relevant text is removed (Section 5.6.1.1). Subgroup immunogenicity analysis for high-risk subjects in the PPI is added (Section 5.7.1.4). Subgroup analyses for United States only, is added (Section 5.7.2). It is clarified how to calculate the Frailty Index at the timepoints Year 1, Year 2 or Year 3, if the SF-36 and the EQ-5D data are not available at those timepoints (Section 5.7.5.2). For completeness, additional categories are added (Sections 5.7.6, 5.7.7). A note is added for the IED CCD to clarify that ‘OR’ in this definition is an ‘inclusive OR’ (Section 6.2.1). Notes are added for the UTI CCD to clarify how fever is defined and how risk factors for cUTI are defined (Section 6.2.2).	

Table 1: SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
		An additional Age classification is added to Table 13(Section 6.4). Minor grammatical, formatting, or spelling changes were made.	
5.0		The original second primary endpoint (IED in blood/sterile sites) has become the sole primary endpoint. The original first primary endpoint (IED in blood/sterile sites/urine) has become the first secondary endpoint (Sections 1, 2, 3, 5.3, 5.4, 6.2.1). The interim analyses (frequency, number of events, significance level,..) are updated according to the changes in the primary endpoint (Sections 1, 2, 5.8). As a result of updates to the primary endpoint, corresponding increases in sample size (+1200 participants) and follow-up duration (+1 year) have been made (Sections 1.2, 3, 5.8.4). With extension of follow-up, immunogenicity samples in the immunogenicity subset will also be collected and analyzed at Year 4 (Sections 1.1,5.7.1). With extension of follow-up and corresponding addition of an ePRO timepoint at 4 years post-vaccination, degree of frailty 4 years post-vaccination will also be assessed and compared between the ExPEC9V and placebo participants (Sections 1.1, 5.7.5). With discontinuation of site study-specific assessments for UTI/ABP events, the definition of follow-up time for UTI-related endpoints has been updated (Sections 5.4.1.3, 5.5.3). With the removal of whole genome sequencing from the serotyping decision tree for primary endpoint definition, the agglutination assay alone will be used for serotype determination. For IED events, this agglutination assay will be run twice and a third time if there is no concordance between these two initial results. If 2 out of 3 concordant results are not obtained, the isolate will be classified as non-typeable. For pyelonephritis, UTI and APB events, agglutination will be performed only once (Section 5.1.4).	Alignment with PA8 (9 August 2024)

Table 1: SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
		The wording of an exploratory objective was updated to state that the efficacy of ExPEC9V will be evaluated in the prevention of the first IED caused by ExPEC9V O-serotypes resistant to ≥ 1, ≥ 2 and ≥ 3 antibiotic classes rather than antibiotics (Section 1.1, 5.5). MRU and PRO are analyzed only for IED/Pyelonephritis/UTI caused by EXPEC9V O-serotypes instead of for any <i>E. coli</i> serotype (Sections 1.1, 5.7.3, 5.7.4). The exploratory endpoint on the use of tokenization and generation of real-world data among US participants was removed. Appendix 6.6 (Prior and Concomitant Medication) is updated to include wording on the use of other <i>E. coli</i> vaccines.	
		Wording is added to clarify what will happen when the study is stopped for futility (Section 1.2). If confirmed misconduct has been detected at a specific site, it is specified how data of this site will be handled in the analysis (Section 5.1). Additional sensitivity analyses are added (Section 5.1). For secondary endpoints related to <i>E. coli</i>, it is stated that if central lab confirmation is not available then local lab confirmation is sufficient to classify an event as being due to <i>E. coli</i>. In cases of discrepancies between the local and central lab bacterial identification, only central lab results will be used (Section 5.1.4). It is stated that an IED event will be classified as an IED in blood, or an IED in sterile sites, or an IED in urine only, according to the classification by the IEAC (Section 5.1.4). Rules are added to handle the serotyping results in case of missing serotyping data for a clinical sample source of interest, in case of discrepancies between the serotyping results of different clinical sample sources and in case of discrepancies between the serotyping results of the same clinical sample source (Section 5.1.4).	Updates and clarifications

Table 1: SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
		Additional exploratory analyses of multidrug resistant (MDR) <i>E. coli</i> and resistance to antibiotics per WHO “Access/Watch/Reserve” classification have been included (Section 5.5). For multi-enrollers, it is specified that appropriate tables and listings for the safety analysis will be provided (Section 5.6). As the time period reporting of the SAEs may be different in different countries/territories (up to 6 months after vaccination or the whole study period), the SAE tables will cover the time period up to Day 181 (instead of the whole study period). Listings of the SAEs will cover the whole study period (Section 5.6.1.1). Additional subgroup analyses are added (Section 5.7.2). In the analysis of ePROs, it is stated that ePROs that are triggered erroneously will not be part of the analysis (Section 5.7.4).	
		Throughout the SAP, minor corrections and changes to make the SAP consistent throughout. Minor grammatical, formatting, or spelling changes were made.	Minor errors and inconsistencies were noted.
6.0	14 August 2024	Change approval date of protocol amendment 8 from 23 August 2024 to 9 August 2024	Update
7.0	12 September 2024	Section 5.1.5 is added to clarify how data from duplicate participants (individuals randomized more than once into a study of ExPEC9V) will be handled in the E.mbrace study. Section 5.2 is updated to give more details on the disposition for duplicate participants.	As the sponsor is aware that a number of individuals have been randomized more than once at different sites in an ExPEC9V study (duplicate participants), adjusted data handling and reporting for such cases are needed
		Specific reporting for duplicate participants as described in this version of the SAP will be done in the final analysis (or in the early efficacy analysis). For Safety Interim Analyses for the IDMC and for the Futility Analysis, the reporting as described in SAP Amendment 5 will be used.	
		In Section 5.1.4, instructions for final O-serotyping determination are added in case the agglutination assay detects multiple serotypes.	Extra instructions
		Clarification on the Go/No Go approach during the futility analysis when both rules are fulfilled is added (see Section 5.8.3).	Clarification

Table 1: SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
		Section 5.9 is added to give details on the End-Of-Study analysis.	
		Terminology updates are done for the estimand sections for the key secondary and exploratory endpoints.	Terminology
8.0	6 May 2025	Based on the outcome of the first interim analysis for the primary endpoint (futility) the IDMC recommended, and the Sponsor agreed, to stop the study for futility. All participants will be followed up for at least 6 months post-vaccination. Two efficacy evaluations will be performed, main efficacy analyses and an end-of-study efficacy analysis. A single immunogenicity analysis will be performed, based on available data. There are no changes to safety analysis Therefore, updates to the analysis plan are made and are described below	IDMC recommendation and /Sponsor decision to stop the study for futility
		The main efficacy analyses will include IEDs, pyelonephritis and UTIs events with onset on or before the announcement of the futility outcome (13 February 2025).	Maintain interpretability of the study by avoiding potential biases in event reporting that could occur following announcement of the futility analysis outcome.
		For the main efficacy analysis, comprehensive serotyping of <i>E. coli</i> isolates for UTIs will be discontinued	At the time of the first interim analysis, sufficient UTI events have been serotyped for a robust analysis of vaccine efficacy for UTI prevention.
		For IED/pyelonephritis/UTI events with onset after futility decision (Feb 13 2025), comprehensive serotyping of <i>E. coli</i> isolates will be discontinued.	Due to futility outcome, the study and program will be discontinued, only available data will be included in the end of study analysis.
		Discontinuation of IEAC case review for suspected IED and pyelonephritis. Parallel analyses will be performed: (a) analysis of IED/Pyelonephritis that have been adjudicated (b) analysis of IED/Pyelonephritis using the Clinical Case Definition (programmatic determination of endpoint)	There is a high degree of correlation between the adjudication outcome and application of the clinical case definition for both IED and pyelonephritis
		Discontinuation of IEAC review of death cases for determination of IED-related deaths. Instead,	There is a high degree of correlation between

Table 1: SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
		determination of deaths as IED-related will be per PI assessment.	adjudication outcome of IED-related death and PI-assessment of IED related death.
		A cumulative immunogenicity analysis of the immunogenicity subset (PPI and FAS) will be performed using available ECL and MOPA. For the FAS analysis, the immunogenicity data collected at the time of the interim immunogenicity analysis (based on the first 200 subjects) will be included. Completion of comprehensive testing is discontinued. Immunogenicity assessments will be performed with available data.	Comprehensive immunogenicity testing is discontinued, as sufficient information on immune response to vaccination is already generated. Blood draws for immunogenicity sampling following the announcement of the study are discontinued due to unfavorable risk/benefit for participant.
		ePRO analysis of surveys (SF-36 and EQ-5D-5L) completed before futility analysis will be conducted with available data. Analysis of ePRO surveys that have been completed after the announcement of the futility analysis outcome will be discontinued.	Avoid potential biases that could derive from participant knowledge of the futility analysis outcome.
		Frailty index analysis will be performed only up until the announcement of the futility outcome (13 February 2025).	Frailty index analysis requires elements from the SF-36 and EQ-5D-5L, which will no longer be analyzed after 13 February 2025 due to concerns of bias, therefore frailty index analysis must also be discontinued at the same date.
		MRU analysis will be limited to evaluation of hospitalization data	As futility was demonstrated, a more detailed analysis of medical resource utilization is not required, as differences in outcome are not expected.
		Mortality assessment will be limited to all cause-events. IED-mortality per vaccine group will only be assessed if there are a minimum of 6 IED-related deaths available for analysis.	Too small number of IED-related mortality events to enable meaningful analysis.
		Reduced scope of exploratory objectives.	Exploratory objectives may or may not be assessed, and given futility outcome, the scope has been reduced
		Reduced number of sensitivity analyses. If performed, the sensitivity analyses will only be performed on primary endpoint and first secondary endpoint.	As futility of the vaccine candidate was demonstrated, more detailed sensitivity analyses are not deemed scientifically useful.

Table 1: SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
			Per SAP version 7, sensitivity analysis are not required.
		UTI and pyelonephritis will be analyzed separately.	To align with Protocol Amendment 8

1. INTRODUCTION

This statistical analysis plan (SAP) of this study contains the definitions of analysis sets, derived variables, and statistical methods that will be used to evaluate the safety, reactogenicity, immunogenicity and efficacy of the ExPEC9V vaccine candidate for the prevention of invasive *Escherichia coli* disease (IED: previously referred to as invasive extraintestinal pathogenic *Escherichia coli* [ExPEC] disease) in adults aged 60 years and older with a history of urinary tract infection (UTI) in the past 2 years.

This SAP will include the details of the statistical analysis of the data collected in each of the interim analyses, as well as the end of study analysis. The tables, listings and figures (TLF) required for each analysis will be described in separate data presentation specifications (DPS) documents.

This document was originally developed in line with the Clinical Trial Protocol (CTP) Amendment 8 approved on 9 August 2024.

On 11 February 2025, the results of the planned first interim analysis (IA) (futility) of VAC52416BAC3001 on the primary endpoint, prevention of first IED event with microbiological confirmation from blood or other sterile sites caused by ExPEC9V O-serotypes, were presented to the independent data monitoring committee (IDMC) by the independent statistical support group (SSG). This planned IA did not demonstrate sufficient efficacy on the primary endpoint; hence the IDMC recommended that the study be stopped for futility (See IDMC Charter). The IDMC also reviewed cumulative safety data, and no safety concerns were raised. Therefore, the sponsor announced on 13 February 2025 that the study would be prematurely discontinued. The results of this first interim analysis will be provided in the appendix of the CSR. Knowledge of the results of the futility analysis outcome on 13 February 2025 has the potential to influence conduct of the study as there is a potential for less rigorous/comprehensive event reporting by sites. To minimize bias in analyses, efficacy events before or on the clinical cutoff date of 13 Feb 2025 will be analyzed separately and will be considered for the main efficacy analysis. The main analysis for efficacy will include all data from events with onset on or before 13 February 2025. The end of study efficacy analysis will include the data collected during the entire study period. The analysis of safety and the analysis of immunogenicity will be performed on the entire study period.

Furthermore, with the VAC52416BAC3001 trial discontinuation, there are no plans to pursue the development of the ExPEC9V candidate vaccine, nor to submit a registrational file to any authority. Certain activities may be discontinued such as adjudication of IED, pyelonephritis and deaths by the IEAC (Independent Endpoint Adjudication Committee), comprehensive serotyping of *E. coli*, comprehensive immunogenicity testing, etc. as these do not add scientific value in the absence of vaccine efficacy.

Accordingly, this document is updated to account for changes in planned analyses in the context of early study termination for futility of the vaccine candidate.

1.1. Objectives and Endpoints

The table below defines the objectives and endpoints of the study.

Based on the outcome of the first interim analysis (futility), the study is discontinued. The objectives and endpoints below are based on VAC52416BAC3001 clinical trial protocol amendment version 8. The objectives/endpoints have not been modified as the result of premature study termination. Changes to the analysis are detailed in specific sections of the SAP.

Table 2: Objectives and Endpoints

Objectives	Endpoints
Primary	
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event, with microbiological confirmation from blood or other sterile sites, caused by ExPEC9V O-serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75	First IED event^a, with microbiological confirmation from blood or other sterile sites^b, excluding IED cases with microbiological confirmation from urine only, caused by ExPEC9V O-serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75
Secondary	
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event, with microbiological confirmation from blood, other sterile sites, or urine, caused by ExPEC9V O-serotypes^c	First IED^{a d}, event, with microbiological confirmation from blood, other sterile^b sites or urine, caused by ExPEC9V O-serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of all IEDs (including multiple IEDs per participant) caused by ExPEC9V O-serotypes^c	All IEDs^d (including multiple IEDs per participant) caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first hospitalized IED event caused by ExPEC9V O-serotypes^c	First hospitalized IED event^d caused by ExPEC9V O-serotypes

^a Refer to Section 6.2.1 for the IED clinical case definition.

^b Refer to Section 6.9 for the sterile sites' definition.

^c Key secondary efficacy endpoints, part of the confirmatory testing strategy.

^d Indicated secondary endpoints related to IEDs will include events with *E. coli* microbiological confirmation from blood, other sterile sites, or urine.

Table 2: Objectives and Endpoints

Objectives	Endpoints
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event meeting criteria for sepsis caused by ExPEC9V O- serotypes^c	First IED event^d meeting criteria for sepsis caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first bacteremic (ie, microbiological confirmation from blood) IED event caused by ExPEC9V O- serotypes^c	First bacteremic IED event caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first pyelonephritis event caused by ExPEC9V O- serotypes^c	First pyelonephritis event caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first urinary tract infection (UTI) event caused by ExPEC9V O- serotypes^c	First UTI event^e caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of all UTIs (including multiple UTIs per participant) caused by ExPEC9V O- serotypes^c	All UTIs^e (including multiple UTIs per participant) caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event caused by <i>E. coli</i>^c	First IED event^d caused by <i>E. coli</i>
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first pyelonephritis event caused by <i>E. coli</i>^c	First pyelonephritis event caused by <i>E. coli</i>
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first UTI event caused by <i>E. coli</i>^c	First UTI event^e caused by <i>E. coli</i>

^e UTI and ABP events that occur after implementation of Protocol Amendment 8 will no longer be included in analyses.

Table 2: Objectives and Endpoints

Objectives	Endpoints
To evaluate the immunogenicity of ExPEC9V in the Immunogenicity Subset	Antibody titers to vaccine O-serotype antigens and exotoxin A derived from <i>Pseudomonas aeruginosa</i> (EPA) in the Immunogenicity Subset, as determined by multiplex electrochemiluminescent (ECL)-based immunoassay and antibody titers to vaccine O-serotype antigens, as determined by multiplex opsonophagocytic killing assay (MOPA) on Day 1 (pre-vaccination), Day 30, Day 181, Year 1, Year 2, Year 3 and Year 4
To evaluate the safety and reactogenicity of ExPEC9V	- Solicited local and systemic AEs (collected until 14 days post-vaccination [from Day 1 to Day 15] in the Safety Subset) - Unsolicited AEs (collected until 29 days post-vaccination [from Day 1 to Day 30] in all participants) - Serious adverse events (SAEs)^f in all participants

^f All SAEs until the end of Day 181 and only SAEs related to study vaccine, study procedures, or non-investigational sponsor products from Day 182 until the end of the study. Additional SAEs may be reported per local regulatory requirements.

Table 2: Objectives and Endpoints

Objectives	Endpoints
- To evaluate the preservation of health status and health-related quality of life (HRQoL) of ExPEC9V compared to placebo as measured by the Short Form-36 (SF-36) survey and the 5-level EuroQol 5-dimension questionnaire (EQ-5D-5L) descriptive system - To evaluate the impact of IED, caused by ExPEC9V O-serotypes, on physical and mental health, and overall HRQoL as measured by the SF-36 and the EQ-5D-5L - To evaluate the impact of pyelonephritis, caused by ExPEC9V O-serotypes, on physical and mental health, and overall HRQoL as measured by the SF-36 and the EQ-5D-5L - To evaluate the impact of UTI^e, caused by ExPEC9V O-serotypes, on physical and mental health, and overall HRQoL, as measured by the SF-36 and the EQ-5D-5L	SF-36 and EQ-5D-5L responses at scheduled time points
To assess the degree of frailty in participants who received ExPEC9V versus placebo at baseline, Year 1, Year 2, Year 3, Year 4 and at the time of an IED	Frailty Index as a measure of frailty at baseline, Year 1, Year 2, Year 3, Year 4 and at the time of an IED^d
To determine the medical resource utilization (MRU) for IED, UTI and acute bacterial prostatitis (ABP) events caused by ExPEC9V O-serotypes	- MRU for IED events^d caused by ExPEC9V O-serotypes - MRU for UTI events^e (Immunogenicity Subset only) and ABP events^e (Immunogenicity Subset only) caused by ExPEC9V O-serotypes - Hospitalization and length of stay in hospital, including ICU hospitalization and ICU length of stay, for IED^d, UTI^e or ABP^e events caused by ExPEC9V O-serotypes
To determine the mortality associated with IED	IED-related^d and all-cause mortality
Exploratory	
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event caused by ExPEC9V O-serotypes resistant to ≥ 1, ≥ 2, and ≥ 3 antibiotic classes	First IED event caused by ExPEC9V O-serotypes showing resistance to ≥ 1, ≥ 2, and ≥ 3 antibiotic classes in the microbiological profile

Table 2: Objectives and Endpoints

Objectives	Endpoints
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of first uncomplicated, complicated, and recurrent UTI events caused by ExPEC9V O-serotypes	- First uncomplicated UTI event^e caused by ExPEC9V O-serotypes - First complicated UTI event^e caused by ExPEC9V O-serotypes - First recurrent UTI (rUTI)^e event caused by ExPEC9V O-serotypes - First recurrent uncomplicated UTI event^e caused by ExPEC9V O-serotypes
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first ABP event caused by ExPEC9V O-serotypes	First ABP event^e caused by ExPEC9V O-serotypes
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first ABP event caused by <i>E. coli</i>	First ABP event^e caused by <i>E. coli</i>
To evaluate antibody titers to vaccine O-serotype antigens and EPA in participants with IED	Antibody titers to vaccine O serotype antigens and EPA, as determined by multiplex ECL-based immunoassay and antibody titers to vaccine O-serotype antigens, as determined by MOPA assays in participants with IED, with sera obtained at the time of diagnosis of the suspected IED
To evaluate the severity of IED	Severity of IED (presence of sepsis or septic shock) in participants with IED
To identify a correlate of protection for the vaccine-mediated immune response associated with vaccine efficacy (VE)	Antibody titers for ExPEC9V determined by multiplex ECL-based immunoassay and MOPA assays at Day 1 pre-vaccination, Day 30 and at the time of diagnosis of the suspected IED (all participants) or at the time of an UTI or ABP event (Immunogenicity Subset only), in association with VE
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first invasive disease event caused by <i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>) and the first <i>P. aeruginosa</i> UTI/ABP event	- First invasive disease event caused by <i>P. aeruginosa</i> - First <i>P. aeruginosa</i> UTI event^e - First <i>P. aeruginosa</i> ABP event^e

Table 2: Objectives and Endpoints

Objectives	Endpoints
Exploratory (added in SAP)	
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event, with microbiological confirmation from urine only, caused by ExPEC9V O-serotypes	First IED event, with microbiological confirmation from urine only, excluding IED cases with microbiological confirmation from blood or other sterile sites, caused by ExPEC9V O-serotypes

1.2. Study Design

This is a randomized, double-blind, placebo-controlled, parallel group, multicenter, interventional Phase 3 study to be conducted in approximately 19,800 medically stable adults aged ≥ 60 years and with a history of UTI in the past 2 years.

This study incorporates an IED case-driven inferentially seamless group sequential design.

The target number of participants to be enrolled is approximately 19,800. Note that over enrollment may be allowed if required by local health authorities for the purpose of local health authority consideration. When the first 200 vaccinated participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3), or discontinued earlier, an immunogenicity interim analysis will be conducted and the sponsor will review summary statistics of the immunogenicity data by study vaccination group (at this stage, the sponsor will remain blinded to individual study vaccine allocation). Enrollment will not be paused during this immunogenicity interim analysis. The purpose of this immunogenicity interim analysis is to obtain an initial result of the immune response, no decision will be taken on the remainder of the study and therefore no type I error adjustment is required. In addition, once the first 500 randomized participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3), or discontinued earlier, the Independent Data Monitoring Committee (IDMC) will review unblinded safety data. In case of serious safety concerns during this IDMC safety review, the IDMC may recommend the sponsor to stop the study. The safety data will include SAEs and unsolicited AEs from all participants and solicited AEs from participants in the Safety Subset. According to the ratio of the Safety Subset versus the initial total sample size of the study ($4,000/18,556=21.6\%$), approximately 108 participants are targeted in the Safety Subset among the first 500 randomized participants. Enrollment will be paused after randomization of the 500th participant until the IDMC issues a recommendation. Thereafter, an IDMC safety review will be conducted at least every 6 months. When 21 of 53 (40%) vaccine serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sites) have been observed, an interim analysis for the primary endpoint (futility) will be performed by the Statistical Support Group. Based on the outcome of this interim analysis, the IDMC may recommend stopping enrollment of participants and stopping the study (in case of futility) or to continue the study. In the event of the study being stopped for futility, all participants will be followed up for at least 6 months post-vaccination. The futility analysis will be based on the Go/No Go approach. In addition, if the event rate is lower or higher than expected, a blinded sample size re-estimation (ie,

based on the event rate combining all participants from both the ExPEC9V and placebo groups) may be performed at the time of this interim analysis by the sponsor. A second interim analysis for the primary endpoint (efficacy/futility) will be conducted when 37 of 53 (70%) vaccine-serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sites) have been observed. The final analysis for the primary endpoint will occur when 53 vaccine serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sites) have been observed in the study or at the latest when the last participant has been followed up for 48 months post-vaccination.

The efficacy analyses for the primary endpoint will use the α -spending approach. For all the interim analyses an independent Statistical Support Group (SSG) will perform the interim analyses and will present the results to the IDMC (at the immunogenicity interim analysis the SSG will also prepare the immunogenicity tables for the study team).

For further details on the interim analyses, refer to Section 5.8.

More details about the study design are given in the Clinical Trial Protocol, Section 4.1.

The study consists of screening, randomization, and vaccination for each participant on Day 1. Selected screening procedures may be performed up to 8 days prior to vaccination on Day 1. If screening procedures are split over different days, eligibility criteria must be rechecked, pre-vaccination vital signs and pre-vaccination blood samples for immunogenicity should be collected on the day of vaccination (Day 1). Each participant will be followed for a maximum of approximately 48 months (1,461 days; 4 years) post-vaccination. At screening/Day 1, demographic data, Short Physical Performance Battery (SPPB) score and medical conditions of interest will be collected. All participants will be enrolled and randomized in parallel in a 1:1 ratio to either ExPEC9V or placebo and receive the study vaccine on Day 1. Central randomization will be implemented in this study. Participants will be randomly assigned to 1 of 2 study vaccination groups based on a computer-generated randomization schedule prepared before the study by or under the supervision of the sponsor. The randomization will be stratified according to participant age (≥ 60 to < 65 years of age versus ≥ 65 to < 75 years of age versus ≥ 75 years of age) and according to the presence of at least one risk factor for complicated UTIs (cUTIs) at baseline and done in blocks to ensure balance across arms. A maximum of 20% of the study population will be enrolled in the ≥ 60 to < 65 years of age stratum.

An IDMC and Independent Endpoint Adjudication Committee (IEAC) will be commissioned for this study.

Quality Tolerance Limit (QTL) parameters and thresholds are defined and will be monitored in this study. QTL parameters will be summarized. More details are described in the Integrated Analytical Risk-Based Monitoring (iARBM) Plan.

1.2.1. Update post first interim (futility) analysis

Based on the outcome of the first interim analysis (futility), the study will be discontinued. Hence, the second interim analysis (at 37 IED events) and final analysis (at 53 IED events) will not be performed. Instead, the main efficacy analyses will include all data from events with onset on or before the futility announcement (13 February 2025). The end of study efficacy analysis will include the data collected during the entire study period.

Please note that on 13 February 2025, the Sponsor discontinued enrolment prematurely due to the outcome of the futility analysis. All ongoing participants who have been followed for at least 6 months post-vaccination (Day 181) will be withdrawn from the study. All ongoing participants who have not yet reached the Day 181 visit as of 13 February 2025, will be followed for at least 6 months post-vaccination before withdrawal from the study.

2. STATISTICAL HYPOTHESES

The primary efficacy endpoint will evaluate the incidence of IED at least 29 days post vaccination (from Day 30) of ExPEC9V versus placebo until the end of the follow-up period for IED in the per protocol efficacy (PPE) population according to the clinical case definition described in [Appendix 2 Section 6.2.1](#).

For the primary endpoint, the following null and alternative hypotheses on VE at pre-specified 2.5% one-sided significance level with appropriate control of the type 1 error for interim monitoring will be used:

Hypothesis (1):

- H_0 : VE of ExPEC9V against placebo $\leq 20\%$
versus
- H_a : VE of ExPEC9V against placebo $> 20\%$

A hierarchical testing strategy is employed whereby if the primary endpoint hypothesis testing reaches significance, the secondary endpoints will be evaluated (see Section 2.1). For the key secondary objectives related to IED, the following null and alternative hypotheses will be used:

Hypothesis (2):

- H_0 : VE of ExPEC9V against placebo $\leq 0\%$
versus
- H_a : VE of ExPEC9V against placebo $> 0\%$.

For the key secondary objectives related to UTI and pyelonephritis, the following null and alternative hypotheses will be used:

Hypothesis (3):

- H_0 : VE of ExPEC9V against placebo $\leq 10\%$
versus

- H_a : VE of ExPEC9V against placebo >10%.

2.1. Hierarchical Testing Strategy

A hierarchical testing strategy¹⁰ will be applied to the following endpoints:

Primary:

1. First IED with microbiological confirmation from blood or other sterile sites, excluding IED cases with microbiological confirmation from urine only, caused by ExPEC9V O-serotypes

Secondary:

1. First IED, with microbiological confirmation from blood, other sterile sites, or urine, caused by ExPEC9V O-serotypes
2. All IEDs (including multiple IEDs per participant) caused by ExPEC9V O-serotypes
3. First hospitalized IED event caused by ExPEC9V O-serotypes
4. First IED event meeting criteria for sepsis caused by ExPEC9V O-serotypes
5. First bacteremic IED event caused by ExPEC9V O-serotypes
6. First pyelonephritis event caused by ExPEC9V O-serotypes
7. First UTI caused by ExPEC9V O-serotypes
8. All UTIs (including multiple UTIs per participant) caused by ExPEC9V O-serotypes
9. First IED event caused by *E. coli*
10. First pyelonephritis event caused by *E. coli*
11. First UTI caused by *E. coli*

In this testing strategy, the first key secondary endpoint (first IED event, with microbiological confirmation from blood, other sterile sites or urine, caused by ExPEC9V O-serotypes) will only be tested when the primary endpoint shows statistical significance (at the interim or final analyses). If the first key secondary endpoint shows statistical significance, the other secondary endpoints will be tested according to the above hierarchical testing strategy. The second key secondary endpoint will include all ExPEC9V IED (with microbiological confirmation from blood, other sterile sites or urine) events with onset of at least 29 days after vaccination (PPE) until the end of the follow-up period.

If the first secondary endpoint is tested, it will be tested using the Pocock boundary using a 1-sided significance level of 0.0162, regardless of whether this test occurs at the interim or final analysis. This testing strategy is graphically presented in [Figure 1](#). The significance level of 0.0162 represents a Pocock boundary with 2 interim looks at information fractions 0.7 and 1.

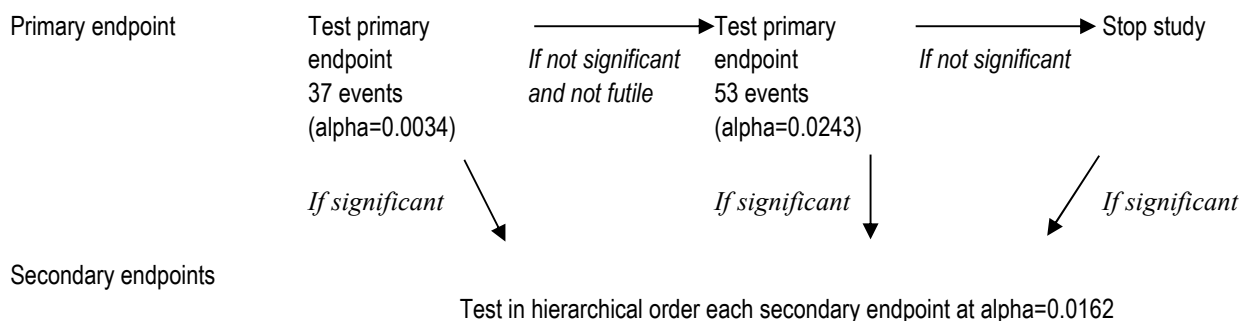
If the lower limit of the $(1-2\alpha)\%$ confidence interval (CI) for the first key secondary endpoint is above 0%, VE will have been demonstrated for this endpoint and the next key secondary endpoint

will be tested using the same strategy. For the secondary endpoints related to pyelonephritis and UTI, a lower limit of the $(1-2\alpha)\%$ CI of 10% will be used (see Section 2).

Applying the Pocock boundary to the key secondary endpoints is an optimal allocation of the significance level for the hierarchical testing strategy as the minimal significance level to be used at an unspecified timepoint is maximized.¹⁰

Note that for the key secondary objectives related to IED that are part of the hierarchical testing strategy, microbiological confirmation can be obtained from blood, other sterile sites or urine, except for the bacteremic IED objective where microbiological confirmation must be obtained from blood (see Section 5.4.1). These secondary objectives will also be analyzed using exploratory endpoints with microbiological confirmation from blood or other sterile sites (see Section 5.5).

Figure 1: Hierarchical Testing Strategy for Primary and Secondary Endpoints



The primary endpoint will use 1-sided significance levels 0.0034 and 0.0243 at the interim and the final analysis, respectively. The secondary endpoints will use 0.0162 as a 1-sided significance level irrespective of when they are tested; however, they will only be tested once the primary endpoint is significant.

3. SAMPLE SIZE DETERMINATION

3.1. Total Sample Size

This is an event (ExPEC9V serotype-specific IED) driven study. The total number of first occurrences of IED and the total number of participants required to observe these IEDs are determined from the following assumptions.

- 1) VE of 70% for the primary endpoint
- 2) Incidence Rate of IED in the placebo group at 0.17 per 100 person-years
- 3) ExPEC9V serotype coverage of 65%
- 4) One sided type 1 error rate at 0.025
- 5) Power to reject the null hypothesis that the true VE is at most 20% at 90%
- 6) Randomization ratio for ExPEC9V:Placebo at 1:1
- 7) Yearly overall dropout rate of 10%
- 8) Average duration of study for each participant 3.5 years

Under these assumptions, an estimated total number of 53 events for the primary endpoint, from approximately 19,800 participants are required. The statistical power takes into consideration that a futility interim analysis after 21 events, an efficacy interim analysis after 37 events and a final analysis after 53 events will be performed. Since futility has been reached, no further analyses based on IED event accrual will be performed.

More details on the interim analyses are given in Section 5.8 and Appendix 8.

3.2. Immunogenicity Subset

The antibody immune response to ExPEC9V or placebo determined from both multiplex ECL-based immunoassay and MOPA assays will be evaluated from a total of approximately 1,200 participants (approximately 600 ExPEC9V participants and 600 placebo participants). Due to local requirements, over enrollment may occur for the Immunogenicity Subset.

As described in the CTP, Section 9.2.2, these 1,200 participants are sufficient to provide blood samples to evaluate serotype-specific immune response mediated by a single vaccination of ExPEC9V or placebo, as measured by multiplex ECL-based immunoassay and MOPA assays.

In addition, for those participants in this Immunogenicity Subset that develop UTI or ABP during the follow-up period, data on medical resources utilization (MRU) will be collected after each episode.

In context of the futility outcome, blood sampling for immunogenicity will be discontinued, as it is no longer in the best interest of participant (this includes assessments at scheduled visits, e.g. Day 30, in the immunogenicity subset, and during suspected IED events). Consequently, comprehensive immunogenicity testing may not be performed as initially planned: the immunogenicity analysis will be done on the PPI and FAS, based on available data. Similarly, MRU analyses will be limited in scope and based on available data (up to futility announcement).

3.3. Safety Subset

The Safety Subset includes approximately 4,000 participants, with approximately 2,000 ExPEC9V participants and 2,000 placebo participants. Due to local requirements, over enrollment may occur for the Safety Subset. The analysis of solicited adverse events will be restricted to the Safety Subset. Solicited adverse events, collected through an eDiary will be recorded from time of vaccination until 14 days following vaccination only for participants included in the Safety Subset.

In context of the futility outcome, there are no changes to the safety procedures, and all participants will be followed for at least 6 months post-vaccination. The safety analysis will be done on the FAS, based on available data during the entire study period.

4. POPULATIONS (ANALYSIS SETS) FOR ANALYSIS

The study vaccination assignment will follow the as treated principle. That is, all participants will be analyzed according to the actual study vaccination received. The different Analysis Sets that will be used in the analysis are shown in Table 3.

Table 3: Analysis Sets

Analysis Set	Description
Enrolled	The enrolled analysis set will include all participants who signed the informed consent form.
Randomized	The randomized analysis set will include all participants who were randomized, i.e. had a valid randomization issued by the interactive web response system (IWRS) implemented in this study.
Full Analysis Set (FAS)	The FAS will include all participants with documented vaccine administration, regardless of the occurrence of protocol deviations. This is the safety population. The analysis of solicited AEs will be restricted to a subset (Safety Subset) of the FAS.
Per Protocol Efficacy (PPE)	The PPE set will include all randomized and vaccinated participants for whom efficacy data concerning endpoint measures are available excluding participants with major protocol deviations expecting to impact the efficacy outcomes (see Appendix 5). Participants will be excluded from the date of the major protocol deviation onwards. Participants who receive other <i>E. coli</i> vaccines* will be excluded from the PPE population from the date of vaccination with the other <i>E. coli</i> vaccine onwards. The PPE population is the primary efficacy population.
Per Protocol Immunogenicity (PPI)	The PPI population will include all randomized and vaccinated participants, who are part of the Immunogenicity Subset and for whom immunogenicity data are available, excluding participants with major protocol deviations expected to impact the immunogenicity outcomes (see Appendix 5). Participants will be excluded from the date of the major protocol deviation onwards. Participants who receive other <i>E. coli</i> vaccines^g will be excluded from the PPI population from the date of vaccination with the other <i>E. coli</i> vaccine onwards. For the MOPA immunogenicity analysis, blood samples taken - since PA6 - of participants who are on antimicrobial prophylaxis at the time of the blood sampling, will be excluded from the PPI. All blood samples taken before PA6 and after the start of prophylactic antibiotics treatment will be withdrawn from the MOPA analysis. The PPI population is the primary immunogenicity population.

^g *E. coli* vaccines include, and are not limited to, immunoactive agents used for rUTI prophylaxis and containing *E. coli* immunogens, eg, inactivated whole bacterial lysates of *E. coli* (eg, Uromune®) or lyophilized preparation of *E. coli* membrane proteins (eg, Uro-vaxom®).

5. STATISTICAL ANALYSES

5.1. General Considerations

Data from all study sites will be pooled together for analysis. If confirmed misconduct has been detected at a specific site, data from this site may be excluded from the analysis and presented separately, as appropriate. The primary efficacy population is the PPE set, safety population is the FAS and primary immunogenicity population is the PPI set.

As the public announcement on 13 February 2025 of the futility of the vaccine candidate and the early termination of the study has the potential to introduce bias in data collection of subsequent events, analyses have been structured to preserve the rigor in scientific interpretation of the study as follows:

- The Main Efficacy Analyses will provide the information available at the time of futility announcement. These are analyses for efficacy events (IED, pyelonephritis and UTI/ABP) with onset on or before 13 February 2025 and will be performed using both the PPE and the FAS. Ongoing subjects with no events on or before 13 February 2025 will be censored on 13 February 2025 for these analyses.
- For the primary endpoint and secondary endpoints related to IED and pyelonephritis, 3 parallel efficacy analyses will be conducted, first an analysis for the PPE based on IED/Pyelonephritis events that were assessed and confirmed by the IEAC. A second analysis for the PPE will be based on the efficacy events that are consistent with the protocol clinical case definition (programmatic approach). Furthermore, a third analysis of these efficacy events according to the clinical case definition will be done on the FAS.
- For UTI related endpoints, 2 parallel analyses will be conducted, both based on the clinical case definition for UTI, for the PPE and the FAS respectively.
- The End of Study Efficacy Analysis will assess efficacy events that occurred throughout the entire study period, according to the clinical case definition. This analysis will be limited to IED and Pyelonephritis events “caused by *E. coli*”, as comprehensive serotyping will be discontinued after 13 February 2025. UTI events will not be analyzed, because at time of discontinuation of the study, the majority of sites had Protocol amendment 8 implemented and therefore reporting of UTI events had also previously ceased. This end of study analysis will be done on the FAS only, and with descriptive analyses only. No statistical hypothesis testing will be done.
- The Safety Analysis will be performed using the FAS throughout the entire study period.
- The Immunogenicity Analysis will be performed using both the PPI and the FAS throughout the entire study period.

The IEAC will review data collected from suspected *E. coli* confirmed IED and *E. coli* confirmed pyelonephritis cases and based on this data, assign each case according to their clinical judgement and the clinical case definitions. In addition, the source of infection, deaths attributable to IED or to other causes and the severity of IED cases provided by the investigator will be verified and confirmed by the IEAC. Re-adjudication may occur if new information becomes available. The IEAC's assessment is considered the definitive classification of the case.

As the Sponsor elected to terminate the study early due to futility, IEAC review of suspected *E. coli* confirmed IEDs, pyelonephritis and deaths will be discontinued. As described above, in the Main Efficacy Analyses (including only events with onset on or before 13 February 2025), 3 parallel analyses will be performed: 1) using the IEAC assessment for the PPE, 2) using the clinical case definition of the events for the PPE and 3) using the clinical case definition of the events for the FAS. The clinical case definition for the different events is described in Appendix 2. Note that in the analysis based on the IED CCD, the source of infection of an IED (urinary tract, gastrointestinal tract, respiratory tract, or other) will be as per PI assessment of “primary source of infection” in the eCRF.

Death cases that have not yet been reviewed by the IEAC will be determined as attributable to IED per PI assessment in the eCRF.

For the primary and secondary endpoints involving *E. coli* identification from blood or sterile sites, all IED cases (including those with multiple pathogens) will be included in the efficacy analyses.

For the secondary endpoints involving *E. coli* identification from urine only (including culture-confirmed IED and pyelonephritis), cases with multiple pathogens will only be considered an endpoint if per the IEAC judgement, *E. coli* was the only clinically significant pathogen contributing to the IED or pyelonephritis. The IEAC review has been discontinued due to early termination of the study. In the Main Efficacy Analysis based on the clinical case definitions (for the PPE and the FAS) for secondary endpoints involving *E. coli* identification from urine only, the following rules will be implemented for assessment of multiple pathogens:

- If during the event there are no additional pathogens reported in urine or in other sterile sites, it will be considered that *E. coli* was the only clinically significant pathogen contributing to the IED or pyelonephritis event. Such events will be included in secondary endpoint analyses.
- If during the event additional pathogens are reported in urine or in other sterile sites, it will be considered that *E. coli* was not the only clinically significant pathogen OR it is unclear if *E. coli* was the only clinically significant pathogen contributing IED or pyelonephritis events. Such events will be excluded from secondary endpoint analyses.

For secondary endpoints involving first *E. coli* (of any serotype, irrespective of ExPEC9V), local lab identification of *E. coli* is considered sufficient for including an event in the analysis, including cases where an isolate is not available for microbiological analyses at the central lab.

For UTI (uncomplicated UTI, complicated UTI) and ABP related endpoints, cases according to the clinical case definition with only *E. coli* (and no other pathogens in urine) will be included in the analysis.

As the Sponsor elected to terminate the study early due to futility, changes to the planned sensitivity analyses may be performed, either analyses will not be done or may be reduced in scope. For instance, evaluations may be performed for the primary endpoint and the first secondary endpoint only, in FAS population using data available up until 13 February 2025. No sensitivity analyses will be performed for the end of study analyses. In addition, analyses will be performed

only if a reasonable number of events is added. The following sensitivity analyses may be performed for the primary and for the first secondary endpoint:

- An analysis which includes IED cases in urine, for which the IEAC members are unable to determine if *E. coli* was the only clinically significant pathogen.
- An analysis of IED cases with septic shock in which septic shock will be defined programmatically as administration of vasopressors and a serum lactate level greater than 2 mmol/L (18mg/dL).
- An analysis of first IED event with onset at least 14 days after vaccination (from Day 15).
- An analysis of cases with discrepant serotype determination either from the same clinical sample source or from different clinical sample source where one of the isolates has a serotyping determination of non-ExPEC9V and another isolate is ExPEC9V, the serotype determination from that sample source will be classified as non-ExPEC9V

As appropriate, continuous variables will be summarized with mean, standard deviation, first quartile, median, third quartile, interquartile range, minimum and maximum; categorical variables will be summarized with frequency and percentage.

5.1.1. Study Phases

Study Day 1 corresponds to the date the participant was vaccinated.

A baseline (or reference) value will be defined, unless otherwise specified as the value of the last available assessment prior to the vaccination on Day 1. Change from baseline will be defined as (post-baseline value – baseline value).

Study day or relative day is defined as follows:

- Study Day = visit date – date of Day 1 + 1; if visit date $\geq$ 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).

The safety analysis will present all results by phase (see Section 5.1.2). Immunogenicity results will be presented per scheduled time point as appropriate. Listings will be shown per phase and time point, as appropriate.

5.1.2. Phase Definitions

The phases in the study will be constructed as follows:

Table 4: Phase Definitions

Phase	Phase #	Period	Period #	Interval	
				From	To
Screening	1			Date and time of signing the informed consent form	One minute prior to start of 29 days post-vaccination period
29 days post-vaccination ¹	2	29 days post-vaccination 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) c) 23:59 at the database cut-off for any interim analysis
Follow-up 1 (Day 31- Day 181)	3			00:00 at Day 31	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 on 180 days after the first vaccination (23:59 of day of vaccination + 180 days) c) 23:59 at the database cut-off for any interim analysis
Follow-up 2 (from Day 182)	4			00:00 at Day 182	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of Day 1096 visit c) 23:59 at the database cut-off for any interim analysis

¹ For solicited AEs, the period will be labeled “14 days post-vaccination”.

5.1.3. Immunogenicity Visit Windows

For immunogenicity summaries and tabulations per scheduled time point, assessments will be allocated to an analysis day based on the planned visit as captured in the CRF (see [Table 5](#)). Immunogenicity samples will also be collected from participants at the time of suspected IED diagnosis (+48 hours window) and at one-year post-IED identification (± 14 days window) or at the end of the study (whichever comes first), as well as from participants in the Immunogenicity Subset at the time of diagnosis (+48 hours window) of suspected UTI (including pyelonephritis) or ABP. Visits that are out of the protocol-defined visit windows will not be included in the immunogenicity summaries and tabulations per time point in the PPI analysis. However, they may be included in the analysis of the FAS.

Table 5: Immunogenicity Scheduled Visit Windows

Clinic visit	Subset	Analysis day	Window	Purpose
Visit 1	All	Day 1		Pre-vaccination blood draw
Visit 3	All	Day 30	± 7 days	29 days post-vaccination
Visit 5	Immunogenicity	Day 181	± 14 days	180 days post-vaccination
Visit 6	Immunogenicity	Day 366	± 14 days	1 year post-vaccination
Visit 7	Immunogenicity	Day 731	± 28 days	2 years post-vaccination
Visit 8	Immunogenicity	Day 1096	± 28 days	3 years post-vaccination
Visit 9	Immunogenicity	Day 1461	± 28 days	4 years post-vaccination

5.1.4. Microbiological Assessments

The source of microbiologic confirmation (blood, sterile sites, or urine) for IED cases will be determined by the IEAC or determined programmatically. Programmatic determination of microbiologic source of an IED will be as per “source of specimen collection” of the positive *E. coli* cultures. See sections 5.1 and 6.10 for further details.

In cases of discrepancies between the local and central lab bacterial identification, only central lab results will be used.

The ExPEC9V O-serotype(s) (i.e., O1, O2, O4, O6, O15, O16, O18, O25 and O75) determination is assessed by the agglutination assay.

Isolates characterized by the central lab as *E. coli sensu stricto*, as well as species comprising or previously comprising *Escherichia clades* I through V, will be serotyped and, if confirmed to be an ExPEC9V O-serotype, will be included in the statistical analyses for relevant ExPEC9V endpoints.

All *E. coli* isolates from an episode will be characterized independently of the clinical sample source from which *E. coli* was identified (e.g. blood, urine, abscess, etc.). Only isolates from the Day 1 or Unscheduled visit folders in RAVE will be used for serotype determination of a clinical event. Isolates reported only in the Day 29/Day of Resolution folders in RAVE will not be used for serotype determination of a clinical event.

During a suspected IED event, all *E. coli* isolates that are available for microbiologic analyses at the central lab will have agglutination performed twice. Concordant O-serotype results will be reported as the specific ExPEC9V O-serotype or negative for all ExPEC9V typing antisera. In cases where the 2 initial agglutination results are discordant, agglutination will be performed a third time. If 2 out of 3 concordant results are not obtained, the isolate will be classified as non-typeable. If in the 2 agglutination runs multiple serotypes are detected within a sample, then no third run will be done, and the isolate will be classified as non-typeable.

During a suspected pyelonephritis, UTI, or ABP event, *E. coli* isolates that are available for microbiologic analyses at the central lab will have agglutination testing performed only once. If the test detects multiple serotypes within a sample, the isolate will be classified as non-typeable.

In context of the futility announcement, the study sites will continue with all study procedures except for blood draws for immunogenicity sampling which are discontinued due to unfavorable risk/benefit for patient. In case of a newly reported IED event, blood and/or urine culture may be performed by treating physician for diagnostic/treatment purposes. Study sites are encouraged to retrieve an isolate and/or urine sample, if reasonable feasible. Comprehensive serotyping is discontinued.

For IED serotype determination, the following sequential rules will be applied:

1. If a valid serotyping result is available from at least one isolate derived from blood, then the serotyping result from the blood-derived isolate will be used.
2. If a valid serotyping result is not available from a blood-derived isolate, but there is a valid serotyping result from a sterile site, then the sterile site isolate will be used.
3. If a valid serotyping result is not available from isolates derived from either blood or sterile sites, but there is a valid serotyping result available from a urine derived-isolate, then the serotyping result from the urine derived-isolate will be used.
4. If no valid serotyping result is available, the case will be excluded from the statistical analysis of endpoints related to ExPEC9V O-serotypes, and only included in the secondary endpoints “caused by *E. coli*”.

For pyelonephritis, UTI and ABP events, the serotyping determination result must be from a urine-derived isolate.

If multiple positive *E. coli* cultures were reported by the local lab, but one or more of the isolates was not available for central lab microbiological analyses for agglutination testing, or no valid agglutination testing result was returned, then the culture source (blood, sterile site, or urine) will dictate the hierarchy of serotype determination for the event as described above.

See Section 5.1.4.1 for rules dictating handling of discrepant serotyping results for isolates derived from the same sample source during a single clinical event.

As the Sponsor elected to terminate the study early due to futility and made an announcement of this decision on 13 February 2025, the following changes in central laboratory microbiologic analyses will be implemented:

- For all IED and pyelonephritis events per the clinical case definition with onset on or before 13 February 2025, serotype determination will be completed, if feasible, and included in analyses.
- For UTI events with onset on or before 13 February 2025, available serotyping data will be used for analyses.
- IED, pyelonephritis and UTI events with onset after 13 February 2025 may not undergo serotype determination. Available serotyping data testing will be used for analyses.
- For IED, pyelonephritis and UTI events, available antimicrobial susceptibility testing may be used for analyses.

5.1.4.1. Handling of Discrepant Serotyping Results for Isolates Derived from the Same Sample Source Type during a Clinical Event

If during a clinical event multiple isolates derived from the same sample source are assessed via the agglutination assay, there is potential for discrepancy in the serotyping results (e.g. one blood-derived isolate is ExPEC9V and a second blood-derived isolate is non-ExPEC9V or non-typeable).

If this occurs, then the following sequential rules will be applied for sample source serotype determination:

1. If at least one of the isolates from the same clinical sample source has a valid serotyping determination of ExPEC9V, the serotype determination from that sample source will be classified as ExPEC9V.
2. If none of the isolates from the same clinical sample source has a valid serotyping determination of ExPEC9V, but at least one of the isolates has a classification of non-ExPEC9V, the serotype determination from that sample source will be classified as non-ExPEC9V.
3. If none of the isolates from the same clinical sample source has a valid serotyping determination of either ExPEC9V or non-ExPEC9V, but at least one of the isolates has a classification of non-typeable, the serotype determination from that sample source will be classified as non-typeable.
4. If none of the isolates from the same clinical sample source has a valid serotyping result, the serotype determination from that sample source will be excluded in the statistical analysis of endpoints related to ExPEC9V O-serotypes.

5.1.5. Duplicate Participants

The sponsor has defined “duplicate participants” as individuals who enroll and are randomized more than once into a study of ExPEC9V. This typically occurs when individuals visit more than one study site, and at each site are assigned participant IDs, randomized, and administered blinded study vaccine (active/placebo).

The sponsor considers “cross-study duplicates” to be individuals randomized at least once in more than one study of ExPEC9V (e.g.: E.mbrace in addition to another clinical study of ExPEC9V). Cross study duplicates may be included in pooled analyses of ExPEC9V, but as cross study duplicates are out of scope for statistical analyses of E.mbrace, these analyses are not described in this SAP.

A duplicate participant is treated in all analyses as a unique individual with multiple participant IDs assigned to an individual person. Any randomization into an ExPEC9V study after the first randomization into an ExPEC9V study is considered a major protocol deviation impacting efficacy and immunogenicity. Therefore, for individuals confirmed to be duplicate participants, only data from the first randomization until the second randomization will be included for the Per Protocol Efficacy Set and Per Protocol Immunogenicity Set. To avoid confounding, data from the second study vaccination (second participant ID assigned, and any additional participant IDs assigned)

onwards will be excluded from the efficacy analyses based on the PPE and the immunogenicity analyses based on the PPI.

In analyses based on the Full Analysis Set, the following guidelines will be used:

- A duplicate participant will be classified per the treatment group (active/placebo) of his or her first randomization into the study.
- For the demographic and baseline characteristics: only data collected during the first randomization in the study will be used.
- For the efficacy analysis based on the FAS: for duplicate participants the follow-up time is taken from the first vaccination until the event of interest or until the final (most recent) discontinuation date of the duplicate participant from the study. The clinical event of a duplicate participant will be classified per the treatment group (active/placebo) of his or her first randomization into the study.
- For the safety analysis: the safety data will be presented as each unique person (duplicate individual) reported to have received at least one dose of blinded study vaccine. Additional safety assessments may be performed to account for adverse events that occur following administration of multiple study vaccines.
- For the immunogenicity analyses based on the FAS (Immunogenicity Subset): the immunogenicity data of the first randomization in the Immunogenicity Subset will be used in tables and figures. Note that the duplicate participants will be classified under the treatment allocation (active/placebo) of the first randomization in the study. For example, if a participant was first randomized in the general subset and received placebo, and later he or she was randomized in the immunogenicity subset and received active vaccine, then in this case, he or she will be classified as having received placebo. Additional listings may be provided with the full immunogenicity profile of all duplicate participants (eg: not restricted to the Immunogenicity Subset).

5.2. Participant Dispositions

The following disposition categories will be summarized by study vaccination group and overall:

- participants screened
- participants screen failed including the main reason for screen failure participants vaccinated and not randomized
- participants randomized and not vaccinated
- participants vaccinated with incorrect vaccine
- participants in the enrolled, randomized, FAS, PPE and PPI analysis sets (and reasons for not being in the PPE or PPI)
- participants who completed/discontinued study (for FAS and PPE)
- reasons for discontinuation or termination

Also, the number of participants and percentage per phase and per subset will be tabulated.

To provide transparency as to study vaccine exposure, a table with the number of individuals with study vaccination administration will be provided. This table will also include the number of individuals with multiple study vaccines administered (duplicate participants) that may have been administered different combinations of vaccination schemes (eg Active+Active, Active+Placebo,).

See [Appendix 4](#) for a description of the demographic and baseline characteristics that will be tabulated for the participants in the FAS, PPE, Safety Subset and PPI.

5.3. Primary Efficacy Endpoint Analysis

5.3.1. Definition of Endpoint

The analysis of the primary endpoint will evaluate:

the number of participants with at least 1 IED event, with microbiological confirmation in blood or other sterile sites, excluding IED cases with microbiological confirmation from urine only, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set.

The clinical case definition of IED is given in the [Appendix 2](#).

5.3.2. Estimands

5.3.2.1. Primary Estimand

The five primary estimand attributes applicable to the primary endpoint are defined by the following (per-protocol analysis):

- Population: Adults aged 60 years and older with a history of UTI in the past 2 years
- Study interventions: ExPEC9V compared to Placebo
- Endpoint:
 - First IED caused by ExPEC9V O-serotypes with microbiological confirmation in blood or other sterile sites, excluding IED cases with microbiological confirmation from urine only, with onset at least 29 days after vaccination (from Day 30)
- Intercurrent event:
 - Death (non-IED): while alive
 - Major protocol deviations impacting efficacy: while without major protocol deviations (e.g. receiving immune-modifying drugs or vaccines, see [Appendix 5](#)). Efficacy data from participants with major protocol deviations potentially impacting efficacy will be excluded from the date of the major protocol deviation onwards.

- Population-level summary: Vaccine Efficacy is defined by $(1-RR) \times 100\%$, where RR is the incidence rate ratio of IED in the ExPEC9V group relative to the placebo group (see [Appendix 8](#)).

5.3.2.2. Supplementary Estimand

The following attributes for the supplementary estimand will be changed compared to the attributes of the primary estimand (FAS analysis):

- Endpoint:
 - First IED caused by ExPEC9V O-serotypes with microbiological confirmation in blood or other sterile sites, excluding IED cases with microbiological confirmation from urine only, with onset from vaccination (from Day 1)
- Intercurrent event:
 - Death (non-IED): while alive
 - Major protocol deviations impacting efficacy: treatment policy. Major protocol deviations impacting efficacy will be ignored.

5.3.3. Analysis Methods

For the Main Efficacy Analyses (including only events with onset on or before 13 February 2025), 3 parallel analyses will be done for the primary endpoint:

1. based on the IEAC assessment (PPE)
2. based on the Clinical Case definition (PPE)
3. based on the Clinical Case definition (FAS)

As serotyping for IED events that occur after 13 February 2025 is not intended to be performed, an End-of-Study efficacy analysis will not be done for the primary endpoint.

Vaccine efficacy will be estimated by 1 minus the ratio of the incidence rate of cases of IED in the ExPEC9V group compared to the placebo group (see [Appendix 8](#)). An exact Poisson regression, stratified for the stratification factors used for randomization, i.e., age (≥ 60 to < 65 years of age versus ≥ 65 to < 75 years of age versus ≥ 75 years of age) and the presence of at least one risk factor for cUTIs at baseline (see [Appendix 4](#)) will be fitted with the event rate, defined as the number of cases over the follow-up time (offset) as dependent variable and the study vaccination group as independent variable. VE is calculated by exponentiating the coefficient corresponding to the vaccination group. The exact 2-sided $(1-2\alpha)\%$ CI of VE will be constructed for the interim and final analyses. The nominal $\alpha(s)$ will be defined at the interim and final analyses using the α -spending approach (see Section [5.8.3](#)). If the lower limit of the CI is higher than 20%, this will suggest that VE for the primary endpoint has been demonstrated.

The follow-up time is taken into account. For participants with an IED event, the follow-up time is defined as the time between vaccination and the occurrence of the first event (calculated as date of the occurrence of the first event – date of vaccination + 1). For participants without an IED event, it is the time between vaccination and last visit (or last contact for participants that discontinued the study, including lost to follow-up; calculated as date of last visit/contact – date of vaccination + 1). For the main efficacy analyses calculation of follow-up time, ongoing participants with no events with onset on or before 13 February 2025 will be censored at 13 February 2025.

The onset of an IED event will be defined as the date of the first culture with microbiological confirmation of *E. coli* for a participant with signs or symptoms of suspected IED.

If participants have multiple IED events during their follow-up period, the analysis of the primary endpoint will only take the first ExPEC9V IED with microbiological confirmation in blood or other sterile sites into account, ignoring ExPEC9V IED events with only microbiological confirmation in urine. The second secondary endpoint will evaluate all IEDs (including multiple IEDs per participant) caused by ExPEC9V O-serotypes (see Section 5.4 for a description of the other secondary endpoints).

IEDs are temporally bounded between Day 1 (beginning) and Day 29 post-IED (or the day of resolution of IED signs/symptoms, whichever comes first). IED events cannot overlap in time. If new clinical suspicion of an IED occurs within this defined window (between Day 1 and Day 29/Day of IED resolution), clinical details will be reported to the adjudicators as part of the initial IED case, it will not be considered a new IED event.

In case of multiple isolates per episode, all isolates will be characterized, and all results will be taken into account for final IED case evaluation (see also Section 5.1.4).

For the primary endpoint, efficacy analyses will be performed on the PPE incorporating the randomization stratification factors (age and presence of at least one risk factor for cUTI) as collected in the database (which may differ to the strata as recorded in IWRS). See Table 15 in Appendix 4 for the risk factors that will be taken into account for the analysis.

The number of events and the incidence rate for each population (#of events/#person-years of follow-up) will be tabulated by study vaccination group.

The time to onset of the first IED event will be graphically summarized using cumulative incidence (Kaplan-Meier) plots in the PPE from Day 30 and in the FAS from Day 1.

To assess potential time-effects of VE, the method of Gilbert et al. (2002)⁹ will be used (if the number of events allows to perform the estimation) to estimate VE over time with pointwise and simultaneous 95% CI. VE is based on the log hazard ratio over time ($VE = 1 - \exp[\log \text{hazard ratio}]$) and the hazard function in each study vaccination group is estimated using nonparametric kernel estimates. The window of the kernel smoother will be based on the number of events observed.

The study will be successful if the primary endpoint has shown statistical significance.

Additional supportive analyses will include the following:

- An analysis using an exact binomial CI, based on the PPE set and FAS.
- An analysis where VE will be estimated with an associated two-sided confidence interval (Wald test) based on the hazard ratio obtained from a Cox proportional hazards regression model. The analysis will be stratified for age and presence of at least one risk factor for cUTI (as collected, see [Table 15](#) in [Appendix 4](#)).
- An analysis based on the PPE set with the stratification factors age and presence of at least one risk factor for cUTI as stratified (instead of as collected) in case there are many discrepancies between as stratified and as collected.

5.4. Secondary Efficacy Endpoints Analysis

5.4.1. Key Confirmatory Secondary Efficacy Endpoints

5.4.1.1. Definition of Endpoints

For the definition of a confirmed IED/Pyelonephritis/UTI, see [Section 6.2](#).

For a confirmed IED/Pyelonephritis/UTI to be caused by ExPEC9V O-serotypes, the O-serotyping by the agglutination assay has to be available and the IED/Pyelonephritis/UTI has to be confirmed as being O1, O2, O4, O6, O15, O16, O18, O25, or O75 positive. For the secondary endpoints caused by *E. coli* (see [Table 6](#) endpoints 9, 10 and 11), local lab confirmation of *E. coli* (without serotyping performed by the central lab) is sufficient.

As the primary endpoint also the secondary endpoints efficacy analyses will be performed on the PPE incorporating the randomization stratification factors (age and presence of at least one risk factor for cUTI) as collected in the database (which may differ to the strata as recorded in IWRS). See [Table 15](#) in [Appendix 4](#) for the risk factors that will be taken into account for the analysis.

Table 6: List of Secondary Endpoints

Endpoint	Analysis Variable
1. First IED event ^{a,d} with microbiological confirmation from blood, other sterile ^b sites or urine, caused by ExPEC9V O--serotypes.	The total number of IED events with microbiological confirmation in blood, other sterile sites or urine, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
2. All IEDs ^d (including multiple IEDs per participant) caused by ExPEC9V O-serotypes	The total number of IED events with microbiological confirmation in blood, other sterile sites or urine, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
3. First hospitalized IED event ^d caused by ExPEC9V O-serotypes	The number of participants with at least 1 hospital admission as inpatient due to an IED event with microbiological confirmation in blood, other sterile sites or urine, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
4. First IED event ^d meeting criteria for sepsis caused by ExPEC9V O-serotypes	The number of participants with at least 1 IED event with microbiological confirmation in blood, other sterile sites or urine, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 documented with an acute change in total Sequential Organ Failure Assessment (SOFA) score of ≥ 2 points from baseline and deemed secondary to the IED with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
5. First bacteremic IED event caused by ExPEC9V O-serotypes	The number of participants with at least 1 IED event, with microbiological confirmation in blood, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
6. First pyelonephritis event caused by ExPEC9V O-serotypes	The number of participants with at least 1 pyelonephritis event, with microbiological confirmation in urine, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE
7. First UTI ^c caused by ExPEC9V O-serotypes	The number of participants with at least 1 UTI event, with microbiological confirmation in urine, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
8. All UTIs ^c (including multiple UTIs per participant) caused by ExPEC9V O-serotypes	The total number of UTI events with microbiological confirmation in urine, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
9. First IED event ^d caused by <i>E. coli</i>	The number of participants with at least 1 IED event with microbiological confirmation in blood, other sterile sites or urine, caused by <i>E. coli</i> with onset at least 29 days after

Table 6: List of Secondary Endpoints

	vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
10. First pyelonephritis event caused by <i>E. coli</i>	The number of participants with at least 1 pyelonephritis event, with microbiological confirmation in urine, caused by <i>E. coli</i> with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
11. First UTI ^c caused by <i>E. coli</i>	The number of participants with at least 1 UTI event, with microbiological confirmation in urine, caused by <i>E. coli</i> with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set

5.4.1.2. Estimands

5.4.1.2.1. Key Secondary Estimand

The five estimand attributes for each of the endpoints are defined by the following:

- Population: Adults aged 60 years and older with a history of UTI in the past 2 years
- Study interventions: ExPEC9V compared to Placebo
- Endpoint:
 - First IED event caused by ExPEC9V O-serotypes with microbiological confirmation in blood, other sterile sites or urine,
 - Number of IED events in each participant caused by ExPEC9V O-serotypes with microbiological confirmation in blood, other sterile sites or urine
 - First hospitalized IED event caused by ExPEC9V O-serotypes with microbiological confirmation in blood, other sterile sites or urine
 - First IED event meeting criteria for sepsis caused by ExPEC9V O-serotypes with microbiological confirmation in blood, other sterile sites or urine
 - First bacteremic IED event caused by ExPEC9V O-serotypes with microbiological confirmation in blood
 - First pyelonephritis event caused by ExPEC9V O-serotypes with microbiological confirmation in urine
 - First UTI caused by ExPEC9V O-serotypes with microbiological confirmation in urine
 - Number of UTI events in each participant caused by ExPEC9V O-serotypes with microbiological confirmation in urine
 - First IED event caused by *E. coli* with microbiological confirmation in blood, other sterile sites or urine

- First pyelonephritis event caused by *E. coli* with microbiological confirmation in urine
- First UTI caused by *E. coli* with microbiological confirmation in urine
- For these endpoints, events will be taken into account with onset at least 29 days after vaccination (from Day 30).
- Intercurrent event:
 - Death (non-IED): while alive
 - Major protocol deviations impacting efficacy: while without major protocol deviations (e.g. receiving immune-modifying drugs or vaccines, see [Appendix 5](#)). Efficacy data from participants with major protocol deviations potentially impacting efficacy will be excluded from the date of the major protocol deviation onwards.
- Population-level summary: Vaccine Efficacy (see Section [5.3.2.1](#)).

5.4.1.2.2. Supplementary Estimand

The following attributes for the supplementary estimand will be changed compared to the attributes of the key secondary estimand (FAS analysis):

- Endpoint: events will be taken into account with onset from vaccination (from Day 1)
- Intercurrent event:
 - Death (non-IED): while alive
 - Major protocol deviations impacting efficacy: treatment policy. Major protocol deviations impacting efficacy will be ignored.

5.4.1.3. Analysis Methods

A similar Poisson regression model as for the primary endpoint will be used for the analyses of the key secondary efficacy endpoints.

Following the futility announcement, the Main Efficacy Analyses (including only events with onset on or before 13 February 2025), 3 parallel analyses will be done for the secondary endpoints related to IED and pyelonephritis:

1. based on the IEAC assessment (PPE)
2. based on the Clinical Case definition (PPE)
3. based on the Clinical Case definition (FAS)

For the endpoints related to UTI, 2 parallel analyses will be done:

1. based on the Clinical Case definition (PPE)
2. based on the Clinical Case definition (FAS)

Note also that for UTIs, event reporting was discontinued in Protocol Amendment 8, and at the time of study discontinuation, the majority of study sites had implemented PA8 already.

As serotyping of events that occur after 13 February 2025 is not planned, the End-of-Study efficacy analysis cannot be done for the 9V events, but only for IED and pyelonephritis according to the CCD that are “caused by *E. coli*”. This analysis will be done for the FAS.

The follow-up time for IED or Pyelonephritis related endpoints is defined as the time between day of vaccination until the onset day of the first endpoint of interest or until the day of the last visit or last contact for participants that discontinued the study, including lost to follow-up, whichever comes first. For the Main Efficacy Analyses calculation of follow-up time, ongoing participants with no events with onset on or before 13 February 2025 will be censored at 13 February 2025.

As of Protocol Amendment 8, site-specific procedures concerning UTI/ABP have been discontinued, therefore the analysis of the UTI related endpoints (secondary endpoints 7 and 11), will not consider the UTIs occurring after the implementation of Protocol Amendment 8. Further, the participants enrolled after Protocol Amendment 8 will not be included in this analysis. The follow-up time for these endpoints is defined as follows: for participants experiencing a UTI before the implementation of Protocol Amendment 8, the follow-up time for UTI is taken from the date of vaccination till the onset date of his/her first UTI episode. For participants without a UTI episode, the follow-up time is taken from the date of vaccination until the date of the last visit or last contact for participants that discontinued the study or date of implementation of Protocol Amendment 8 in the participant’s country/territory (whichever comes first). A listing with the UTIs reported after the implementation of PA8 may be provided.

For the secondary endpoint where all IEDs (including multiple IEDs per participant) are considered, the follow-up time is defined for all participants as the time between vaccination and last visit or last contact for participants that discontinued the study, including lost to follow-up (calculated as date of the last visit/contact – date of vaccination + 1). For the Main Efficacy Analyses calculation of follow-up time for all ongoing participants (with or without an event), the end date will be set at 13 February 2025.

For the secondary endpoint where all UTIs (including multiple UTIs per participant) are considered, the follow-up time is defined for all participants as the time between vaccination and last visit or last contact for participants that discontinued the study, including lost to follow-up (calculated as date of the last visit/contact – date of vaccination + 1) or date of implementation of Protocol Amendment 8 in the participants’ country/territory (whichever comes first).

The onset of an IED/Pyelonephritis/UTI event will be defined as the date of the first culture with microbiological confirmation of *E. coli* for a participant with signs or symptoms of UTI or suspected IED (see Section 5.3.3 for the definition of onset of overlapping IED events).

The severity and outcome of IED cases will be assessed by recording whether the participant experienced sepsis or septic shock (or neither of those) and by recording any adverse sequelae of IED (e.g. residual organ impairment or accelerated progression of pre-existing chronic conditions). Whether the participant meets criteria for sepsis or septic shock will be verified and confirmed by the IEAC. With discontinuation of IEAC case review, sepsis and septic shock will be defined programmatically (see Appendix 2) for the Main Efficacy Analysis based on the clinical case definitions. The number of confirmed sepsis or septic shock cases among the IED events will be tabulated.

As with IED events, pyelonephritis events are also temporally bounded between Day 1 (beginning) and Day 29 post-UTI (or the day of resolution of UTI signs/symptoms, whichever comes first) (end). Pyelonephritis events cannot overlap in time. If new clinical suspicion of a pyelonephritis occurs between these defined windows (between Day 1 and Day 29/Day of UTI resolution), clinical details will be reported to the adjudicators as part of the initial pyelonephritis case, it will not be considered a new pyelonephritis event.

5.4.2. Supporting Secondary Efficacy Endpoints

IED-related and all-cause mortality rate and IED case fatality rate will also be estimated by vaccination group. IED-related and all-cause mortality rate will be calculated as the number of IED-related or any deaths divided by the total number of person-years of follow-up for all participants in the FAS. The IED case fatality rate (defined as the percentage of IED-related deaths confirmed by the IEAC, among the IED cases) will also be explored. Analysis of the supporting secondary efficacy endpoints will be included in the End of Study Efficacy analysis for the FAS.

With discontinuation of IEAC case review, deaths attributable to IED will be determined by PI assessment. To be considered an IED-related death, the investigator must respond affirmatively (“YES”) to the following questions in the eCRF:

- The event qualifies as: “a suspected IED due to *E. coli* infection only” OR “a suspected IED due to mixed pathogens including *E. coli*”, and
- “In the investigator’s opinion, did the suspected IED or systemic infection lead to death?”

The cause of death will be tabulated by study vaccination group.

If fewer than 6 IED-related deaths are observed, no statistical hypothesis testing of IED-related deaths will be performed.

5.5. Exploratory Efficacy Endpoints Analysis

As the Sponsor elected to terminate the study early due to futility, changes to the exploratory objectives may be performed, either analyses will not be done or may be reduced in scope. The analysis will be conducted using PPE (by applying solely the programmatic CCD) and/or FAS

5.5.1. Definition of Endpoints

Table 7: Definition of Exploratory Endpoints

Endpoint	Analysis Variable
1. First IED event caused by ExPEC9V O-serotypes showing resistance to ≥ 1 , ≥ 2 , and ≥ 3 antibiotic classes in the microbiological profile	The number of participants with at least 1 IED event, with microbiological confirmation in blood, other sterile sites or urine, caused by ExPEC9V O-serotypes showing resistance to ≥ 1 , ≥ 2 , and ≥ 3 antibiotic classes in the microbiological profile with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
2. First uncomplicated UTI event caused by ExPEC9V O-serotypes	The number of participants with at least one uncomplicated UTI event with microbiological confirmation in urine caused by ExPEC9V O-serotypes with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
3. First complicated UTI event caused by ExPEC9V O-serotypes	The number of participants with at least one complicated UTI event with microbiological confirmation in urine caused by ExPEC9V O-serotypes with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
4. First recurrent UTI (rUTI) event caused by ExPEC9V O-serotypes	The number of participants with at least one rUTI event with microbiological confirmation in urine caused by ExPEC9V O-serotypes with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
5. First recurrent uncomplicated UTI event caused by ExPEC9V O-serotypes	The number of participants with at least one recurrent uncomplicated UTI event with microbiological confirmation in urine caused by ExPEC9V O-serotypes with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
6. First ABP event caused by ExPEC9V O-serotypes	The number of participants with at least one ABP event with microbiological confirmation in urine caused by ExPEC9V O-serotypes with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
7. First ABP event caused by <i>E. coli</i>	The number of participants with at least one ABP event with microbiological confirmation in urine caused by <i>E. coli</i> with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
8. First invasive disease event caused by <i>P. aeruginosa</i>	The number of participants with at least one event of systemic bacterial infection confirmed by signs and symptoms as per IED definition and with samples positive for <i>P. aeruginosa</i> with no serotyping required with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set

Table 7: Definition of Exploratory Endpoints

Endpoint	Analysis Variable
9. First <i>P. aeruginosa</i> UTI event	The number of participants with at least one UTI event with samples positive for <i>P. aeruginosa</i> with no serotyping required with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
10. First <i>P. aeruginosa</i> ABP event	The number of participants with at least one ABP event with samples positive for <i>P. aeruginosa</i> with no serotyping required with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
Additional exploratory endpoints include the following IED related secondary and exploratory endpoints where the source of the microbiological confirmation is only from blood or other sterile sites, excluding IED cases with microbiological confirmation from urine only	
11. All IEDs (including multiple IEDs per participant) caused by ExPEC9V O-serotypes	The total number of IED events with microbiological confirmation in blood or other sterile sites, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
12. First hospitalized IED event caused by ExPEC9V O-serotypes	The number of participants with at least 1 hospital admission as inpatient due to an IED event with microbiological confirmation in blood or other sterile sites, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
13. First IED event meeting criteria for sepsis caused by ExPEC9V O-serotypes	The number of participants with at least 1 IED event with microbiological confirmation in blood or other sterile sites, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25 and O75 documented with an acute change in total Sequential Organ Failure Assessment (SOFA) score of ≥ 2 points from baseline and deemed secondary to the IED with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
14. First IED event caused by <i>E. coli</i>	The number of participants with at least 1 IED event with microbiological confirmation in blood or other sterile sites, caused by <i>E. coli</i> with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set
15. First IED event caused by ExPEC9V O-serotypes showing resistance to ≥ 1 , ≥ 2 , and ≥ 3 antibiotic classes in the microbiological profile	The number of participants with at least 1 IED event, with microbiological confirmation in blood or other sterile sites, caused by ExPEC9V O-serotypes showing resistance to ≥ 1 , ≥ 2 , and ≥ 3 antibiotic classes in the microbiological profile with onset at least 29 days after vaccination (from Day 30) in the ExPEC9V group compared to the placebo group in the PPE set

5.5.2. Estimands

5.5.2.1. Exploratory Estimand

The five estimand attributes for each of the endpoints are defined by the following:

- Population: Adults aged 60 years and older with a history of UTI in the past 2 years

- Study interventions: ExPEC9V compared to Placebo
- Endpoint:
 1. First IED event, with microbiological confirmation in blood, other sterile sites or urine, caused by ExPEC9V O-serotypes showing resistance to ≥ 1 , ≥ 2 , and ≥ 3 antibiotic classes in the microbiological profile
 2. First uncomplicated UTI event with microbiological confirmation in urine caused by ExPEC9V O-serotypes
 3. First complicated UTI event with microbiological confirmation in urine caused by ExPEC9V O-serotypes
 4. First rUTI event with microbiological confirmation in urine caused by ExPEC9V O-serotypes
 5. First recurrent uncomplicated UTI event with microbiological confirmation in urine caused by ExPEC9V O-serotypes
 6. First ABP event with microbiological confirmation in urine caused by ExPEC9V O-serotypes
 7. First ABP event with microbiological confirmation in urine caused by *E. coli*
 8. First event of systemic bacterial infection confirmed by signs and symptoms as per IED definition and with samples positive for *P. aeruginosa* with no serotyping required
 9. First UTI event with samples positive for *P. aeruginosa* with no serotyping required
 10. First ABP event with samples positive for *P. aeruginosa* with no serotyping required
 11. Number of IED events in each participant caused by ExPEC9V O-serotypes with microbiological confirmation in blood or other sterile sites
 12. First hospitalized IED event caused by ExPEC9V O-serotypes with microbiological confirmation in blood or other sterile sites
 13. First IED event meeting criteria for sepsis caused by ExPEC9V O-serotypes with microbiological confirmation in blood or other sterile sites
 14. First IED event caused by *E. coli* with microbiological confirmation in blood or other sterile sites
 15. First IED event, with microbiological confirmation in blood or other sterile sites, caused by ExPEC9V O-serotypes showing resistance to ≥ 1 , ≥ 2 , and ≥ 3 antibiotic classes in the microbiological profile

For these endpoints, events will be taken into account with onset at least 29 days after vaccination (from Day 30).

- Intercurrent event:
 - Death (non-IED): while alive
 - Major protocol deviations impacting efficacy: while without major protocol deviations (e.g. receiving immune-modifying drugs or vaccines, see [Appendix 5](#)). Efficacy data from participants with major protocol deviations potentially impacting efficacy will be excluded from the date of the major protocol deviation onwards.
- Population-level summary: Vaccine Efficacy (see Section [5.3.2.1](#)).

5.5.2.2. Supplementary Estimand

The following attributes for the supplementary estimand will be changed compared to the attributes of the exploratory estimand (FAS analysis):

- Endpoint: events will be taken into account with onset from vaccination (from Day 1)
- Intercurrent event:
 - Death (non-IED): while alive
 - Major protocol deviations impacting efficacy: treatment policy. Major protocol deviations impacting efficacy will be ignored.

5.5.3. Analysis Methods

For all exploratory efficacy endpoints, an exact Poisson regression model as described for the primary efficacy endpoint will be performed based on the PPE set and on the FAS.

The onset of an IED/UTI/ABP event will be defined as the date of collection of the first culture with microbiological confirmation of *E. coli* for a participant with signs or symptoms of UTI/ABP or suspected IED.

The follow-up time will be calculated as described in Section [5.4.2](#).

Serotyping confirmation of a case is done by the agglutination assay (see Section [5.4.1](#)).

As the Sponsor elected to terminate the study early due to futility, comprehensive antimicrobial susceptibility testing may not be completed, and assessments may be performed using available data, and may be limited to IED events (PPE using CCD and/or FAS).

For the exploratory endpoints 1 and 15 in [Table 7](#), each isolate will be assessed for resistance to the 21 antibiotics listed in [Table 17](#), using the breakpoints for susceptible, intermediate, and resistant described in the CLSI M100 v31 ([Appendix 11: Antimicrobial Susceptible Testing](#)). As amoxicillin and plasmocin do not have breakpoints for Enterobacterales defined in CLSI M100 v31, antibiotic susceptibility to these two antibiotics will be reported only as their MIC determination.

The 21 antibiotics belong to 12 antibiotic classes, (Table 17). For antibiotic classes for which there is more than one antibiotic tested (eg: carbapenem testing, which includes both imipenem and meropenem), if the isolate is resistant to at least one antibiotic within the class, it will be defined as resistant to that antibiotic class. All 12 antibiotic classes will be used to assess the number of isolates that are fully susceptible and resistant to ≥ 1 , ≥ 2 and ≥ 3 antibiotic classes. Because the breakpoints for colistin do not allow for a classification of "susceptible", an analysis of isolates fully susceptible to all antibiotic classes excluding polymyxin may also be performed.

Multidrug resistant (MDR) *E. coli* is defined as non-susceptibility to ≥ 3 antibiotic classes¹³. Of the 12 antibiotic classes tested, only 10 antibiotic classes are included in the definition of MDR *E. coli* (Table 17). Non-susceptibility to at least one antibiotic within at least three MDR-defined classes will be used to assess the number of isolates that are MDR. Because the breakpoints for colistin do not allow for a classification of "susceptible", an analysis of isolates non-susceptible to ≥ 1 antibiotic class excluding polymyxin may also be performed.

Antibiotics are also grouped based on the WHO Model List of Essential Medicines²³ (Access, Watch, Reserve; Table 17). Additional exploratory analyses related to drug resistance may be performed including, but not limited to, the percent of isolates resistant to WHO Model List of Essential Medicines²³ "Access", "Reserve", and/or "Watch" group antibiotics and the efficacy of the ExPEC9V vaccine within the WHO Model List of Essential Medicines²³ "Access", "Reserve", and/or "Watch" group antibiotics.

Clinical case definitions for uncomplicated UTI, complicated UTI, rUTI, recurrent uncomplicated UTI and ABP are available in Appendix 2.

Participants with UTI/ABP according to the clinical case definition, but with urine samples positive for *P. aeruginosa* (colony forming units [CFU] $\geq 10^5$ /mL) will be reported to have *P. aeruginosa* UTI/ABP. Participants with signs and symptoms of systemic bacterial infection (at least 1 SIRS criteria or an acute change in SOFA score of 2 or more) and with blood or sterile site cultures positive for *P. aeruginosa*, will be defined as invasive disease due to *P. aeruginosa*. Additionally, participants with signs and symptoms with an acute change in SOFA score of 2 or more and with urine culture positive for *P. aeruginosa*, will be defined as invasive disease due to *P. aeruginosa*. No serotyping will be performed for *P. aeruginosa*. Cases with mixed pathogens in the culture results including *P. aeruginosa*, will not be considered for this exploratory endpoint.

5.6. Safety

Safety data will be analyzed per SAP (FAS), throughout the entire study period. The analysis of solicited AEs will be restricted to a subset (Safety Subset) of the FAS. The key safety assessments include adverse events (AEs), physical examinations and vital signs. No formal statistical testing of safety data is planned.

The safety analysis for adverse events will include the descriptive summary of solicited local Adverse Events (AEs), solicited systemic AEs, unsolicited AEs, and SAEs. The overall frequencies per group as well as frequencies according to severity and duration will be calculated

for solicited and unsolicited AEs. Denominator for the percentages is the number of participants in the considered population of the specified group (incidence per 100 participants).

For duplicate participants, separate tables and listings will be provided, as appropriate.

5.6.1. Adverse Events

5.6.1.1. Definitions

Solicited AEs within 14 days following vaccination period shown in the tables and listings will be based on the overall assessment of the investigator, documented in the SDTM Clinical Events (CE) domain. ADaM will be based on CE. For unsolicited AEs, only the AEs within the 29-day period following vaccination will be presented in the safety tables. All other collected unsolicited adverse events will be presented through listings. SAEs will be recorded until the end of Day 181 and only related SAEs related to study interventions, study procedures or non-investigational sponsor products will be recorded from Day 182 until the end of the study. Therefore, the safety tables will report all SAEs from vaccination until Day 181, irrespective of relationship. Tables for related SAEs will cover the whole study period. The SAE listings will include SAEs from the whole study period. SAEs may be reported throughout the whole study period in certain countries/territories per local regulatory requirements.

The number of participants in the Safety Subset will be used as a denominator for the solicited AEs and the number of participants in the FAS will be used as a denominator for the unsolicited AEs and SAEs. Note that the number of participants in the Safety Subset will also be used as a denominator for the unsolicited AEs, as a sensitivity analysis.

The verbatim terms used in the eCRF by investigators to identify AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

Solicited local (at injection site) symptoms will be by definition considered as related to the study vaccine.

The severity of the AEs will be graded as grade 1 to 4. Solicited events that are graded less than grade 1, are not considered as AE. In case no grades are available, the grading of the solicited events will occur according to the grading listed in [Appendix 7](#).

5.6.1.2. Analysis of Adverse Events

Number and percentage of participants 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 (administration site, systemic) and Preferred Term.

For solicited AEs, the following tables will be provided: summary, by worst severity grade, at least grade 3, related (systemic only), time to onset (in days) and duration (in days) for all events. Note: Duration is defined as number of days from the start date of the event until the date of the resolution of the event (i.e., resolution date – start date + 1 day). The time to first onset is defined as (date of first onset – reference date + 1). The reference date is the vaccination date. The time to first onset

of solicited AE is defined as early if the solicited AE occurs on or before Day 5; otherwise, the time to first onset is late.

For unsolicited AEs, the following tables will be provided: summary table (including SAEs, fatal outcome and discontinuation), all events, at least grade 3, related and SAE. Unsolicited AEs may also be presented for Standardized MedDRA Queries (SMQs) to facilitate review of certain classes of unsolicited AEs. The unsolicited AEs tables (summary table, all events, at least grade 3 and related) will also be generated for participants in the Safety Subset.

All IED and UTI/ABP events that meet the definition of an SAE will be reported as SAEs, regardless of whether they are protocol-specific assessments. IED and UTI/ABP events reported as SAEs during the course of the study, will be excluded from any SAE analyses in the clinical study report if they are subsequently found to be caused by *E. coli* or *P. aeruginosa* infection and confirmed to meet the clinical case definition for the primary or secondary efficacy endpoints. These events will be removed at the analysis level from the SAE listings and tables and presented separately in the clinical study report.

Listings and/or participant narratives will be provided as appropriate, for those participants who die, discontinue study due to an AE, experience an SAE, and for duplicate participants.

5.6.1.3. Phase Allocation of Adverse Events

Solicited events are always allocated to the Post-vaccination Period (see [Table 4](#)).

Step 1: Allocation of events to the Post-vaccination Period:

Adverse events in the SDTM database are allocated to phases/periods based on their start date/time (in the remainder of this SAP, the term period will mean any Phase or Period as defined in [Table 4](#), except if specific reference is made to “Post-vaccination Period”). 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 end date, the date is imputed by the cut-off date of the analysis for participants still ongoing in the study, and by the end date of the last period for participants who discontinued or completed the trial. In case of a completely missing start date, the event is allocated to the Post-vaccination Period, except if the end date of the AE falls before the start of the Post-vaccination Period.

Step 2: Combination of events:

Overlapping/consecutive events are defined as events of the same participant 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 phases/period - Screening or Follow-up (defined as non-active periods) - followed by an AE in - Post-vaccination Period (defined as active period) - they are allocated to their respective phases/period 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 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 consecutive 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 duration. All related attributes to the AE/phase/period should also be consistent with the new event.
4. In case a non-active period is followed by another non-active period, and the overlapping/consecutive events start in both periods, they are allocated to the first period and 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. All related attributes to the AE/phase/period should also be consistent with the new event.

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 phase/period allocation), this date is also considered as a complete date.
3. Time is not considered when determining overlap of events.

5.6.1.4. Missing Data

Missing safety data will not be imputed. Participants who do not report an event will be considered as participants without an event. An AE with a missing severity or relationship will be considered as an AE reported but will be considered as not reported for the severity or relationship. 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 of at least grade 3. The analysis of solicited AEs will include the safety data as determined by the investigator.

5.6.2. Vital Signs and Physical Examination Findings

Weight, height and body mass index will be summarized at baseline using descriptive statistics.

Vital signs including temperature, pulse/heart rate, respiratory rate and systolic and diastolic sitting blood pressure will be measured both before vaccination and after the end of the 30-minute observation period. If any clinically significant changes in vital signs are observed, they will be reported as an AE. If any clinically significant changes in vital signs are observed pre-vaccination, they will be reported as medical history.

A full physical examination will be carried out at screening for all participants. At all other visits, a targeted (abbreviated and symptom-directed) examination will be performed for participants in the Safety Subset at the discretion of the investigator, based on clinically relevant issues, clinically relevant symptoms and medical history.

Any clinically relevant abnormalities or changes in severity observed during the review of body systems should be documented in the eCRF as an AE (post-vaccination). Consequently, no physical examination (post-vaccination) specific table or listings will be generated.

The full physical examination at baseline will be documented in the eCRF and summarized in a table and listing.

5.7. Other Analyses

5.7.1. Immunogenicity

Blood samples are to be collected (if feasible) from all participants for immunogenicity at Day 1 (pre-vaccination) and Day 30. Blood will also be drawn from participants at the time of diagnosis of suspected IED and at one-year post-IED identification (or at the end of study whichever comes first). These blood samples will be analyzed to identify a correlate of protection for the vaccine mediated immune response associated with vaccine efficacy (see Section 5.7.1.5).

For participants in the Immunogenicity Subset, there will be additional immunogenicity assessments at Days 181, 366 (Year 1), 731 (Year 2), 1096 (Year 3) and 1461 (Year 4) and at the time of diagnosis of suspected UTI or ABP, which occurred prior to Protocol Amendment 8 implementation. Following the announcement of study futility, blood collection for immunogenicity assessments is not in the best interest of the participant from a risk/benefit perspective. The Sponsor has instructed sites accordingly to discontinue blood draws for immunogenicity during the remainder of study follow up.

The immunogenicity analysis will use the PPI set and the FAS populations. Sensitivity analysis may be performed by repeating the analysis on FAS (restricted to participants in the Immunogenicity Subset). The immunogenicity interim analysis that will be performed on the first 200 vaccinated participants in the study will be performed on the FAS, (participants with major protocol deviations expecting to impact the immunogenicity outcomes may be excluded).

As the Sponsor elected to terminate the study early due to futility, immunogenicity assessments will be performed using available immunogenicity data (ECL and MOPA). Therefore, the immunogenicity data of the first 200 participants may be combined with the data of the immunogenicity subset participants in the analysis.

5.7.1.1. Secondary Endpoints

ExPEC9V serotype antibodies will be measured by multiplex ECL-based immunoassay and MOPA assays. The EPA is measured by multiplex ECL-based immunoassay only.

The immunogenicity endpoints are

- 1) Total serum immunoglobulin G (IgG) antibody titers determined by multiplex ECL-based immunoassay. (The reported values for the ECL assay are arbitrary units/ml but are expressed as titer to align with historical documentation.)
- 2) Functional antibody titers determined by MOPA

As the Sponsor elected to terminate the study early due to futility, immunogenicity assessments will be performed using available immunogenicity data (ECL and MOPA).

5.7.1.2. Parameters

The following measures of immunogenicity will be evaluated at all immunogenicity timepoints.

- 1) Proportion of participants with a ≥ 2 -fold and ≥ 4 -fold increase in serum antibody titers from Day 1 (pre-vaccination)
- 2) Geometric mean titer (GMT)
- 3) Geometric mean of fold change from baseline (Geometric Mean Ratio, GMR), calculated from the ratio of post baseline to baseline value (pre-vaccination).

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

No statistical technique will be used to impute missing immune response data. If a participant's data are missing for any reason, that participant will not be included in that portion of the analysis.

Values below the lower limit of quantification (LLOQ) will be treated differently according to the statistical analysis. For the calculation of GMT, titer values below LLOQ will be imputed to $\frac{1}{2}$ of the LLOQ. For the calculation of GMR, titer values below LLOQ will be imputed to LLOQ.

Values above the upper limit of quantification (ULOQ) will be imputed to ULOQ.

Both LLOQ and ULOQ values will be available in the clinical immunogenicity database.

5.7.1.4. Immune Response Analysis

Analysis of the immunogenicity variables will be performed on the PPI set, focusing on the Immunogenicity Subset. Sensitivity analysis may be performed by repeating the analysis on the FAS (restricted to participants in the Immunogenicity Subset).

No formal hypothesis on immunogenicity will be tested.

The analysis will be presented by serotype, timepoint and study vaccination group (ExPEC9V dose and placebo).

The percentage of participants reaching at least a 2- and 4-fold increase in antibody titer from baseline will be presented. A 2-sided 95% Clopper-Pearson CI will be provided for each percentage.

The following results will be calculated: n, GMT and GMR with the corresponding 95% CI of the actual values and fold changes from baseline, respectively. The GMT (GMR) is obtained by taking the anti- $\log_{10}$ of the mean (fold change) of the $\log_{10}$ transformed values. The CI is calculated as the back-transformed CI of the mean (fold change) of the $\log_{10}$ transformed values. The CI is used to describe the precision of the GMT and GMR. The CI will be based on the t -distribution. If less than 6 participants are observed for a certain subgroup, the CI will not be shown.

The Pearson correlation and a nonparametric version such as the Spearman correlation between multiplex ECL-based immunoassay and MOPA measurements will be calculated at each timepoint and for each serotype. In addition, at each timepoint the Deming regression will be explored to investigate the proportionality between the two assays by evaluating the 90% confidence interval of the slope.

Graphical representations of immunological parameters will be made as applicable. For the graphs, original values will be displayed on the $\log_{10}$ scale, even if they are below the LLOQ or above the ULOQ, if available.

The immunogenicity statistical analysis will also be repeated in the subgroup of the PPI with a high-risk profile ie (1) history of urosepsis or *E. coli* bacteremia prior to randomization or (2) history of inpatient hospitalization in the past 2 years prior to randomization or (3) presence at baseline of at least 1 risk factor for complicated UTI (any toxicity grade).

For duplicate participants, listings of all available immunogenicity data will be created.

5.7.1.5. Correlates of Protection

An attempt will be made to determine a serological correlate of protection for the ExPEC9V vaccine, based on an analysis of protection from IED, UTI/ABP and other endpoints as appropriate, and antibody titers from vaccinated and placebo participants. Statistical modelling of the vaccine-mediated immunogenicity with clinical efficacy will be performed to provide an estimate of this correlate, if feasible. The ability to determine a correlate of protection will be dependent on the specifics of the efficacy and immune responses to the vaccine. The correlate of protection will be investigated using several methods, which may include the A:B model.¹ Furthermore, the relationship between antibody titer data and VE will be presented graphically.^{1,9}

Based on the futility outcome the correlate of protection analysis will not be performed. Instead, an IED case-control study may be done to examine and compare the immunogenicity outcomes. Details of this case-control study will be described in a separate analysis plan.

5.7.2. Definition of Subgroups

The homogeneity of VE on the occurrence of the primary endpoint and the first secondary endpoint may be investigated across subgroups. In the subgroup analyses, estimates and 2-sided 95% CIs for VE will be provided for each subgroup as well as a test for interaction between the subgroup categories and the VE. If less than 6 events are observed for a certain subgroup, the VE will not be shown. These subgroup analyses may also be done for United States only. Given the premature discontinuation of the study due to futility, subgroups will contain fewer numbers of participants with IED events than intended in the original study design; thus, caution in interpretation of results is warranted.

Subgroup analyses for selected immunogenicity and safety tables may also be performed. The subgroups are listed below.

- 1) Sex: Female, Male, Unknown, Undifferentiated
- 2) Age
 - a. Age: ≥ 60 to < 65 years, ≥ 65 to < 75 years, ≥ 75 years
 - b. Age: ≥ 60 to < 65 years, ≥ 65 to < 75 years, ≥ 75 to < 85 years, ≥ 85 years
- 3) Body Mass Index (BMI; calculated as $[\text{weight in kg}]/[\{\text{height in m}\}^2]$): underweight $< 18.5 \text{ kg/m}^2$, normal 18.5 - $< 25 \text{ kg/m}^2$, overweight 25 - $< 30 \text{ kg/m}^2$, obese $\geq 30 \text{ kg/m}^2$
- 4) Race: American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or other Pacific Islander, White, Not Reported, Unknown, Multiple
- 5) Ethnicity: Hispanic or Latino, not Hispanic or Latino, Not Reported, Unknown
- 6) Country/Territory: Denmark, The Netherlands, Spain, United Kingdom, United States, Canada, Japan, Australia, New Zealand, South Korea, Czech Republic, France, Germany, Sweden, Israel, Taiwan, Colombia, Thailand, China, India
- 7) Region: North America [United States, Canada], South America [Colombia], Europe [Denmark, The Netherlands, Spain, United Kingdom, Czech Republic, France, Germany, Sweden], Asia & Pacific [Japan, South Korea, Australia, New Zealand, Israel, Taiwan, Thailand, China, India]
- 8) Risk factor for complicated UTI (with toxicity grade): Yes, No (See [Table 15](#) in [Appendix 4](#))
- 9) Risk factors for complicated UTI (any toxicity grade) (Yes,No) (See [Table 15](#) in [Appendix 4](#))
- 10) SPPB (fit, pre-frail, frail) (see Section [5.7.5.1](#))
- 11) Frailty Index (fit, pre-frail, frail) (see Section [5.7.5.2](#))
- 12) Hospitalization in the last 2 years (Yes, No)
- 13) History of urosepsis/*E. coli* bacteremia (Yes, No)
- 14) Hospitalization in the last 2 years or History of urosepsis/*E. coli* bacteremia or risk factors for complicated UTI (any toxicity grade) (Yes,No)
- 15) Use of prophylactic antibiotics at baseline (Yes,No)

Subgroup analyses for VE may also be visualized with forest plots.

Other demographics or baseline characteristics, e.g., the most prevalent medical conditions of interest (see [Table 14](#)), may also be included in these subgroup analyses and forest plots.

5.7.3. Medical Resource Utilization and Health Economics Analyses

The PPE set will be used to analyze IED and UTI/ABP events for MRU analyses including hospitalizations and the length of stay in the hospital, including ICU hospitalization and ICU length of stay, for IED, UTI or ABP events caused by ExPEC9V O-serotypes.

The number of inpatient hospitalizations and the length of stay in the hospital for IED and UTI/ABP events will be summarized by study vaccination group. This analysis will be done for all hospitalizations combined and for Intensive Care Unit [ICU], Hospital inpatient department [Ward] or Other.

The length of hospitalization stay will be defined as Discharge date – Admission date + 1.

As the Sponsor elected to terminate the study early due to futility, additional assessments of MRU and health economics will not be performed. The statistical analysis will include MRU data for IED events according to the CCD with onset on or before 13 February 2025 for the FAS (Main Efficacy Analyses).

5.7.4. Patient-Reported Outcomes

The analysis of the Patient-Reported Outcomes will be done on the PPE.

Electronic Patient-Reported Outcomes (ePRO) instruments will be completed by all participants at baseline (Pre Assessments and Pre Vaccination), Day 15, Day 30, Day 181, Day 366, Day 731, Day 1096, Day 1461 and the Early Exit visit. HRQoL will also be assessed for participants with confirmed IED cases at the time of diagnosis, at the day of discharge from the hospital or at 29 days after diagnosis and at Day 91 and Day 366 post-IED identification. For participants with confirmed UTI or ABP, HRQoL will be assessed at the time of diagnosis and 29 days after diagnosis. Two ePRO instruments will be completed by participants in the study to measure HRQoL: the Short Form-36 (SF-36) version 2 Acute and EQ-5D-5L. The SF-36 version 2 and EQ-5D-5L will be completed on the electronic clinical outcome assessment device.

For each of the questionnaires, the completion status, the way the questionnaire was taken (paper or device) and the reason for not taking the questionnaire (if applicable) may be summarized by study vaccination group and by timepoint.

Data from ePROs that are triggered erroneously will be ignored in the analysis.

As the Sponsor elected to terminate the study early due to futility, ePRO analysis will be limited to the main efficacy analysis period (data up to 13 February 2025) and performed with available data and for efficacy events according to the CCD (FAS), collected at pre-specified timepoints. In this analysis, only the first efficacy event for a participant will be considered.

5.7.4.1. SF-36

The SF-36 version 2 (acute) is a self-administered, 36-item questionnaire with a recall period for all items of 1 week.¹⁴ The SF-36 was developed as part of the Rand Health Insurance Experiment and consists of 8 multi-item scales:

- Limitations in physical functioning due to health problems (PF)
- Limitations in usual role activities due to physical health problems (RP)
- Bodily pain (BP)
- General mental health, psychological distress and well-being (MH)
- Limitations in usual role activities due to personal or emotional problems (RE)
- Limitations in social functioning due to physical or mental health problems (SF)
- Vitality, energy and fatigue (VT)
- General health perception (GH)

Responses to all individual items are rated on a 3-, 5- or 6-point Likert scale and the 8 multi-item scales are scored from 0 to 100 with higher scores indicating better health. Another algorithm yields 2 summary scores, the Physical Component Summary (PCS) and the Mental Component Summary (MCS). These summary scores are also scaled with higher scores indicating better health but are scored using a norm-based system where linear transformations are performed to transform scores to a mean of 50 and standard deviation of 10, based upon general US population norms.¹⁴ The concepts measured by the SF-36 are not specific to any age, disease, or treatment group, allowing comparison of relative burden of different diseases and the relative benefit of different treatments.²¹ The 8 multi-item subscales are calculated whenever $\geq 50\%$ of the items that comprise the individual subscale are available (nonmissing). Any missing items will be estimated using the average value across the nonmissing items for that subscale. If $< 50\%$ of the items that comprise the subscale is available, the subscale will not be calculated. If any of the individual subscales that comprise the physical component summary score or the mental component summary score are missing, then the physical or mental component summary scores cannot be calculated.

The 8 multi-item scores and 2 summary scores (PCS and MCS) will be derived based on the algorithm and software provided by the developer¹⁸.

The change from baseline for the scores of the 8 multi-item scales and the PCS and MCS scores of the SF-36 will be summarized by timepoint and by study vaccination group.

The non-parametric Wilcoxon rank sum test may be used to compare the scores and change from baseline of each of the 8 multi-item scales and the PCS and MCS scores between the study vaccination groups at each timepoint. The change from baseline over time for the scores of the 8 multi-item scales and the PCS and MCS scores may also be analyzed with a repeated measures linear mixed model²⁰.

For IED, pyelonephritis and UTI cases caused by ExPEC9V O-serotypes, the percentage of participants who had a ≥ 5 -point decrease in PCS or MCS scores at the time of IED, pyelonephritis or UTI, respectively, and at the follow up time points will be summarized by study vaccination group.

As the Sponsor elected to terminate the study early due to futility, SF-36 analysis will be limited to the main efficacy analysis period (with onset on or before 13 February 2025) and performed with available data and for the first efficacy events according to the CCD (FAS); at prespecified timepoints.

5.7.4.2. EQ-5D-5L

The 5-level EuroQol 5-Dimension (EQ-5D-5L) Descriptive System is a self-administered, standardized measure of health status in a wide range of health conditions and treatments. It provides a descriptive profile and a single index value for health status that can be used in the clinical and economic evaluation of health care. The recall period for all items is 'Today'. The EQ 5D-5L consists of the EQ-5D descriptive system and the EQ visual analog scale (EQ-VAS). The EQ-5D descriptive system is comprised of 5 items across the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The EQ-5D-5L uses a 5-point Likert response scale ranging from "No problems" to "Extreme problems". The EQ-5D health states defined by the EQ-5D descriptive system can be converted into a single index value using country specific value sets. The index value facilitates the calculation of quality-adjusted life years (QALYs) that are used to inform economic evaluations of health care interventions.

For the purpose of the analyses in this SAP, the US value set will be used¹⁷. Different country valuations may be used at the country economic evaluation stage.

The EQ-5D also includes a visual analog scale (EQ-VAS) that has endpoints labeled "best imaginable health state" and "worst imaginable health state" anchored at 100 and 0, respectively. Participants are asked to indicate how they rate their own health by indicating the point on the EQ VAS which best represents their own health on that day.

At each of the timepoints, for each of the 5 dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression), the number and percentage of participants at each level of the response scale (no, slight, moderate, severe or extreme/unable) will be summarized by study vaccination group.

The continuous index value and EQ-VAS scores as well as the change from baseline for the index value and EQ-VAS scores will be summarized by timepoint and by study vaccination group. The non-parametric Wilcoxon rank sum test may be used to compare the index value and EQ-VAS score and the change from baseline between the study vaccination groups at each timepoint. The change from baseline over time for the index value and EQ-VAS scores may also be analyzed with a repeated measures linear mixed model²⁰.

The continuous index value will be calculated as follows using the US value set ([Table 8](#)):

Index value = $1 - (\text{MO}_i + \text{SC}_j + \text{UA}_k + \text{PD}_l + \text{AD}_m)$ for health state ijklm.

For IED, pyelonephritis and UTI cases caused by ExPEC9V O-serotypes, the continuous index value and the EQ-VAS score at the time of IED, pyelonephritis or UTI, respectively, and at the follow up time points will be summarized by study vaccination group.

Table 8: US EQ-5D-5L value set parameters

	Mobility (MO)	Self-care (SC)	Usual activities (UA)	Pain/discomfort (PD)	Anxiety/depression (AD)
<i>Slight (2)</i>	0.096	0.089	0.068	0.060	0.057
<i>Moderate (3)</i>	0.122	0.107	0.101	0.098	0.123
<i>Severe (4)</i>	0.237	0.220	0.255	0.318	0.299
<i>Unable/Extreme (5)</i>	0.322	0.261	0.255	0.414	0.321

Table reproduced and adapted from Pickard et al. 2019¹⁷

As an example, for health state 12345, the index value is $1 - 0.089 - 0.101 - 0.318 - 0.321 = 0.171$.

As the Sponsor elected to terminate the study early due to futility, EQ-5D-5L analysis will be limited to the main efficacy analysis period (with onset on or before 13 February 2025) and performed with available data and for the first efficacy events according to the CCD (FAS), at prespecified timepoints.

5.7.5. Frailty Assessments

The frailty assessment analysis will be based on available data up to 13 February 2025 and may be limited to the baseline assessment (FAS). For the evaluation of the frailty for IED events, the analysis may be done, based on the first IED events according to the CCD with onset on or before 13 February 2025 (FAS).

Frailty will be assessed at baseline based on the SPPB score and in addition, the Frailty Index will be calculated using participants self-reported information regarding health status (components of SF-36 and EQ-5D), and baseline health conditions collected through the participant's medical conditions of interest.^{3,4} Frailty Index will also be assessed at Year 1, Year 2, Year 3, Year 4 and at the Early Exit visit, as well as at the time of IED case evaluation (Day 1), and at follow up after IED.

In addition to the Frailty Index, the Charlson Comorbidity Index (CCI) score will be calculated at the same time points as the Frailty Index. Calculations will be based on the data collected through the medical conditions of interest.

5.7.5.1. SPPB

The SPPB assesses lower-extremity function by measures of three separate tests, i.e. standing balance (maximum 4 points), gait speed (maximum 4 points), and ability to rise from a chair (maximum 4 points).⁶ A summary performance score is created by adding the scores for the tests of standing balance, gait speed, and repeatedly rising from a chair. The summary scores range between 0 and 12, with higher scores indicating better performance.

Summary score cut-offs have been defined based on subsequent risk of disability and mortality:

- SPPB 10-12 points fit participant, normal
- SPPB 8-9 points pre-frail participant
- SPPB ≤ 7 points frail participant.

At baseline, the summary performance score (continuous) and the number and percentage of participants at each level of the SPPB scale (“fit”, “pre-frail”, “frail”) will be summarized by study vaccination group.

5.7.5.2. Frailty Index

The Frailty Index will be assessed at Year 1, Year 2, Year 3, Year 4 and at the Early Exit visit, as well as at the time of IED case evaluation (Day 1) and at follow-up after IED.

The specific medical conditions of interest used for the calculation of the Frailty Index score will include: diabetes mellitus, history of myocardial infarction, congestive heart failure, hypertension, history of cerebrovascular disease, COPD, cancer, osteoarthritis or rheumatoid arthritis, gastric or duodenal ulcer, long term disability or handicap, hearing problems, migraine, cataract, and glaucoma (see [Table 14](#) in [Appendix 4](#)).

A total deficit score, ranging from 0 to 43, will be calculated as the accumulation of the individual’s deficits. Relevant medical conditions, as listed above, contribute a maximum score of 14 (1 for each medical condition of interest) and the SF-36 and EQ-5D questionnaires contribute a maximum score of 25 and 4, respectively, as detailed in [Table 9](#).

The Frailty Index is calculated by converting the deficit score into an index from 0 to 1 as follows: Frailty Index = (deficit score/n) where n is the number of non-missing components.

Summary score cut-offs will be defined as in Curran et al. 2019³:

- Frailty Index ≤ 0.08 fit participant, normal
- Frailty Index $0.08 < \leq 0.25$ pre-frail participant
- Frailty Index > 0.25 frail participant.

[Table 9](#) is reproduced and adapted from [Table 1](#) of Curran et al. 2019³ as follows:

- “SF-36 Q3I – Q3J (Physical functioning)” has been changed to “SF-36 Q3A – Q3J (Physical functioning)” to get a maximum contribution of 10 points for this item
- [Table 1](#) of Curran et al. 2019³ uses EQ-5D-3L (3 levels) while in the VAC52416BAC3001 study, EQ-5D-5L (5 levels) are used. Based on the cross-tabulation for EQ-5D-3L and EQ-5D-5L as presented in [Table 2](#) of van Hout et al. 2012¹⁹, the levels Slight, Moderate and Severe in EQ-5D-5L will be given the score of Some in EQ-5D-3L.

If the SF-36 and the EQ-5D-5L data are not available at Year 1, Year 2, Year 3, Year 4 as well as at the time of IED case evaluation (Day 1) and at follow-up after IED, the Frailty Index will not be calculated for those timepoints (ie put as missing).

As the Sponsor elected to terminate the study early due to futility and discontinue analysis of SF-36 and EQ-5D assessments recorded after 13 February 2025, frailty statistical analyses will include data up to 13 February 2025.

Table 9: Detail of SF-36 and EQ-5D Components Contribution to the Frailty Index

Item	Scoring method based on response to question	Maximum Contribution to deficit score
SF-36 Q1 (General Health)	Poor = 1 Fair = 0.5 Good = 0 Very good = 0 Excellent = 0	1
SF-36 Q11A-11D (General Health)	Q11A, Q11C Definitely true = 1 Mostly true = 0.5 Don't know = 0 Mostly false = 0 Definitely false = 0 Q11B, Q11D Definitely true = 0 Mostly true = 0 Don't know = 0 Mostly false = 0.5 Definitely false = 1	4
SF-36 Q3A – Q3J (Physical functioning) (Adapted)	Limited a lot = 1 Limited a little = 0.5 Not limited at all = 0	10
SF-36 Q9A – Q9I (Vitality and Mental health)	Q9A, Q9D, Q9E, Q9H All of the time = 0 Most of the time = 0	9

Table 9: Detail of SF-36 and EQ-5D Components Contribution to the Frailty Index

Item	Scoring method based on response to question	Maximum Contribution to deficit score
	Some of the time = 0 A little of the time = 0.5 None of the time = 1 Q9B, Q9C, Q9F, Q9G, Q9I All of the time = 1 Most of the time = 0.5 Some of the time = 0 A little of the time = 0 None of the time = 0	
SF-36 Q2 Compared to one week before, how did the subject rate his/her health in general?	Much worse = 1 Somewhat worse = 0.5 Same = 0 Somewhat better = 0 Better = 0	1
EQ-5D Mobility	EQ-5D-3L No Problems = 0 Some Problems = 0.5 Confined to bed = 1 Adapted to EQ-5D-5L No Problems = 0 Slight Problems = 0.5 Moderate Problems = 0.5 Severe Problems = 0.5 Unable to walk = 1	1
EQ-5D Anxiety	EQ-5D-3L No Anxiety = 0 Moderate Anxiety = 0.5 Extreme Anxiety = 1 Adapted to EQ-5D-5L No Anxiety = 0 Slightly Anxious = 0.5 Moderately Anxious = 0.5 Severely Anxious = 0.5 Extremely Anxious = 1	1

Table 9: Detail of SF-36 and EQ-5D Components Contribution to the Frailty Index

Item	Scoring method based on response to question	Maximum Contribution to deficit score
EQ-5D Self care	EQ-5D-3L No Problems = 0 Some Problems = 0.5 Inability to wash or dress himself/herself = 1 Adapted to EQ-5D-5L No Problems = 0 Slight Problems = 0.5 Moderate problems = 0.5 Severe problems = 0.5 Inability to wash or dress himself/herself = 1	1
EQ-5D Usual activities	EQ-5D-3L No Problems = 0 Some Problems = 0.5 Inability to perform usual activities = 1 Adapted to EQ-5D-5L No Problems = 0 Slight Problems = 0.5 Moderate problems = 0.5 Severe problems = 0.5 Inability to perform usual activities = 1	1
Total		29

Table reproduced and adapted from Curran et al. 2019³**5.7.5.3. Charlson Comorbidity Index**

The updated CCI consisting of 12 comorbidities will be calculated²⁴. The following 12 comorbidities (and their weights) will be included: congestive heart failure (2), dementia (2), chronic pulmonary disease (1), rheumatologic disease (1), mild liver disease (2), diabetes with chronic complications (1), hemiplegia or paraplegia (2), renal disease (1), any malignancy including leukemia and lymphoma (2), moderate or severe liver disease (4), metastatic solid tumor (6) and AIDS/HIV (4) (see [Table 14](#) in [Appendix 4](#) which medical conditions of interest contribute to each comorbidity).

For an individual participant, the sum of the comorbidities present in that participant multiplied by the respective weight will determine the CCI. The maximum comorbidity score will be 24 (the

following comorbidities are mutually exclusive: mild liver disease and moderate or severe liver disease; and any malignancy and metastatic solid tumor).

5.7.6. Diagnosis of IED and Clinical Data Collection

Based on the futility announcement, the adjudication of efficacy endpoints has been discontinued. Efficacy events may be either confirmed by applying a programmatic clinical case definition or by investigator assessment. The changes in the diagnosis of IED and clinical data collection are described below.

If a participant presents with signs and symptoms (see Section 8.1.1 of the CTP) which the investigator (or medically qualified designee) assesses to be suggestive of a systemic infection, the participant will be considered a suspected IED case.

For the purpose of summarizing the suspected IED cases, the following categories may be used:

1. Suspected systemic infection episodes (opened in the eCRF)
2. Suspected systemic infection episodes (opened in the eCRF) with *E. coli* in blood/sterile sites or *E. coli* in urine
3. IED cases according to the Clinical Case Definition (CCD) or investigator assessment with *E. coli* in blood/sterile sites or *E. coli* in urine (to be reviewed by the IEAC)
 - a. With isolates not sent to central lab
 - b. With isolates received by central lab
 - i. ExPEC9V Serotype
 - ii. 9V-negative
 - iii. Non-typeable
 - iv. Not serotyped
 - c. Cases that meet the programmatically applied CCD
 - d. Reviewed by the IEAC
 - i. Accepted by the IEAC
 - ii. Rejected by the IEAC
4. IED
 - a. IED cases confirmed by the IEAC as meeting the primary endpoint and *E. coli* laboratory confirmed as ExPEC9V O-serotype
 - b. IED cases that meet the programmatically applied primary endpoint and *E. coli* laboratory confirmed as ExPEC9V O-serotype
5. IED cases that meet the secondary endpoints

- a. IED cases confirmed by the IEAC as meeting the primary or first secondary endpoint and *E. coli* laboratory confirmed as ExPEC9V O-serotype
- b. IED cases that meet the programmatically applied primary or first secondary endpoint and *E. coli* laboratory confirmed as ExPEC9V O-serotype
- c. IED cases confirmed by the IEAC as meeting the primary or first secondary endpoint and *E. coli* identified at either the local or central laboratories
- d. IED cases that meet the programmatically applied clinical case primary or first secondary endpoint and *E. coli* identified at either the local or central laboratories

IED cases will also be summarized according to the composites as shown in [Figure 3](#).

- e. *E. coli* bacteremia without sepsis
- f. *E. coli* bacteremia with sepsis
- g. Non-bacteremic *E. coli* in other sterile body sites without sepsis
- h. Non-bacteremic *E. coli* in other sterile body sites with sepsis
- i. *E. coli* urosepsis detected only in urine

The number and percentage of participants in each of the above categories will be summarized by study vaccination group.

The clinical signs and symptoms collected at the time of the diagnosis of IED will be tabulated and presented in decreasing frequency based on the number of participants reported in each symptom.

Systemic inflammatory response syndrome (SIRS), qSOFA and SOFA scores will be summarized by study vaccination group.

Type of infection linked to IED (community-acquired, hospital-acquired, healthcare-associated) and source of infection (urinary tract, gastrointestinal tract, respiratory tract, other body sites) may be tabulated.

Outcome and complications due to IED may be summarized for events that occur on or prior to the futility analysis announcement (13 February 2025).

The number and percentage of participants with overlapping events (e.g., UTI and IED) will also be summarized.

5.7.7. Diagnosis of UTI/ABP and Clinical Data Collection

If a participant has contacted their physician and/or study site with at least 1 of the UTI signs or symptoms (see [Appendix 2](#) Section 6.2.2) or at least 1 of the ABP signs or symptoms (see [Appendix 2](#) Section 6.2.4), the participant will be considered a suspected UTI or ABP case.

For the purpose of summarizing the suspected UTI/ABP cases, the following categories will be used:

1. UTI/ABP episodes (opened in eCRF)
2. UTI/ABP episodes (opened in eCRF) with any pathogen in urine
3. UTI/ABP episodes (opened in eCRF) with *E. coli* in urine
4. UTI/ABP episodes (opened in eCRF) with *E. coli* in urine (CFU $\geq 10^5$)
5. UTI/ABP cases according to the CCD with only *E. coli* (no other pathogen) in urine (CFU $\geq 10^5$)
 - a. With isolate not sent to central lab
 - b. With isolates received by central lab
 - i. ExPEC9V Serotype
 - ii. 9V-negative
 - iii. Non-typeable
 - iv. Not serotyped
6. Pyelonephritis cases according to the CCD or investigator assessment with *E. coli* in urine (to be reviewed by the IEAC)
 - a. With isolate not sent to central lab
 - b. With isolates received by central lab
 - 1) ExPEC9V Serotype
 - 2) Non-ExPEC9V Serotype
 - 3) Non-typeable
 - 4) Not serotyped
 - c. Cases that meet the programmatically applied CCD
 - d. Reviewed by the IEAC
 - 1) Accepted by the IEAC
 - 2) Rejected by the IEAC
7. Pyelonephritis cases that meet the pyelonephritis endpoints
 - a. Pyelonephritis cases confirmed by the IEAC and *E. coli* laboratory confirmed as ExPEC9V O-serotype
 - b. Pyelonephritis cases that meet the programmatically applied clinical case definition and *E. coli* laboratory confirmed as ExPEC9V O-serotype
 - c. Pyelonephritis cases confirmed by the IEAC and *E. coli* identified at either the local or central laboratories
 - d. Pyelonephritis cases that meet the programmatically applied clinical case definition and *E. coli* identified at either the local or central laboratories

The number and percentage of participants in each of the above categories will be summarized by study vaccination group.

The clinical signs and symptoms collected at the time of the diagnosis of UTI or ABP will be tabulated and presented in decreasing frequency based on the number of participants reported in each symptom.

Type of infection linked to UTI/ABP (community-acquired, hospital-acquired, healthcare-associated) may be tabulated.

Outcome and complications due to UTI/ABP will be summarized for events with onset on or before the futility announcement (13 February 2025).

5.7.8. *E. coli* Isolates

The following source and results of bacterial culture of isolates will be summarized on participant-level and isolate-level and may also be summarized by endpoint (e.g., for systemic infections and UTIs).

1. Source of isolates (blood, urine, other specified normally sterile body site)
2. Taxonomy of pathogen (*E. coli* only, *E. coli*/Escherichia Clades I-IV, *P. aeruginosa* only, Multiple pathogens including *E. coli* but not *P. aeruginosa*, Multiple pathogens including *P. aeruginosa* but not *E. coli*, Multiple pathogens including *E. coli* and *P. aeruginosa*, Not *E. coli* / Not *P. aeruginosa*).
3. Other pathogens
4. Bacterial count for urine cultures, CFU/mL ($<10^3$, 10^3 to $<10^5$, $\geq 10^5$)
5. Antimicrobial susceptibility testing results (see Section 5.5.3 for the antibiotic panel which will be used to screen isolates for antimicrobial resistance).

A listing of microbiology test results will also be provided.

The O-serotype distribution and the cumulative vaccine coverage (at participant level) of 9 ExPEC O-serotypes based on O-agglutination assay may be presented by each of the study vaccination groups and overall.

As the Sponsor elected to terminate the study early due to futility, central laboratory microbiology assessments (serotyping and antimicrobial susceptibility testing) will be performed using available data.

5.8. Interim Analyses and Safety Monitoring

An independent data monitoring committee (IDMC) will be established and will have the responsibility to review cumulative unblinded safety data on periodic basis (i.e., at least every 6 months) as well as to review the results of the futility and efficacy analyses. Following each review meeting, the IDMC will recommend to the Sponsor whether to continue the trial unchanged, modify the conduct of the study or terminate the study early. Rules for continuation of the study,

modification or termination of the study as well as how these recommendations will be made to the Sponsor will be outlined in a separate IDMC charter.

The IDMC will be composed of at least 3 external members not associated with the study. Each member is required to be a recognized expert in the field of infectious diseases and related diseases or biostatistics. The Sponsor will also designate an independent unblinded statistical team, the SSG not affiliated with the Sponsor to prepare and provide statistical support to the IDMC. The SSG will program, validate and archive all analysis datasets and statistical outputs related to the IDMC analyses. The IDMC charter provides complete details regarding the composition and governance of the IDMC.

Besides the 6-monthly IDMC safety reviews, the study also incorporates the following pre-planned interim analyses:

1. An Immunogenicity interim analysis (see Section 5.8.1)
2. An IDMC safety review of the first 500 randomized participants in the study (see Section 5.8.2)
3. Interim Analyses for the Primary Endpoint (see Section 5.8.3)

For all the interim analyses, the SSG will perform the interim analyses and will present the results to the IDMC (at the immunogenicity interim analysis the SSG will also prepare the immunogenicity tables for the Study Team).

In addition, if the event rate is lower or higher than expected, a blinded sample size re-estimation (ie, based on the event rate combining all participants from both the ExPEC9V and placebo groups) may be performed by the sponsor at the time of the first interim analysis of the primary endpoint (See Section 5.8.4).

5.8.1. Immunogenicity Interim Analysis

After the first 200 vaccinated participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have reached Day 30 or discontinued earlier, the Study Team will review the summary statistics of the immunogenicity data by study vaccination group to confirm the immunogenicity profile of ExPEC9V. The Study Team will remain blinded to individual study vaccine allocation. Enrollment will not be paused during this immunogenicity interim analysis. This immunogenicity interim analysis will be performed on the FAS (participants with major protocol deviations expecting to impact the immunogenicity outcomes may be excluded). The purpose of this immunogenicity interim analysis is to obtain an initial result of the immune response, no decision will be taken on the remainder of the study and therefore no type I error adjustment is required. The 200 participants included in this interim analysis will continue their follow-up as the other participants and will be included in future futility and efficacy evaluations.

5.8.2. First IDMC Safety Review

The objective of the first IDMC safety review is to assess the safety of one dose of ExPEC9V.

At the first IDMC safety review (when the first 500 randomized participants in the study have completed the Day 30 visit (Visit 3) or have discontinued earlier), the IDMC will review unblinded safety data. The safety data will include serious adverse events and unsolicited adverse events from all participants and solicited adverse events from participants in the Safety Subset. Enrollment will be paused after randomization of the 500th participant until the IDMC issues a recommendation. Thereafter, an IDMC safety analysis (including solicited and unsolicited AEs, SAEs, laboratory data, and any other data applicable for the study) will be conducted at least every 6 months. More details can be found in the IDMC charter.

5.8.3. Interim Analyses for the Primary Endpoint

Details of the interim analyses for the primary endpoint, including operating characteristics, are presented in [Table 10](#).

Table 10: Criteria of Interim and Final Analyses for the Primary Endpoint

Analysis	Total planned events	Alpha (1-sided) ^b	Futility ^a			Efficacy				
			Cases in vaccine group	Prob under H ₀	Prob under H _a	Cases in vaccine group	VE	(1-2α)% CI	Prob under H ₀	Prob under H _a
IA1 (futility)	21 (40%)	-	≥ 9 (≤ 25.0% VE)	64.0%	3.5%	-				
IA2 (efficacy/futility)	37 (70%)	0.0034	≥ 17	5.1%	0.0%	≤ 8	≥ 72.4%	[20.1%-92.5%]	0.3%	50.86%
Final analysis	53 (100%)	0.0243				≤ 16	≥ 56.8%	[20.1%-77.6%]	2.0%	39.1%
										Global power 89.9%

CI=confidence interval; IA=interim analysis; NA = not applicable; Prob under H₀=probability to reach futility or efficacy under the null hypothesis (VE=20%); Prob under H_a: probability to reach futility or efficacy under the alternative hypothesis (VE=70%); VE=vaccine efficacy.

^a Go/No Go decision criteria: Go = P(VE > 0%) > 80%; No Go = P(VE < 70%) > 90%

^b User-defined alpha-spending function

When 21 of 53 (40%) vaccine-serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sites) have been observed, a first interim analysis of the primary endpoint (non-binding futility) will be performed. The futility analysis will be based on the Go/No Go approach. By allowing the enrollment to stop in the event of futility, participants will be prevented from receiving an ineffective vaccine or a vaccine inferior to placebo. The study will continue to enroll the remaining participants if there is at least 80% probability that VE>0%. Futility will be declared if there is at least 90% probability that VE<70%. If the rule to declare futility (P(True VE<70%)>90%) and the rule to continue (P(True VE>0%)>80%) are both fulfilled, this would lead to a “Pause” situation in the “Go/No Go” framework. However, it was decided that this would also be classified as a “Go” situation. The

IDMC may recommend termination of the study for futility. This will not affect the overall Type 1 error. The IDMC recommendation with regard to futility at the time of this interim analysis will be communicated to the sponsor for further evaluation. If the recommendation from the IDMC is to continue the study, the results will not be shared with the sponsor by the IDMC. The posterior probabilities of $P(\text{True VE} > 0\%)$ or $P(\text{True VE} < 70\%)$ will be determined using a Bayesian approach. Of the 21 expected total number of cases, let x (or $21-x$) be the number of cases observed in the vaccine (or placebo) group. Let p be the probability of a case belonging to the vaccine group ($p = r \cdot (1 - \text{VE}) / (1 + r \cdot \text{VE})$, $r = \text{follow-up time in the vaccine group} / \text{follow-up time in the control group}$, see also [Appendix 8](#)) where $\text{VE} = 0\%$ or $\text{VE} = 70\%$. The variable x is a binomial random variable with parameters p and $n = 21$. Assume that the prior distribution of p is a Beta distribution with both shape parameters equal 0.5. The posterior distribution of p given the value of x is then Beta with shape parameters $a = (\text{\#events in the vaccine group} + 0.5)$ and $b = (\text{\#events in the placebo group} + 0.5)$. The different probabilities of $P(\text{True VE} > 0\%)$ or $P(\text{True VE} < 70\%)$ for different values of x , when the total number of events is 21 and $r = 1$, are shown in [Table 11](#).

Table 11: Go/NoGo Criteria at First Interim Analysis of the Primary Endpoint

#V (x)	#P (21-x)	Observed VE	P(True VE > 0%)	P(True VE < 70%)	Go/NoGo
0	21	100.0%	100.0%	0.1%	GO
1	20	95.0%	100.0%	1.2%	GO
2	19	89.5%	100.0%	6.0%	GO
3	18	83.3%	100.0%	17.0%	GO
4	17	76.5%	99.8%	34.6%	GO
5	16	68.8%	99.3%	55.0%	GO
6	15	60.0%	97.7%	73.5%	GO
7	14	50.0%	93.8%	86.6%	GO
8	13	38.5%	86.3%	94.3%	GO ^a
9	12	25.0%	74.3%	97.9%	NO GO
10	11	9.1%	58.6%	99.4%	NO GO

#V: Number of events in the vaccine group; #P: Number of events in the placebo group; VE: Vaccine Efficacy; $P(\text{True VE} > 0\%)$: Probability that True VE is greater than 0% using a Beta distribution $B(\#V + 0.5; \#P + 0.5)$; $P(\text{True VE} < 70\%)$: Probability that True VE is less than 70% using a Beta distribution $B(\#V + 0.5; \#P + 0.5)$

^a When 8 events are in the vaccine group, the rule to declare futility ($P(\text{True VE} < 70\%) > 90\%$) and the rule to continue ($P(\text{True VE} > 0\%) > 80\%$) are both fulfilled. In the Go/NoGo framework this would be a "Pause" situation. However, it was decided that this would also be classified as a "Go" situation.

At the first interim analysis (IA1, see [Table 10](#)), the study might be stopped for futility if the VE (point estimate) is $\leq 25.0\%$ (see also [Table 11](#)), ie, ≥ 9 events in the ExPEC9V group (non-binding futility). At the first interim analysis of the primary endpoint, there is no possibility to declare early superiority of this primary endpoint. It is possible that when triggering this interim analysis, more events have occurred and might be included in the analysis; however, the Go/No Go criteria will not change, and the posterior probabilities will be calculated with the actual observed number of events.

A second interim analysis of the primary endpoint (efficacy/futility) will be conducted when 37 of 53 (70%) vaccine serotype IED events according to the primary endpoint have been observed. At the time of this interim analysis early superiority may be declared. At the second interim analysis (IA2), the nominal α will be fixed at 0.0034 (1-sided). Using this nominal α , superiority will be declared if ≤ 8 (out of 37) events are observed in the ExPEC9V group (ie, if the VE [point estimate]

is $\geq 72.4\%$). In the unlikely event that there are ≥ 17 events in the ExPEC9V group at the time of the second interim analysis, the study might be stopped for futility. It is possible that when triggering this interim analysis, more events have occurred and might be included in the analysis, however, the nominal α will not change.

The final analysis of the primary endpoint will occur when 53 vaccine serotype IED events with microbiological confirmation from blood or other sterile sites have been observed. If the final analysis is performed with 53 events and the second interim analysis with 37 events, the nominal 1-sided significance level of 0.0243 will be used (if the number of events is different from 37 at the second interim analysis or 53 at the final analysis, the final nominal α will depend on the observed number of events and will be determined using the α -spending approach) and superiority will be declared if ≤ 16 (out of 53) events (i.e. if the VE [point estimate] is $\geq 56.8\%$) are observed in the ExPEC9V group.

Table 12 shows some operating characteristics for different true VE assumptions, including VE of 30% and 50%.

Table 12: Operating Characteristics Under Different True VE Assumptions for Primary Endpoint

	Full study	IA 1 (21 events)		IA 2 (37 events)		Final analysis (53 events)	
True VE	Overall power	Superiority	Futility	Superiority	Futility	Success	Failure
0.0%	0.3%	0.0%	80.8%	0.0%	5.5%	0.2%	13.4%
10.0%	0.8%	0.0%	73.5%	0.1%	5.6%	0.7%	20.1%
20.0%	2.3%	0.0%	64.0%	0.3%	5.1%	2.0%	28.6%
30.0%	6.4%	0.0%	52.1%	1.0%	4.1%	5.4%	37.5%
40.0%	16.2%	0.0%	38.3%	3.1%	2.6%	13.1%	43.0%
50.0%	35.7%	0.0%	24.0%	8.7%	1.2%	27.0%	39.1%
60.0%	64.5%	0.0%	11.6%	22.9%	0.3%	41.6%	23.6%
70.0%	89.9%	0.0%	3.5%	50.8%	0.0%	39.1%	6.5%
80.0%	99.2%	0.0%	0.4%	84.9%	0.0%	14.4%	0.3%
90.0%	100.0%	0.0%	0.0%	99.5%	0.0%	0.5%	0.0%

IA = interim analysis; VE = vaccine efficacy

5.8.4. Blinded Sample Size Re-estimation

If the event rate is lower or higher than expected (21 events are expected to be reached after 44.5 months of study start), a blinded sample size re-estimation may be performed at the time of the first interim analysis of the primary endpoint (21 events). At the time of this interim analysis and before unblinded data of the interim analysis is sent to the IDMC for efficacy evaluation, the study team will first perform the sample size re-estimation based on the incidence rate of the combined study vaccination groups (blinded, thus placebo and vaccine groups pooled and therefore no alpha adjustment is required).

If the incidence rate is lower than expected, the sample size will not be increased by more than 50%. At the time of the first interim analysis of the primary endpoint (21 events), the sponsor will decide whether to increase the sample size of the study and/or extend the follow-up period of participants in the study (e.g., from 4 to 5 years).

5.9. End-of-study analysis

A descriptive end-of-study efficacy analysis will include the data collected during the entire study period.

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1: List of Abbreviations

ABP	acute bacterial prostatitis
ADaM	Analysis Data Model
AE	adverse event
ATC	anatomic and therapeutic class
BMI	Body Mass Index
CCD	Clinical Case Definition
CCI	Charlson Comorbidity Index
CFU	colony forming units
CI	confidence interval
CTP	clinical trial protocol
DPS	data presentation specifications
<i>E. coli</i>	<i>Escherichia coli</i>
ECL	electrochemiluminescent
eCRF	electronic case report form
EPA	exotoxin A derived from <i>Pseudomonas aeruginosa</i>
ExPEC	Extraintestinal pathogenic <i>Escherichia coli</i>
EQ-5D-5L	5-level EuroQol 5-Dimension questionnaire
FAS	full analysis set
FDA	Food and Drug Administration
GMT	Geometric Mean Titer
HPF	high-power field
ICF	informed consent form
IDMC	Independent Data Monitoring Committee
IEAC	Independent Endpoint Adjudication Committee
IED	Invasive Extraintestinal Pathogenic <i>Escherichia coli</i> Disease
IgG	serum immunoglobulin G
iARBM	Integrated Analytical Risk-Based Monitoring
IRR	incidence rate ratio
IWRS	Interactive web response system
LLOQ	lower limit of quantification
MCS	Mental Component Summary
MedDRA	Medical Dictionary for Regulatory Activities
MIC	Minimum inhibitory concentration
MOPA	multiplex opsonophagocytic killing assay
MRU	Medical resource utilization
NA	not applicable
PCS	Physical Component Summary
PP	per protocol efficacy analysis set
PPE	per protocol efficacy analysis set
PPI	per protocol immunogenicity analysis set
qSOFA	Quick sequential organ failure assessment
QTL	Quality Tolerance Limit
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SDTM	Study Data Tabulation Model
SE	standard error
SF-36	Short Form-36: 36-item short form health survey

SIRS	Systemic inflammatory response syndrome
SMQ	Standardised MedDRA Queries
SOFA	Sequential organ failure assessment
SPPB	Short Physical Performance Battery
TLF	tables, listings and figures
ULOQ	upper limit of quantification
UTI	urinary tract infection
VE	vaccine efficacy

6.2. Appendix 2: Clinical Case Definition of Endpoints

6.2.1. IED

A participant will be considered to have IED if the below criteria are met and the diagnosis is confirmed by the IEAC. Data packages for evaluation by the IEAC will be sent for all cases which are considered as suspected IED (both those confirmed by the investigator as meeting the clinical case definition as well as those where IED cannot be excluded) and which include confirmation of presence of *E. coli* in relevant cultures (including cases of mixed pathogens). The cases of invasive disease due to *P. aeruginosa* will not be sent to the IEAC for confirmation of the diagnosis recorded by the investigator. The package will consist of all available clinical data collected by Day 29 post-IED identification or by the day of resolution of IED signs and symptoms (whichever comes first). During IEAC review, the IEAC reviewers need to decide if the collected data are pertinent to be included in the case definition evaluation for the same IED event.

With discontinuation of IEAC case review, IED events will be confirmed as endpoints using application of the clinical case definition. For both the primary and secondary endpoints related to IED, parallel analyses will be conducted: 1) an analysis based on IED events that were assessed and confirmed by the IEAC, and 2) an analysis based on IED events that are consistent with the protocol clinical case definition (programmatically confirmed).

An adult participant diagnosed with IED is any participant with:

- signs and symptoms of systemic bacterial infection as indicated by the presence of any of the following:
 - temperature $<36.0^{\circ}\text{C}$ (96.8°F) or $>38.0^{\circ}\text{C}$ (100.4°F),
 - tachycardia (HR) >90 bpm,
 - tachypnea (RR) >20 breaths/minute or $\text{PaCO}_2 < 32$ mm Hg
 - WBC count <4 or $>12 \times 10^9/\text{L}$ or $\geq 10\%$ immature (band) forms,
- OR
- an acute change in total SOFA score of ≥ 2 points from baseline

AND

- microbiological confirmation by culture of:

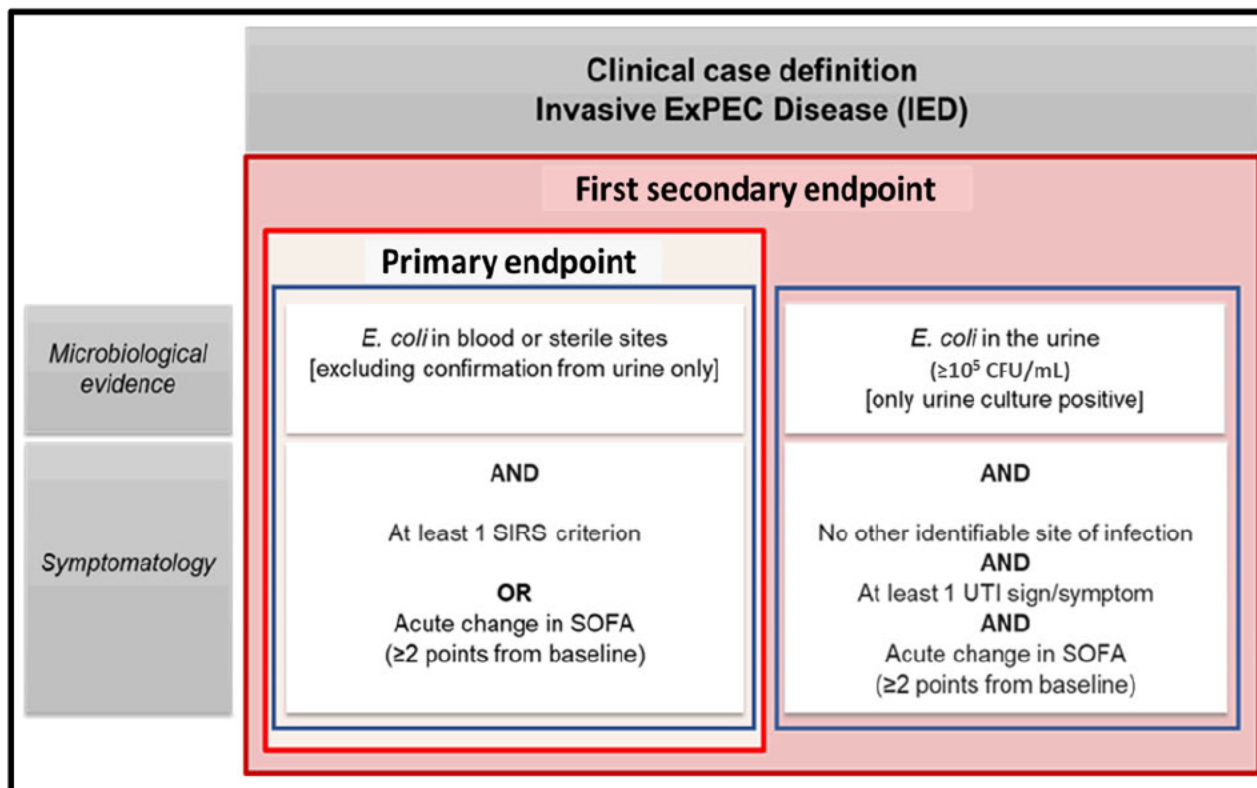
- *E. coli* in blood or in any other sterile site^h (e.g. cerebrospinal fluid [CSF], pleura),

AND/OR

- *E. coli* in urine ($\geq 10^5$ CFU/mL) with UTI signs/symptomsⁱ and with no other identifiable site of infection and with an acute change in total sequential organ failure assessment (SOFA) score of ≥ 2 points from baseline.

A schematic diagram of the IED clinical case definition is depicted in [Figure 2](#).

Figure 2: Schematic Diagram of IED Clinical Cases



CFU=colony-forming units; *E. coli*=Escherichia coli; IED=invasive extraintestinal pathogenic Escherichia coli disease; SIRS=systemic inflammatory response syndrome; SOFA=sequential organ failure assessment; UTI=urinary tract infection.

When a participant is assessed for the first time (e.g. at the moment of emergency room evaluation or hospital admission), the baseline SOFA score will be considered as 0, unless the medical history indicates otherwise. In case multiple SOFA scores are available before the onset of systemic infection, the acute change will be counted from the baseline score defined as the lowest score

^h Refer to [Appendix 9](#) for the sterile sites' definition

At least one of the UTI signs/symptoms ([Appendix 2](#) Section 6.2.2) over the last 72 hours preceding the diagnosis of systemic infection.

over the last 72 hours preceding the diagnosis of systemic infection, ie, the time when the first positive culture was obtained that is attributable to the same IED event.

For the primary endpoint, IED events with microbiological confirmation from blood or other sterile sites will be taken into account, excluding IED cases with microbiological confirmation from urine only.

Secondary and exploratory endpoints related to IEDs will include events with *E. coli* microbiological confirmation from blood, other sterile sites, or urine (unless otherwise indicated per the Objectives and Endpoints table in Section 1.1). IED cases will also be summarized according to the composites as shown in Figure 3.

Figure 3: Clinical Case Definition: Invasive *E. coli* Disease (IED) Mapping to Composites

<i>E. coli</i> bacteremia without sepsis	<i>E. coli</i> bacteremia with sepsis	<i>E. coli</i> infection in other sterile body sites without sepsis	<i>E. coli</i> infection in other sterile body sites with sepsis	<i>E. coli</i> urosepsis with detection in urine only
<i>E. coli</i> in blood	<i>E. coli</i> in blood	<i>E. coli</i> in other sterile body sites	<i>E. coli</i> in other sterile body sites	<i>E. coli</i> in the urine ($\geq 10^5$ CFU/mL) [only urine culture positive]
AND No acute change in SOFA (≥ 2 points from baseline) AND At least 1 SIRS criterion	AND Acute change in SOFA (≥ 2 points from baseline)	AND No acute change in SOFA (≥ 2 points from baseline) AND At least 1 SIRS criterion	AND Acute change in SOFA (≥ 2 points from baseline)	AND No other identifiable source of infection AND At least 1 UTI sign/symptom AND Acute change in SOFA (≥ 2 points from baseline)

6.2.2. Pyelonephritis

If a participant has contacted their healthcare provider and/or study site with signs and symptoms of pyelonephritis, the participant will be defined as having a confirmed UTI endpoint if the criteria outlined below are met and confirmed by the IEAC for cases of pyelonephritis. For cases of UTI see Section 6.2.3, for ABP see Section 6.2.4.

Data packages for evaluation by the IEAC will be sent for all cases of *E. coli* confirmed pyelonephritis. Data will include all available clinical data collected by Day 29 post-pyelonephritis diagnosis or by the day of resolution of UTI signs and symptoms (whichever comes first). For pyelonephritis, the IEAC will review all the clinically diagnosed cases of pyelonephritis with microbiological confirmation of *E. coli* (regardless of meeting protocol criteria) as well as the cases that would be identified based on the protocol case definition. Only pyelonephritis cases confirmed by the IEAC will be used in the efficacy analysis.

An event is considered a confirmed pyelonephritis endpoint if an adult with documented pyuria (WBC count ≥ 10 cells/mm³ in a non-centrifuged urine or ≥ 5 WBC per high-power field [400x enlargement] [WBC/HPF] in urine sediment) and microbiological confirmation by culture of *E. coli* in urine ($\geq 10^5$ CFU/mL) presents with at least 2 of the following signs and symptoms:

- fever (temperature $\geq 38.0^{\circ}\text{C}$ [as measured by the participant or health care professional]), or chills, or rigors, or warmth (warmth only if associated with evidence of fever),
- flank pain, or
- tenderness in the costovertebral angle on physical examination, or
- nausea or vomiting.

Note: Fever is taken from the CE domain as well as from the VS domain.

6.2.3. UTI

An event is considered a confirmed UTI endpoint if an adult with documented pyuria (WBC count ≥ 10 cells/mm³ in a non-centrifuged urine or ≥ 5 WBC per high-power field [400x enlargement] [WBC/HPF] in urine sediment) and microbiological confirmation by culture of *E. coli* in urine ($\geq 10^5$ CFU/mL) presents with one of the following definitions for signs and symptoms:

- for uncomplicated UTI, at least 2 of the following signs or symptoms:
 - dysuria,
 - urinary frequency,
 - urinary urgency, or
 - suprapubic pain.
- for complicated UTI (cUTI):
 - at least 2 of the following signs or symptoms:
 - fever (temperature $\geq 38.0^{\circ}\text{C}$ [as measured by the participant or health care professional]), or chills, or rigors, or warmth (warmth only if associated with evidence of fever),
 - dysuria, urinary frequency or urinary urgency,
 - lower abdominal pain or pelvic pain, or
 - nausea or vomiting,

Note: Fever is taken from SDTM CE domain as well as from the SDTM VS domain.

AND,

- at least 1 of the following complicating factors:
 - history of urinary retention (in male participants),
 - current indwelling urinary catheter,
 - obstructive uropathy, or
 - any relevant functional or anatomical abnormality.

Note: All risk factors for complicated UTI (with toxicity grade) (see [Table 15](#) of [Appendix 4](#)) recorded at baseline, are considered as “Any relevant functional or anatomical abnormality”.

- for recurrent UTI (rUTI):
 - recurrence of symptomatic UTIs with a frequency of 2 UTIs in 6 months, or
 - recurrence of symptomatic UTIs with a frequency of at least 3 UTIs in 1 year.
 - For rUTI caused by ExPEC9V O-serotypes, only UTIs caused by ExPEC9V O-serotypes will be taken into account for the frequency of 2 UTIs in 6 months or at least 3 UTIs in 1 year.
- for recurrent uncomplicated UTI:
 - recurrence of uncomplicated UTIs with a frequency of 2 UTIs in 6 months, or
 - recurrence of uncomplicated UTIs with a frequency of at least 3 UTIs in 1 year.
 - For recurrent uncomplicated UTI caused by ExPEC9V O-serotypes, only uncomplicated UTIs caused by ExPEC9V O-serotypes will be taken into account for the frequency of two UTIs in six months or at least 3 UTIs in one year.

As an exploratory endpoint, participants meeting the above definition of UTI but with urine samples positive for *P. aeruginosa* ($\geq 10^5$ CFU/mL) will be reported to have *P. aeruginosa* UTI. No serotyping will be performed for *P. aeruginosa*. Cases with mixed pathogens with the presence of *P. aeruginosa* will not be considered for this exploratory endpoint.

6.2.4. ABP

If a participant has contacted their health care provider and/or study site with signs and symptoms of ABP, the participant will be defined as having a confirmed ABP endpoint if the below criteria are met.

An adult with documented pyuria (WBC count ≥ 10 cells/mm³ in non-centrifuged urine or ≥ 5 WBC/HPF [400x enlargement] in urine sediment) and microbiological confirmation by culture of *E. coli* in urine ($\geq 10^5$ CFU/mL) with fever (temperature $\geq 38.0^\circ\text{C}$ [as measured by the participant or health care professional]) and an acute onset of the following:

- irritative voiding symptoms:
 - dysuria,
 - urinary frequency,
 - urinary urgency,

OR
- obstructive voiding symptoms:
 - hesitancy,

- incomplete voiding,
- straining to urinate,
- weak stream,

AND

- suprapubic, rectal, or perineal pain.

6.2.5. Sepsis

Sepsis is defined as life-threatening organ dysfunction due to dysregulated host response to infection. A case of IED will be defined as meeting criteria for sepsis if there is a documented acute change in total Sequential Organ Failure Assessment (SOFA) score of ≥ 2 points from baseline and deemed secondary to the IED.

6.2.6. Septic Shock

Septic shock occurs in a subset of patients with sepsis and comprises an underlying circulatory and cellular/metabolic abnormality that is associated with increased mortality. A case of IED will be defined as leading to septic shock if the following criteria are documented:

- persisting hypotension requiring vasopressors to maintain a mean arterial pressure of 65 mm Hg or higher, and
- a serum lactate level greater than 2 mmol/L (18 mg/dL) despite adequate volume resuscitation

6.3. Appendix 3: Changes to Protocol-Planned Analyses

This is Amendment 7 of the SAP. It is aligned with Protocol Amendment 8 (Amendment 8, EDMS-ERI-206001377, 11.0, approved on 9 August 2024).

6.4. Appendix 4: Demographics and Baseline Characteristics

The following demographic and baseline characteristics will be summarized.

Table 13, presents a list of the demographic variables and baseline characteristics that will be summarized by study vaccination group and overall, for the FAS, PPE, Safety Subset and PPI. Demographic variables and baseline characteristics will also be summarized by region, age and risk factor for complicated UTI using the FAS.

Table 13: Demographic Variables or Baseline Characteristics

Continuous Variables:	Summary Type
Age (years)	Descriptive statistics (N, mean, standard deviation [SD], median and range [minimum and maximum]).
Weight (kg)	
Height (cm)	
Body Mass Index (BMI) (kg/m ²)	
SPPB summary score (see Section 5.7.5.1)	
CCI (see Section 5.7.5.3)	
Categorical Variables	

Age (≥ 60 to < 65 years, ≥ 65 to < 75 years, ≥ 75 years)	Frequency distribution with the number and percentage of participants in each category.
Age (≥ 60 to < 65 years, ≥ 65 to < 75 years, ≥ 75 to < 85 years, ≥ 85 years)	
Sex (Female, Male, Unknown, Undifferentiated)	
Race ^a (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or other Pacific Islander, White, Not Reported, Unknown, Multiple)	
Ethnicity (Hispanic or Latino, not Hispanic or Latino, Not Reported, Unknown)	
BMI ([underweight < 18.5 kg/m ² , normal 18.5 – < 25 kg/m ² , overweight 25 – < 30 kg/m ² , obese ≥ 30 kg/m ²])	
Country/Territory (Denmark, The Netherlands, Spain, United Kingdom, United States, Canada, Japan, Australia, New Zealand, South Korea, Czech Republic, France, Germany, Italy, Sweden, Israel, Taiwan, Colombia, Thailand, Saudi Arabia, China, India)	
Region (North America [United States, Canada], South America [Colombia], Europe [Denmark, The Netherlands, Spain, United Kingdom, Czech Republic, France, Germany, Italy, Sweden], Asia & Pacific [Japan, South Korea, Australia, New Zealand, Israel, Taiwan, Thailand, Saudi Arabia, China, India])	
Medical conditions of interest (see Table 14)	
Risk factor for complicated UTI (with toxicity grade) (Yes, No) (see Table 15)	
Risk factor for complicated UTI (any toxicity grade) (Yes, No) (see Table 15)	
SPPB (fit, pre-frail, frail) (see Section 5.7.5.1)	
Antimicrobial prophylaxis for UTI at baseline (Yes, No)	
Frailty Index (fit, pre-frail, frail) (see Section 5.7.5.2)	
Hospitalization in the last 2 years (Yes, No)	
History or urosepsis/ <i>E. coli</i> bacteremia (Yes, No)	
Hospitalization in the last 2 years or history or urosepsis/ <i>E. coli</i> bacteremia or risk factors for complicated UTI (any toxicity grade) (Yes, No)	

^a If multiple race categories are indicated, the Race is recorded as 'Multiple'

Table 14 shows the medical conditions of interest that will be captured at baseline and at different timepoints during the study. The most prevalent medical conditions of interest will also be summarized in the baseline characteristics.

The medical conditions of interest and medical conditions required for assessment of the Charlson Comorbidity Index and the Frailty Index are presented by organ system in Table 14.

Table 14: Medical Conditions of Interest, Medical Conditions of Interest for the Charlson Comorbidity Index, and Frailty Index

Organ system	Medical conditions of interest	CCI term (weight)	Medical conditions of interest for Frailty Index
Urinary tract disorders	Medullary sponge kidney	Renal disease (1)	
	Acute Renal Injury	Renal disease (1)	
	Chronic kidney disease	Renal disease (1)	
	Hydronephrosis	Renal disease (1)	
	Neurogenic Bladder		
	Obstructive Uropathy		

Table 14: Medical Conditions of Interest, Medical Conditions of Interest for the Charlson Comorbidity Index, and Frailty Index

Organ system	Medical conditions of interest	CCI term (weight)	Medical conditions of interest for Frailty Index
	Urinary fistula		
	Ureteric strictures		
	Urethral Strictures		
	Urinary Retention		
	Urinary Tract Obstruction		
	Urine incontinence		
	Urolithiasis		
	Vesicoureteral Reflux		
	Voiding dysfunction		
	Interstitial cystitis		
	Chronic prostatitis		
	Benign Prostatic Hypertrophy		
	Acute prostatitis		
	Pelvic prolapse		
	Pyelonephritis		
	Nephrolithiasis		
Surgical and medical procedures	Cystostomy		
	Nephrostomy		
	Urostomy		
	Nephrostomy tube placement		
	Renal transplant	Renal disease (1)	
	Ureteric stent		
	Urinary catheterization		
	Urological intervention		
Respiratory disorders	COPD or other chronic pulmonary disease	Chronic pulmonary disease (1)	Has chronic bronchitis or emphysema
Nervous system disorders	Dementia	Dementia (2)	
	Migraine		Has migraine headache
	Mental disorder		
	Hearing problems		Has hearing problem
Malignancies	Prostatic Adenocarcinoma		Has cancer
	Tumours of the urinary tract	Any malignancy including leukemia and lymphoma (2)	Has cancer
	Any malignancy, including lymphoma and leukemia, except malignant neoplasm of skin, tumors of urinary tract and prostatic adenocarcinoma	Any malignancy including leukemia and lymphoma (2)	Has cancer
	Metastatic solid tumour	Metastatic solid tumor (6)	Has cancer
Musculoskeletal and connective tissue disorders	Arthritis	If toxicity grade ≥ 3 Rheumatologic disease (1)	Has arthritis or rheumatism
Metabolic disorders	Diabetes mellitus	If toxicity grade ≥ 2	Has diabetes mellitus

Table 14: Medical Conditions of Interest, Medical Conditions of Interest for the Charlson Comorbidity Index, and Frailty Index

Organ system	Medical conditions of interest	CCI term (weight)	Medical conditions of interest for Frailty Index
		Diabetes with chronic complications (1)	
Infections	Urosepsis		
	<i>E. coli</i> bacteremia		
	HBV infection	If toxicity grade ≤ 2 Mild liver disease (2)	
	HCV infection	If toxicity grade ≤ 2 Mild liver disease (2)	
	HIV infection	AIDS/HIV (4)	
Immune system disorders	Anaphylactic and anaphylactoid responses		
	Cachexia		
	Long term Disability or Handicap; Hemiplegia or paraplegia; Immobility	Hemiplegia or paraplegia (2)	Has long-term disability or handicaps
	Major organ transplantation		
Gastrointestinal disorders	Anorectal Conditions		
	Fecal Incontinency		
	Peptic ulcer		Has stomach or intestinal ulcers
	Enterovesical fistula		
	Cholangitis	If toxicity grade ≤ 2 Mild liver disease (2)	
	Cholelithiasis	If toxicity grade ≤ 2 Mild liver disease (2)	
	Chronic Liver Disease	If toxicity grade ≤ 2 Mild liver disease (2) If toxicity grade > 2 Moderate or severe liver disease (4)	
Eye disorders	Cataract		Has cataract
	Glaucoma		Has glaucoma
Cardiovascular disorders	Congestive Heart Failure	Congestive Heart Failure (2)	Has heart failure
	Cerebrovascular accident		Suffers from the effect of stroke
	Central venous catheterization		
	Peripheral vascular disease		
	Hypertension		Has high blood pressure
	Myocardial infarction		Has history of myocardial infarction

The medical conditions of interest considered for eligibility as additional IED risk factors (with their requisite timing relative to randomization) and considered risk factors for complicated UTI (with their requisite toxicity grade for stratification purposes) are presented in [Table 15](#).

For eligibility purposes under Inclusion Criterion 11, select medical conditions of any toxicity grade qualify as additional IED risk factors for study eligibility; however, they are required to have occurred within a specific timing relative to randomization as indicated in the middle column of [Table 15](#).

For stratification purposes at randomization, select medical conditions are only considered complicated UTI risk factors when they are present at a toxicity grading of 2 or above, as indicated in the rightmost column of [Table 15](#). Refer to [Table 16](#) for guidelines on toxicity grading.

Table 15: Medical Conditions Considered Complicated UTI (cUTI) Risk Factors for Inclusion Criteria 11 and Requisite Toxicity Grading for Signant Health/IWRS Stratification

Medical Conditions	Timing of Medical Condition Relative to Randomization for Consideration as a cUTI Risk Factor for Inclusion Criterion 11	Toxicity Grading Required for Answering "yes" to the Signant Health/IWRS Stratification Question for cUTI Risk Factor Presence
Medullary sponge kidney	Present at baseline	If toxicity grade ≥ 2
Acute Renal Injury	Present at baseline, must be eligible per Inclusion Criterion 6 and Exclusion Criterion 4	If toxicity grade ≥ 2
Chronic kidney disease	Present at baseline	If toxicity grade ≥ 2
Hydronephrosis	Present at baseline	Any toxicity grade
Neurogenic Bladder	Present at baseline	If toxicity grade ≥ 2
Obstructive Uropathy	Present at baseline	Any toxicity grade
Ureteric strictures	Present at baseline	If toxicity grade ≥ 2
Urethral Strictures	Present at baseline	If toxicity grade ≥ 2
Urinary Retention	Present at baseline	Any toxicity grade
Urinary Tract Obstruction	Present at baseline	If toxicity grade ≥ 2
Urine incontinence	Present at baseline	If toxicity grade ≥ 2
Urolithiasis	Present at baseline	If toxicity grade ≥ 2
Vesicoureteral Reflux	Present at baseline	If toxicity grade ≥ 2
Voiding dysfunction	Present at baseline	If toxicity grade ≥ 2
Benign Prostatic Hypertrophy	Present at baseline	If toxicity grade ≥ 2
Pelvic prolapse	Present at baseline	If toxicity grade ≥ 2
Cystostomy	Present at baseline	Any toxicity grade
Nephrostomy	Present at baseline	Any toxicity grade
Urostomy	Present at baseline	Any toxicity grade
Nephrostomy tube placement	Present at baseline	Any toxicity grade
Renal transplant	Present at baseline, must be eligible per Exclusion Criterion 8	Any toxicity grade
Ureteric stent	Present at baseline	Any toxicity grade
Urinary catheterization	Present at baseline	Any toxicity grade
Nephrolithiasis	Present at baseline	If toxicity grade ≥ 2
Prostatic Adenocarcinoma	Current or prior, must be eligible per Exclusion Criterion 6	If toxicity grade ≥ 2

Table 15: Medical Conditions Considered Complicated UTI (cUTI) Risk Factors for Inclusion Criteria 11 and Requisite Toxicity Grading for Signant Health/IWRS Stratification

Medical Conditions	Timing of Medical Condition Relative to Randomization for Consideration as a cUTI Risk Factor for Inclusion Criterion 11	Toxicity Grading Required for Answering "yes" to the Signant Health/IWRS Stratification Question for cUTI Risk Factor Presence
Tumours of the urinary tract	Current or prior, must be eligible per Exclusion Criterion 6	If toxicity grade ≥ 2
History of pyelonephritis	>14 days prior to randomization	If toxicity grade ≥ 2

The following general guidelines will be applied (modified from FDA toxicity grading table based on Renal and Urinary Conditions of the CTCAE v5.0¹⁵) for medical conditions of interest:

Table 16: General Guidelines for Toxicity Grading

Grade 1	Mild	Aware of symptoms but easily tolerated; Does not interfere with activity; clinical or diagnostic observations only; intervention NOT indicated.
Grade 2	Moderate	Notable symptoms; Requires modification in activity or use of medications; (minimal, local or noninvasive intervention indicated).
Grade 3	Severe	Incapacitating symptoms; Inability to do work, or usual activities; hospitalization or prolongation of existing hospitalization indicated.
Grade 4	Potentially life-threatening	Life-threatening consequences; urgent intervention indicated.

6.5. Appendix 5: Protocol Deviations

Major protocol deviations will be summarized.

In general, the following list of major protocol deviations may have the potential to impact participants' rights, safety or well-being, or the integrity and/or result of the clinical study. Participants with major protocol deviations will be identified prior to database lock and the participants with major protocol deviations will be summarized by category.

- Developed withdrawal criteria but not withdrawn
- Entered but did not satisfy criteria
- Received a disallowed concomitant treatment
- Received wrong treatment or incorrect dose
- Other

Protocol deviations that influence efficacy or immunogenicity will be flagged in a listing.

In addition, major protocol deviations and eligibility violations will also be summarized by study vaccination group. Protocol deviations for this study include, but are not limited to, the following (see also the Major Protocol Deviation Criteria Form for this study for more details):

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication/vaccination
- Any other deviation that presents significant risk or safety concerns to the patient

6.6. Appendix 6: Prior and Concomitant Medications

The analysis of concomitant therapies will be done using the World Health Organization (WHO) drug coded terms.

Relevant pre-study specific therapies (i.e., non-steroidal anti-inflammatory drugs, corticosteroids, antihistamines, vaccinations, antivirals, and all antibacterials [including antibiotics and urinary tract antiseptics]) administered up to 30 days before dose of study vaccine must be recorded at screening.

Concomitant therapies associated with an SAE will be collected and recorded in the electronic case report form (eCRF) from informed consent form (ICF) signature through the end of the study.

Concomitant therapies associated with unsolicited AEs will be collected and recorded in the eCRF from the time of vaccine administration through 29 days after vaccination (Day 30) or until clinical resolution or stabilization. For participants in the Safety Subset, concomitant therapies associated with solicited AEs will be collected and recorded in the eCRF from the time of vaccine administration through 14 days after vaccination (Day 15) or until clinical resolution or stabilization.

Concomitant medications associated with suspected IED or pyelonephritis episodes will be recorded for all participants experiencing such an episode. As of Protocol Amendment 8, concomitant medications associated with treatment of suspected UTI or ABP episodes will no longer be recorded. Chronic use of antibacterials [including antibiotics and urinary tract antiseptics] (e.g., for UTI prophylaxis) may be initiated during study participation, if medically indicated. Chronic use of antibacterials [including antibiotics and urinary tract antiseptics], for any indication, is to be recorded for the entire duration of the study.

Use of licensed *E. coli*^g vaccines is prohibited. If *E. coli* vaccines other than ExPEC9V/study vaccination are administered during the study period due to medical necessity, their use should be recorded and will be considered a protocol deviation. Participants who receive other *E. coli* vaccines will be excluded from the per protocol efficacy (PPE) population and the per protocol immunogenicity (PPI) population.

Use of licensed *E. coli* vaccines must always be recorded as concomitant therapy for the entire duration of the study.

Chronic or recurrent use of systemic corticosteroids^j, is prohibited during the study and within 3 months before the planned administration of the study vaccine. A substantially immunosuppressive steroid dose is considered to be ≥ 2 weeks of daily receipt of 20 mg or more of prednisone or equivalent. The definition of recurrent corticosteroid use is at the discretion of the investigator.

^j Note: Ocular, topical, intra-articular or inhaled steroids are allowed.

Administration of antineoplastic and immunomodulating agents or radiotherapy is prohibited during the study and within 6 months before the planned administration of the study vaccine. If any of these agents are indicated in a disease setting, these must take priority over the study vaccine.

The sponsor must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited therapies are administered. All such instances will be recorded as a protocol deviation, but this will not exclude the concerned participant from further study participation, unless other conditions apply (see CTP Section 7.2); however, the participant may be excluded from the PPE and/or PPI populations (see CTP Section 9.3). Any medication (whether licensed or experimental) that is prescribed by a treating physician as a treatment of any condition, will have priority over protocol restrictions.

Based on their start and stop date, concomitant therapies will be reported in each applicable phase.

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

- In case of partial start or stop dates, the concomitant therapy records will be allocated to phases 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 phases, and the concomitant therapy record will be allocated to the phase(s) where these date parts match.
- In case of a completely missing end date, the concomitant therapy will be considered as ongoing at the end of the trial.

There will be special attention to any systemic use of analgesics/antipyretics, started 14 days following vaccination (00:00 of day of vaccination + 14 days). Following ATC/DD codes will be used for this: N02A (OPIOIDS) and N02B (OTHER ANALGESICS AND ANTIPYRETICS), M01A (ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS, NON-STEROIDS) and M01B (ANTIINFLAMMATORY/ANTIRHEUMATIC AGENTS IN COMBINATION) (ATC/DD Index²²). The classes will be added in a footnote in all related tables and listings. For the use of analgesics/antipyretics which are taken on the day of vaccination, an exception is made in case the time is before vaccination. In this case, the concomitant medication is also allocated to the Post-vaccination Period.

6.7. Appendix 7: Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

The laboratory values provided in the tables below serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

If a laboratory value falls within the grading as specified below but also within the local laboratory normal limits, the value is considered to be normal and will be reset to grade 0.

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4) **
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia				Insulin requirements or hyperosmolar coma
Fasting – mg/dL	100 – 110	111 – 125	> 125	
Random – mg/dL	110 – 125	126 – 200	> 200	
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – Hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – Hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – Hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – Hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125 – 129 mEq/L) should be recorded as a grade 4 hyponatremia event if the participant had a new seizure associated with the low sodium value.

*** ULN is the upper limit of the normal range.

Note: Grade 0 is assigned to a lab assessment when the lab test is described in the table, but the lab value is not assigned a grade 1 or higher.

Hematology *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm ³	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm ³	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm ³	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm ³	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm ³	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm ³	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)
International Normalized Ratio (INR)***	1.1 to < 1.5 x ULN	1.5 to < 2.0 x ULN	2.0 to < 3.0 x ULN	≥ 3.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** ULN is the upper limit of the normal range.

*** For INR, the values in the table are based on the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, 2014 (version 2.0)

Note: Grade 0 is assigned to a lab assessment when the lab test is described in the table, but the lab value is not assigned a grade 1 or higher.

Urine *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) - red blood cells per high power field (rbc/hpf)	1 - 10	11 - 50	> 50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Note: Grade 0 is assigned to a lab assessment when the lab test is described in the table, but the lab value is not assigned a grade 1 or higher.

Vital Signs *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) ** (°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

* Participant 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 participant populations, for example, conditioned athletes.

Note: Grade 0 is assigned to a vital sign assessment when the vital sign is described in the table, but the vital sign value is not assigned a grade 1 or higher.

Ranges to convert FDA scale to SI units

Ranges to convert FDA scale to SI units		Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Albumin (g/L)	Hypo- albuminemia	28 - 31	25 - 27	< 25	
Eosinophils (10 ⁹ /L)		0.65 - 1.5	1.501 - 5.0	> 5.0	
Hemoglobin for male (g/L)		125 - 135	105 - 124	85 - 104	< 85
Hemoglobin for female (g/L)		110 - 120	95 - 109	80 - 94	< 80
Hemoglobin change from baseline (g/L)		Any decrease – 15	16 - 20	21 - 50	> 50
Lymphocytes (10 ⁹ /L)		0.75 - 1.0	0.5 - 0.749	0.25 - 0.499	< 0.25
Neutrophils (10 ⁹ /L)		1.5 – 2.0	1.0 – 1.499	0.5 - 0.999	< 0.5
Platelets (10 ⁹ /L)		125 – 140	100 – 124	25 – 99	< 25
Protein (g/L)	Hypo- proteinemia	55 – 60	50 – 54	< 50	
WBC (10 ⁹ /L)	Increase	10.8 – 15	15.001 – 20	20.001 – 25	> 25
	Decrease	2.5 – 3.5	1.5 – 2.499	1.0 – 1.499	< 1.0

Other Conversions

Blood, Serum, or Plasma Chemistries *		Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)	Conversion Factor
Glucose (mmol/L)	Hypoglycemia	3.61 – 3.83	3.05 – 3.60	2.50 – 3.04	< 2.50	/18.01477
	Hyperglycemia – Fasting	5.55 – 6.11	6.12 – 6.94	> 6.94		
	Hyperglycemia – Random	6.11 – 6.94	6.94 – 11.10	> 11.10		
Blood urea nitrogen (mmol/L)		8.2 – 9.3	9.4 – 11.1	> 11.1		
Creatinine (μmol/L)		133 – 150	151 – 177	178 – 221	> 221	/0.01131
Calcium (mmol/L)	Hypocalcemia	2.00 – 2.10	1.87 – 1.99	1.75 – 1.86	< 1.75	/4
	Hypercalcemia	2.62 – 2.74	2.75 – 2.87	2.88 – 3.00	> 3.00	/4
Magnesium (mmol/L)	Hypomagnesemia	0.53 – 0.62	0.45 – 0.52	0.37 – 0.44	< 0.37	/2.43072
Phosphorus (mmol/L)	Hypophosphatemia	0.74 – 0.81	0.65 – 0.73	0.52 – 0.66	< 0.52	/3.09693

Blood, Serum, or Plasma Chemistries *		Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)	Conversion Factor
Cholesterol (mmol/L)		5.20 – 5.43	5.44 – 5.82	> 5.82		
Coagulation						
Fibrinogen (μmol/L)	Increase	11.76 – 14.70	14.71 – 17.65	> 17.65		
	Decrease	4.41 – 5.88	3.68 – 4.40	2.94 – 3.67	< 2.94	/34

* Depending upon the laboratory used, reference ranges, eligibility ranges and grading may be split out by sex and/or age.

6.8. Appendix 8: Statistical Methodology for Sample Size Calculations

Vaccine efficacy will be estimated by 1 minus the ratio of the incidence rate of cases of IED in the ExPEC9V group compared to the placebo group.

Vaccine efficacy can be estimated by:

$$VE = 1 - \frac{n_1/N_1}{n_2/N_2} = 1 - \frac{n_1}{r * n_2}$$

Where:

n_1 = number of cases in the vaccinated group

N_1 = follow-up time in the vaccinated group

n_2 = number of cases in the control group

N_2 = follow-up time in the control group

$r = N_1/N_2$.

Conditionally to the total number of cases $n = n_1 + n_2$ and r , let p denote the proportion of cases in the vaccine group. Then, vaccine efficacy can be rewritten as:

$$VE = 1 - \frac{n_1}{n} * \frac{n}{r * (n - n_1)} = 1 - \frac{p}{r * (1 - p)}$$

Further, note that n_1 is binomially distributed $B(n, p)$. Therefore, there is a monotonic link between VE, the vaccine efficacy, and p , the proportion of cases in the vaccine group among the total cases.

The confidence interval (CI) for vaccine efficacy can then be derived from the exact CI for p based on the exact Poisson regression.^{1,15}

This methodology has already been used in other Phase 3 vaccine efficacy studies.¹²

Sample size calculation

To determine the number of events that is needed, standard software, like PASS 15 can then be used.

The null hypothesis $VE \leq 20\%$ can be translated into $H_0: p \geq 0.4444$ (using the above formula). For the power calculations of the primary endpoint, the $VE=70\%$ translates into $p=0.2308$. Hence, using PASS 15 (Tests for one proportion), it can be seen that 53 events are needed to reach a statistical power of at least 90% using a 1-sided 0.025 significance level.

		Proportion Given H0	Proportion Given H1	Difference	Target	Actual	Reject H0
Power	n	P0	P1	P1-P0	Alpha	Alpha	If R ≤
0.91459	53	0.4444	0.2308	-0.2136	0.0250	0.0242	16

The number of participants can then be determined taking into account the incidence rate in the placebo group (~0.17%), the incidence rate in the vaccine group (~0.051%, assuming 70% vaccine

efficacy) and yearly dropout rate (10%). The average follow-up for a participant was assumed to be 3.5 years.

$$N = 53 / [(0.0017 + 0.0017 * (1 - 0.70)) / 2] / (0.90^{3.5}) / 3.5 = 19,815.$$

Hence, approximately 19,815 participants are planned to be enrolled into the study.

6.9. Appendix 9: Sterile Sites' Definition

Normally sterile sites:

- Blood
- Cerebrospinal fluid
- Pleural fluid (i.e., chest fluid, thoracentesis fluid)
- Peritoneal fluid (i.e., abdominal fluid, ascites)
- Pericardial fluid
- Bone, including bone marrow
- Joint fluid (i.e., synovial fluid, fluid, needle aspirate, or culture of any specific joint such as knee, ankle, elbow, hip, wrist)
- Muscle, fascia, or tendon
- Internal body sites (i.e., lymph node, brain, heart, liver, spleen, vitreous fluid, kidney, pancreas, ovary, vascular tissue)
- Bronchoalveolar lavage fluid

For the definition of normally sterile sites, the following specimen sources are excluded:

- Abscess
- Wound
- Gallbladder, bile, biliary tract
- Urinary tract
- Respiratory tract (unless it was obtained by bronchoalveolar lavage)

6.10. Appendix 10: Primary Endpoint based on IEAC Adjudication

The IEAC will review data collected from suspected *E. coli* confirmed IED cases and based on this data, assign each case according to the clinical case definition. In case of multiple isolates per participant, all isolates will be characterized, and all results will be taken into account for final IED case evaluations by the IEAC. Cases with multiple pathogens in the urine culture results will not be considered if the systemic infection cannot be attributed only to *E. coli*. All cases with multiple pathogens including *E. coli* in the culture results from blood or any other sterile site will be included in the efficacy analysis of the primary endpoints.

For IED, the following questions that the IEAC needs to respond to will lead to an endpoint with microbiological confirmation in blood/sterile site or to an endpoint with microbiological confirmation in urine only as shown below:

1. Based on your clinical judgment and the clinical case definition, do you think this case represents an IED that meets the IED endpoint? (Yes or No)
2. The case is microbiologically confirmed in (multiple answers possible):
 - a. blood (bacteremic)
 - b. other sterile sites
 - c. urine
3. In my clinical judgment, for this case (only 1 answer possible)
 - a. *E. coli* was the only clinically significant pathogen contributing to the IED
 - b. *E. coli* was not the only clinically significant pathogen OR it is unclear if *E. coli* was the only clinically significant pathogen contributing to the IED.

Primary endpoint (with microbiological confirmation from blood or other sterile sites, excluding IED cases with microbiological confirmation from urine only, caused by ExPEC9V O-serotypes):

- Question 1 = “Yes” AND Question 2 includes “a” or “b”

Secondary endpoint (with microbiological confirmation from blood, other sterile sites or urine, caused by ExPEC9V O serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75):

- Question 1 = “Yes” AND Question 2 includes “a” or “b”
- Question 1 = “Yes” AND Question 2 includes only “c” AND Question 3 = “a”

Following discontinuation of IEAC review of suspected IED cases, confirmation of IED events as primary endpoints will also be performed by applying statistical programming for the clinical case definition with identification of *E. coli* in either blood or sterile sites.

The ExPEC9V O-serotype(s) (i.e., O1, O2, O4, O6, O15, O16, O18, O25 and O75) need to be confirmed by the agglutination assay.

6.11. Appendix 11: Antimicrobial Susceptible Testing

Table 17: List of antibiotics tested in VAC52416BAC3001

Antibiotic Class ¹	Antibiotics Tested	CLSI M100 v31 Breakpoints (µg/mL)			Included in MDR Classification ²	WHO Model List of Essential Medicines 23rd List (2023) ²³
		Susceptible	Intermediate	Resistant		
Aminoglycoside	Amikacin	≤16	32 [^]	≥64	Yes	Access
	Gentamicin	≤4	8 [^]	≥16	Yes	Access
	Plasomicin ⁴	n/a	n/a	n/a	Yes	Reserve
Anti-pseudomonal penicillins/BLI	Piperacillin/Tazobactam	≤16/4	32/4 - 64/4 [^]	≥128/4	Yes	Watch
Carbapenem	Imipenem	≤1	2 [^]	≥4	Yes	Watch
	Meropenem	≤1	2 [^]	≥4	Yes	Watch
Cephalosporin (third/fourth generation)	Cefepime	≤2	n/a	≥16	Yes	Not applicable
	Cefiderocol ⁴	≤4	8 [^]	≥16	Yes	Reserve
	Cefotaxime	≤1	2 [^]	≥4	Yes	Watch

Table 17: List of antibiotics tested in VAC52416BAC3001

Antibiotic Class ¹	Antibiotics Tested	CLSI M100 v31 Breakpoints (µg/mL)			Included in MDR Classification²	WHO Model List of Essential Medicines 23rd List (2023)²³
		Susceptible	Intermediate	Resistant		
	Ceftazidime	≤4	8 [^]	≥16	Yes	Watch
	Ceftazidime/Avibactam ⁴	≤8/4	n/a	≥16/4	Yes	Reserve
	Ceftolozane/Tazobactam ⁴	≤2/4	4/4 [^]	≥8/4	Yes	Reserve
Fluroquinolones	Ciprofloxacin	≤0.25	0.5 [^]	≥1	Yes	Watch
Folate pathway inhibitors	Trimethoprim/sulfamethoxazole	≤2/38	n/a	≥4/76	Yes	Access
Glycylcyclines	Tigecycline ⁵	≤2	4	≥8	Yes	Not applicable
Monobactam	Aztreonam	≤4	8 [^]	≥16	Yes	Not applicable
Nitrofurantoin	Nitrofurantoin	≤32	64	≥128	No	Access
Penicillins	Amoxicillin	n/a	n/a	n/a	No	Access
	Amoxicillin/Clavulanate	≤8/4	16/8 [^]	≥32/16	Yes	Access
Polymixin	Colistin	n/a	≤2	≥4	Yes	Reserve
Tetracycline	Doxycycline	≤4	8	≥16	No	Access

Table 17: List of antibiotics tested in VAC52416BAC3001

Antibiotic Class ¹	Antibiotics Tested	CLSI M100 v31 Breakpoints (µg/mL)			Included in MDR Classification²	WHO Model List of Essential Medicines 23rd List (2023)²³
		Susceptible	Intermediate	Resistant		

¹ Antibiotic classes are defined based on "antimicrobial category" in [Table 3](#) of Magiorakos et al¹³. As Amoxicillin, Nitrofurantoin and Doxycycline are not classified in Magiorakos et al¹³, these agents are not included in MDR categorization.

² Multidrug-resistance (MDR) is defined as non-susceptibility to at least one agent in three or more MDR antimicrobial classes. As Nitrofurantoin and Doxycycline are not classified in Magiorakos et al¹³, these agents are not included in MDR categorization.

³ Not applicable indicates the antibiotic was not included in the July 2023 WHO Model List of Essential Medicines²³.

⁴ Antibiotic is not mentioned in Magiorakos et al¹³. Antibiotic class has been assigned based on mechanism of action.

⁵ Breakpoints for tigecycline are taken from the FDA Susceptibility Test Interpretive Criteria (STIC) website⁷.

[^] Intermediate range for the applicable antibiotic is based on the known ability of this agent to concentrate in the urine

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