Safety and immunogenicity of a vaccine for extra-intestinal pathogenic *Escherichia coli* (ESTELLA): a phase 2 randomised controlled trial

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Summary

Background ExPEC4V (JNJ-63871860) is a bioconjugate vaccine, containing O-antigens from *Escherichia coli* serotypes O1A, O2, O6A, and O25B, developed for the prevention of invasive extra-intestinal pathogenic *E coli* (ExPEC) disease. We aimed to assess safety, reactogenicity, and immunogenicity of ExPEC4V in healthy adults.

Methods In this phase 2 randomised, double-blind placebo-controlled study, we recruited healthy adults (≥18 years with a body-mass index of 35 kg/m² or less) between Nov 16, 2015, and Aug 8, 2017, and randomly assigned them to receive a single dose of ExPEC4V (antigen O1A:O2:O6A:O25B content 4:4:4:4 μg [group 1]; 4:4:4:8 μg [group 2], 8:8:8:8 μg [group 3], 8:8:8:16 μg [group 4], or 16:16:16:16 μg [group 5]) or placebo. The primary objectives were evaluation of the safety, tolerability, and immunogenicity of ExPEC4V and determination of its dose-dependent immunogenicity 15 days after vaccination by ELISA in individuals who had received at least one vaccination dose. Antibody titres and safety evaluation were used to select two ExPEC4V doses for assessment up to day 360. This trial is registered at ClinicalTrials.gov, number NCT02546960.

Findings Of 848 enrolled participants, 843 (99%) received the ExPEC4V vaccine (757) or placebo (86) and were included in the safety analysis. Of 757 participants vaccinated with ExPEC4V, 222 (29%) had a solicited local adverse event and 325 (43%) had any solicited systemic adverse event, compared with 11 (13%) and 30 (35%) of 86 participants in the control group. Symptoms were mild-to-moderate. The most frequently reported solicited local adverse event was pain or tenderness (205 [27·1%] of 757 in combined ExPEC4V groups) and the most frequently reported solicited systemic adverse event was fatigue (208 [27·6%] of 757). Only 13 (2%) of 843 had a grade 3 event. At day 15, 80% or more of all participants achieved a two times or greater increase in serotype-specific IgG antibodies (except O25B at the lowest dose, 103 [72%] of 144). At day 360, 66% (95% CI 56·47–74·33) of participants in group 2 and 71% (62·13–78·95) of participants in group 4 selected for long-term follow-up maintained a two times or greater increase in serotype-specific antibody compared with baseline.

Interpretation EXPEC4V seemed well tolerated and elicited robust and functional antibody responses across all serotypes, doses, and age groups. For the two dosages evaluated (4:4:4:8 μg and 8:8:8:16 μg), the immune response persisted for 1 year.

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Introduction

Extra-intestinal pathogenic *Escherichia coli* (ExPEC) is the most common Gram-negative bacterial pathogen in humans.[1] It causes diverse and serious invasive diseases across all age groups, including urinary tract infections (UTIs), bacteraemia, neonatal meningitis, surgical site infections, abdominal and pelvic infections, and nosocomial pneumonia.[1–5] *E coli* bacteraemia affects 30–60 individuals per 100 000 over the course of a lifetime with 13–19% case fatality, but the incidence is higher in infants (<12 months of age) and older adults (≥65 years of age), reaching more than 400 per 100 000 in adults aged 85 years or older.[2,3] The expansion of multidrug-resistant clones, such as the fluoroquinolone-resistant ExPEC sequence type 131, contributes to increased treatment failure, prolonged hospitalisation, and increased mortality.[6]

A vaccine to prevent invasive ExPEC disease, including multidrug-resistant infections, is urgently needed. The ExPEC O-antigen, a component of the surface lipopolysaccharide, is a promising vaccine target that is immunogenic in humans, and has conveyed protection against lethal challenge in preclinical models.[7] ExPEC4V is a four-valent vaccine comprised of the *E coli* O1A, O2, O6A, and O25B antigens bioconjugated to the protein carrier *Pseudomonas aeruginosa* exoprotein A, and is being evaluated for the prevention of invasive ExPEC disease. In a phase 1 study, ExPEC4V (containing 4 μg of each polysaccharide) showed a favourable safety profile and induction of functional antibodies in healthy women

Research in context

Evidence before this study

Extra-intestinal pathogenic *Escherichia coli* (ExPEC) causes most urinary tract infections, is the second most common cause of neonatal bacteraemia and meningitis, and is a leading cause of adult invasive ExPEC disease, particularly bacteraemia and sepsis. The risk of developing ExPEC bacteraemia and sepsis increases with age. Furthermore, the increase in multidrug resistance (ie, resistance to two or more antibiotic classes) among ExPEC strains constitutes a major obstacle to successful treatment. No approved vaccine against ExPEC exists. To assess previous evidence on vaccines for the prevention of invasive ExPEC disease, we searched for publications on PubMed published between Jan 1, 1980, and July 1, 2018, using the search terms ["extra-intestinal pathogenic *Escherichia coli*"] OR ["extra-intestinal pathogenic *E coli*" OR "ExPEC" OR "Invasive *E coli*" OR "*E coli* sepsis" OR "*E coli* bacteraemia" OR "*E coli* bloodstream infection" OR "*E coli* pyelonephritis" OR "*E coli* severe UTI" OR "*E coli* surgical site infection" OR "*E coli* severe infection"] AND ["vaccine"], applying filters of ["clinical trial"] AND ["humans"]. Two O-polysaccharide conjugate vaccines were studied in the 1990s. One of these two vaccines underwent early safety and immunogenicity testing in humans with no serious reactions. However, both candidates have since been discontinued. Only two publications were identified from 2000 onwards. They describe phase 1b studies of the bioconjugate vaccine containing O-antigens of four *E coli* serotypes (ExPEC4V) that preceded the phase 2 study described here. These phase 1b, placebo-controlled trials suggested that ExPEC4V was well tolerated (no vaccine related serious adverse events) and induced significant total and functional antibody responses against all vaccine serotypes. We identified no other vaccine candidates in clinical development (human testing) targeting the aforementioned conditions.

Added value of this study

Our study supports the phase 1b findings showing that the parenteral multivalent *E coli* O-antigen bioconjugate vaccine, EXPEC4V, elicited robust and functional antibody responses across the four vaccine serotypes, doses, and age groups. Two dosages (4:4:4:8 µg and 8:8:8:16 µg) showed immune responses for 1 year. All of the doses studied were well tolerated, although a dose-related trend in the incidence of local and systemic symptoms did occur.

Implications of all the available evidence

Our study has shown that ExPEC4V vaccine is immunogenic, with a dose-dependent vaccine immune response observed with ELISA. Functional activity of the antibodies was shown with an ExPEC4V-optimised opsonophagocytic killing assay. Analysis of the immunogenicity data over 1 year after vaccination with ExPEC4V has shown the durability of the immune response and follow-up for a further 3 years will provide information on the duration of immune response.

with a history of recurrent UTI. Although the vaccine did not decrease the incidence of UTIs caused by vaccine preventable serotypes of ExPEC compared with placebo, the vaccine group had significantly fewer UTIs caused by *E coli* of any serotype.[8] To optimise the immune response to ExPEC4V, we aimed to evaluate the safety, tolerability, and immunogenicity of five formulations of ExPEC4V compared with placebo in healthy participants.

See Online for appendix

Methods

Study design and participants

ESTELLA was a phase 2, double-blind, randomised placebo-controlled dose-range study done at 17 sites across the USA. Participants were healthy adult volunteers aged 18 years or older, with a body-mass index of 35 kg/m² or less. Pregnant women were excluded. Full eligibility criteria are shown in the appendix.

The study was split into two parts, a double-blind part from day 1 to day 360 with vaccination on day 1 for all participants (reported here), and a single-blind follow-up part until year 4 for selected participants. Recruitment commenced on Nov 16, 2015, and the data lock point for the final analysis reported here was Aug 8, 2017. Study follow-up is ongoing. During part 1, participants were stratified into two groups by age, 18–49 years (N=275 planned) and 50 years or older (N=560 planned), and randomly assigned to receive a single vaccine dose of one of five ExPEC4V dosages, with antigen O1A:O2:O6A:O25B contents of 4:4:4:4 µg (group 1), 4:4:4:8 µg (group 2), 8:8:8:8 µg (group 3), 8:8:8:16 µg (group 4), and 16:16:16:16 µg (group 5), or placebo. Enrolment of participants was staggered; 55 participants aged 18–49 years were initially vaccinated and 65 participants aged 50 years or older were vaccinated in a stepwise, dose-escalating procedure to the next higher dose (appendix). After each part, safety data up to day 8 was reviewed by an independent data monitoring committee. Enrolment only continued if vaccination in the previous part was deemed safe and well tolerated. All participants were followed for safety for 180 days after vaccination.

On the basis of day 15 immunogenicity and day 30 safety data, participants in groups 2 and 4 (4:4:4:8 µg and 8:8:8:16 µg) and placebo recipients were asked to continue long-term follow-up until year 4 after vaccination (part 2).

All protocol and study documents were reviewed and approved by an ethics committee, and the trial was done in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. All participants provided formal, written consent before taking part in this study.

Randomisation and masking

All participants enrolled in the study were randomly assigned to receive one of the five ExPEC4V dosages

or the placebo 2:2:2:2:2:1 using a computer-generated randomisation schedule.

In part 1, the participants, clinical staff, investigators, and sponsor personnel were blinded to study vaccine allocation, except for the designated pharmacist or qualified staff member with primary responsibility for study vaccine preparation. Sponsor personnel involved in the analysis of the primary data were unblinded at the time of the primary analysis, but primary analysis data were not communicated to the investigators or site staff. Part 2 is single-blind and only study participants remain blinded to the vaccine received.

Procedures

Each vaccine polysaccharide O-antigen (O1A, O2, O6A, and O25B) was covalently linked to glycosylation sites of exoprotein A biosynthetically in a genetically engineered *E coli* strain. The conjugate O-antigen construct was then purified from the *E coli* periplasm, eliminating contamination from the lipopolysaccharide fraction, which in turn allowed for large-scale production of a high-purity vaccine.[9] Placebo was Tris-buffered saline.

Vaccines were administered as a single intramuscular injection into the deltoid muscle.

Any adverse event occurring within 30 min of vaccination was recorded by study personnel. For 8 days after vaccination, participants recorded solicited adverse events (pain or tenderness, erythema, and induration or swelling at the injection site as well as headache, fatigue, malaise, nausea, and myalgia) in an electronic diary. Additionally, unsolicited adverse events were recorded until day 30. Serious adverse events were recorded until study completion. Blood samples were taken for the assessment of safety and immunogenicity and stool samples for exploratory assessments of the effect of vaccination on intestinal flora.

For all participants, IgG antibodies for each of the four vaccine serotypes were measured on days 1 (before vaccination), 15, and 30 by ELISA as previously described.[4] All participants in groups 2 and 4 and all placebo recipients had blood collected for ELISA on days 180 and 360.

Functional antibacterial antibodies were measured using an opsonophagocytic killing (OPK) assay,[4,5] in a subset of 60% of participants randomly selected across all groups on days 1 and 15, and in groups 2 and 4 and placebo recipients on days 30, 180, and 360. With the use of conjugate vaccines, an OPK titre of 8 or more is considered protective for pneumococcal invasive disease,[6] and for meningococcal invasive disease, a serum bactericidal assay titre of 4 or more is considered protective.[7] A minimum protective serum titre for invasive ExPEC disease has not been identified, and we arbitrarily used a conservative OPK titre threshold of 100 as one indication of a response to vaccination, and which we consider is likely to be linked to protection.

Correlation between results from the ELISA and OPK assays was measured by estimation of Pearson correlation coefficients from all tested participants on days 15 and 30 and from participants from groups 2 and 4 and the placebo group on day 360.

Outcomes

The primary study objectives were to evaluate the safety and tolerability of ExPEC4V in terms of solicited and unsolicited adverse events or serious adverse events occurring after vaccination, and to determine its dose-dependent immunogenicity in healthy adults on day 15 after vaccination as measured by ELISA. Secondary endpoints were the evaluation of immunogenicity as measured by OPK assay on day 15, exploration of correlation between ELISA and OPK assay results on day 15, and evaluation of immunogenicity as measured by ELISA on day 30 and 180 (selected groups) and by OPK assay on days 30 and 360 (selected groups).

Stool samples were collected in a subset of 50% of participants selected randomly on day 1 to evaluate the effect of vaccination on pathogens (eg, *Clostridioides difficile* and *Candida* spp) and ExPEC serotypes O1A, O2, O6A, and O25B in the intestinal flora using metagenomics. Metagenomics analyses were done on the stool samples collected from the subset of 50% of randomly selected participants from group 2 and the placebo dose group, taken on days 1 (before vaccination), 30, and 180. In the vaccine group, stools were analysed from 72 participants from day 1, 69 participants from day 30, and 64 from day 180. In the placebo group, stools were analysed from 40 participants from day 1, 39 from day 30, and 36 from day 180.

Changes in the relative abundance of ExPEC4V vaccine serotypes O1A, O2, O6A, and O25B in stools for vaccine and placebo groups on days 1, 30, and 180 were analysed using quantitative PCR (qPCR). Briefly, qPCR primer sequences were identified for *E coli* serotype or sequence type specificity within the designated serotypes or sequence types of interest, which are detailed in the appendix. The four ExPEC serotypes or sequence types were quantified in genomic DNA isolated from human fecal samples.

Larger changes in stool microbiome composition, including of changes in phyla or family, were investigated using 16S RNA analysis. Briefly, a pool containing 16S V4 enriched, amplified, barcoded samples was loaded into a MiSeq (Illumina, San Diego, CA) version 2 reagent cartridge and the amplicons were sequenced for 250 cycles with custom primers designed for 250 bp paired-end sequencing. Quality control and quality assurance metrics were maintained for all sample handling, processing, and storage procedures.

Statistical analysis

The statistical analysis was descriptive, not confirmatory. No formal hypothesis testing was done, and the study was not powered for any formal statistical comparison. A sample size of 150 participants per dose (100 in the

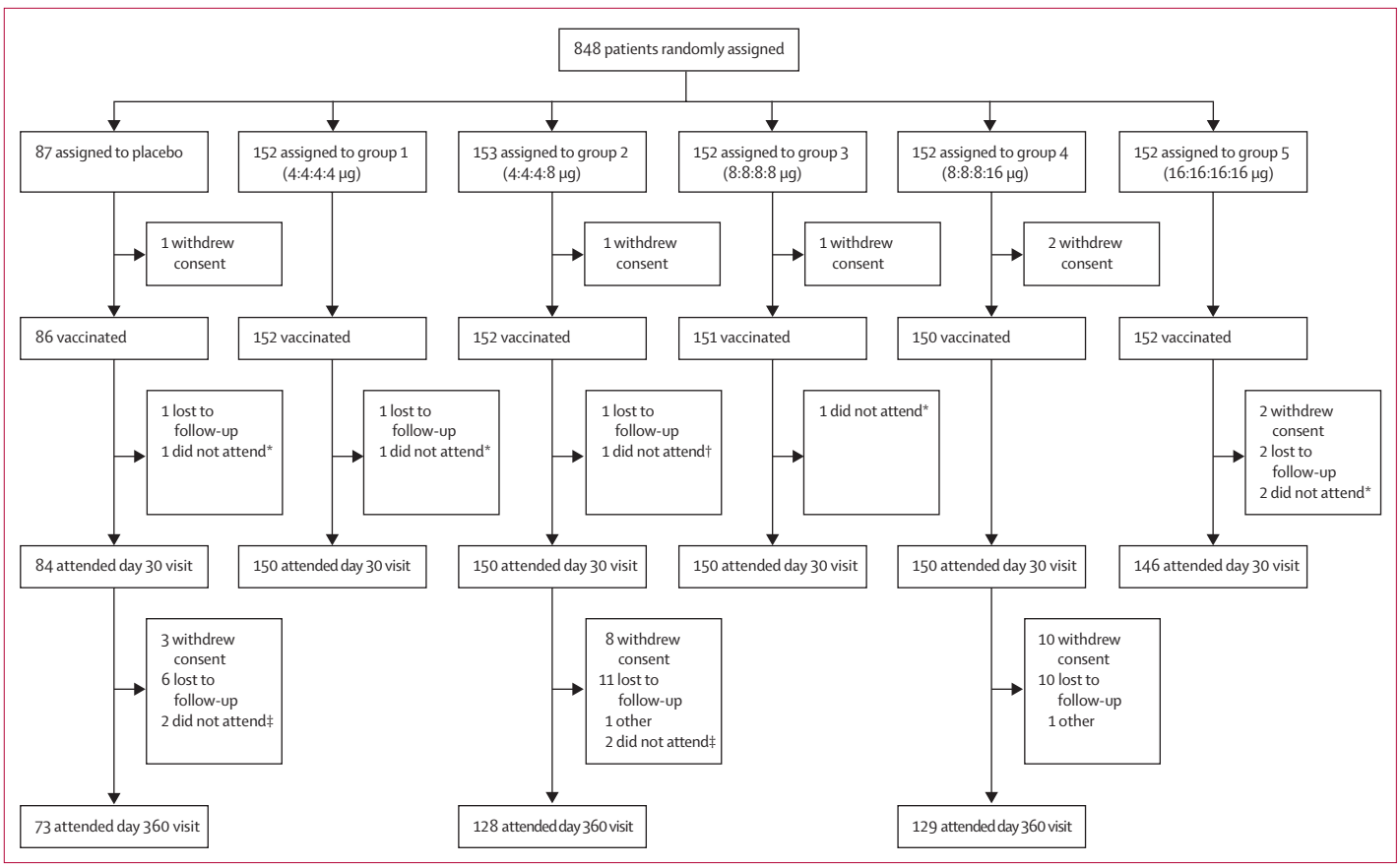

Figure 1: **Study profile**
Antigen 01A:02:06A:025B content of 4:4:4:4 µg (group 1), 4:4:4:8 µg (group 2), 8:8:8:8 µg (group 3), 8:8:8:16 µg (group 4), or 16:16:16:16 µg (group 5). Other reasons were not detailed. *Confirmed as lost to follow-up after day 30. †Confirmed as discontinued owing to physician decision after day 30. ‡ Unknown status, but consent was not gained for long-term follow-up.

≥50-year-old age stratum and 50 in the 18–49-year-old age stratum), was considered adequate to allow detection of common adverse events with a reasonable degree of certainty (>99% probability of detecting adverse events with a 5% rate of occurrence with 150 participants per dose).

Analyses were done using the immunogenicity analysis set (consisting of all participants who were randomly assigned and vaccinated and had at least a baseline antibody titre measurement) and the safety analysis set (consisting of all randomly assigned participants who were vaccinated). Screen failures and randomly assigned participants who were not vaccinated were excluded from the safety analysis set.

The percentage of participants who had at least one occurrence of a solicited or unsolicited adverse event and the median (range) time to onset of any solicited local or systemic adverse event were summarised by treatment group.

A dose-selection immunogenicity algorithm (appendix) was used with day 15 OPK data and day 15 and day 30 ELISA data to identify the dose groups yielding the optimum vaccine-mediated antibody response. For each antigen, several measures of immunogenicity were evaluated, including the proportion of participants with two times or greater increase in serum antibody titres and geometric mean titres. Doses were described for the overall population and by age strata.

This trial is registered with ClinicalTrials.gov, number NCT02546960.

Role of the funding source

The sponsor of the study was involved in all aspects of the study design, data collection, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 848 enrolled participants, 843 (99%) received vaccine or placebo and were included in the safety analysis; 272 (32%) were aged 18–49 years and 571 (67·7%) were aged 50 years or older (figure 1, table 1). 832 participants (98%) were included in the immunogenicity analysis set and 700 (83%) in the

	Placebo (n=86)	Group 1 (n=152)	Group 2 (n=152)	Group 3 (n=151)	Group 4 (n=152)	Group 5 (n=152)	Groups 1–5 combined (n=757)	Total (n=843)
Sex								
Female	41 (48%)	81 (53%)	72 (47%)	78 (52%)	72 (47%)	82 (54%)	385 (51%)	426 (51%)
Male	45 (52%)	71 (47%)	80 (53%)	73 (48%)	80 (53%)	70 (46%)	372 (49%)	417 (49%)
Race								
White	64 (74%)	120 (79%)	117 (77%)	116 (77%)	123 (81%)	112 (74%)	588 (78%)	652 (77%)
Black	20 (23%)	29 (19%)	32 (21%)	31 (21%)	24 (16%)	35 (23%)	151 (20%)	171 (20%)
Asian	1 (1%)	1 (<1%)	0	0	2 (1%)	3 (2%)	6 (<1%)	7 (<1%)
Other	1 (1%)	2 (1%)	3 (2%)	4 (3%)	1 (<1%)	2 (1%)	12 (2%)	13 (2%)
Ethnicity								
Hispanic or Latino	8 (9%)	16 (11%)	16 (11%)	14 (9%)	11 (7%)	15 (10%)	72(10%)	80 (9%)
Not Hispanic or Latino	78 (91%)	132 (87%)	136 (89%)	137 (91%)	138 (91%)	136 (89%)	679 (90%)	757 (90%)
Not reported or unknown	0 (0%)	4 (3%)	0	0	1 (<1%)	1 (<1%)	6 (<1%)	6 (<1%)
Median age (range)	57·0 (21–82)	54·0 (18–79)	56·0 (18–87)	53·0 (19–88)	56·5 (19–85)	55·0 (19–83)	54·0 (18–88)	55·0 (18–88)
Age ≥50 years	62 (72%)	102 (67%)	102 (67%)	102 (68%)	101 (67%)	102 (67%)	509 (67%)	571 (68%)

Values are n (%), unless otherwise specified. Antigen 01A:02:06A:025B content of 4:4:4:4 μg (group 1), 4:4:4:8 μg (group 2), 8:8:8:8 μg (group 3), 8:8:8:16 μg (group 4), or 16:16:16:16 μg (group 5).

Table 1: **Baseline characteristics (total vaccinated cohort)**

	Placebo (n=86)	Group 1 (n=152)	Group 2 (n=152)	Group 3 (n=151)	Group 4 (n=150)	Group 5 (n=152)	Groups 1–5 combined (n=757)	Total (n=843)
Any solicited local adverse event	11 (13%)	41 (27%)	34 (22%)	42 (28%)	47 (31%)	58 (38%)	222 (29%)	233 (28%)
Pain or tenderness	10 (12%)	38 (25%)	30 (20%)	40 (26%)	43 (29%)	54 (36%)	205 (27%)	215 (26%)
Erythema	1 (1%)	5 (3%)	6 (4%)	6 (4%)	7 (5%)	8 (5%)	32 (4%)	33 (4%)
Swelling or induration	0	2 (1%)	3 (2%)	3 (2%)	4 (3%)	8 (5%)	20 (3%)	20 (2%)
Other	1 (1%)	2 (1%)	4 (3%)	2 (1%)	5 (3%)	7 (5%)	20 (3%)	21 (2%)
Median days to onset of any solicited local adverse event (range)	1·0 (1–2)	2·0 (1–8)	2·5 (1–8)	2·0 (1–8)	2·0 (1–8)	6·0 (1–8)	2·0 (1–8)	2·0 (1–28)
Any solicited systemic adverse event	30 (35%)	66 (43%)	54 (36%)	62 (41%)	65 (43%)	78 (51%)	325 (43%)	355 (42%)
Fatigue	13 (15%)	48 (32%)	32 (21%)	47 (31%)	35 (23%)	47 (31%)	209 (28%)	222 (26%)
Headache	16 (19%)	45 (30%)	25 (16%)	27 (18%)	37 (25%)	47 (31%)	181 (24%)	197 (23%)
Nausea	7 (8%)	13 (9%)	9 (6%)	22 (15%)	17 (11%)	26 (17%)	87 (11%)	94 (11%)
Myalgia	10 (12%)	29 (19%)	26 (17%)	32 (21%)	34 (23%)	34 (22%)	155 (20%)	165 (20%)
Malaise	9 (10%)	28 (18%)	18 (12%)	26 (17%)	24 (16%)	36 (24%)	132 (17%)	141 (17%)
Fever (≥38·0°C)	0	1 (<1%)	0	1 (<1%)	0	3 (2%)	5 (<1%)	5 (<1%)
Other	0	0	0	0	2 (1%)	0	2 (<1%)	2 (<1%)
Median days to onset of any solicited systemic adverse events (range)	1·0 (1–8)	2·0 (1–7)	2·0 (1–8)	2·0 (1–8)	1·0 (1–8)	2·0 (1–8)	2·0 (1–8)	2·0 (1–8)

Values are n (%), unless otherwise specified. Antigen 01A:02:06A:025B content of 4:4:4:4 μg (group 1), 4:4:4:8 μg (group 2), 8:8:8:8 μg (group 3), 8:8:8:16 μg (group 4), or 16:16:16:16 μg (group 5). Events were graded on the basis of US Food & Drug Adminstration guidance.[10]

Table 2: **Reported solicited local and systemic adverse events**

per-protocol immunogenicity analysis set. 119 (14%) participants withdrew from the study; 71 (26%) of 275 were aged 18–49 years and 48 (8%) of 573 were aged 50 years or older. Reasons for study withdrawal were similar across the treatment and placebo groups (figure 1). Demographic and baseline characteristics were balanced across study groups (table 1). The median age of the participants was 55·0 years (range 18–88), 426 (51%) of 843 were women, and 652 (77%) were white (table 1).

ExPEC4V was well tolerated by participants with no major safety concerns. The frequency of solicited local adverse events was numerically higher in participants receiving ExPEC4V (41 [27%] of 152 in group 1, 34 [22%] of 152 in group 2, 42 [28%] of 151 in group 3, 47 [31%] of 150 in group 4, and 58 [38%] of 152 in group 5) than in

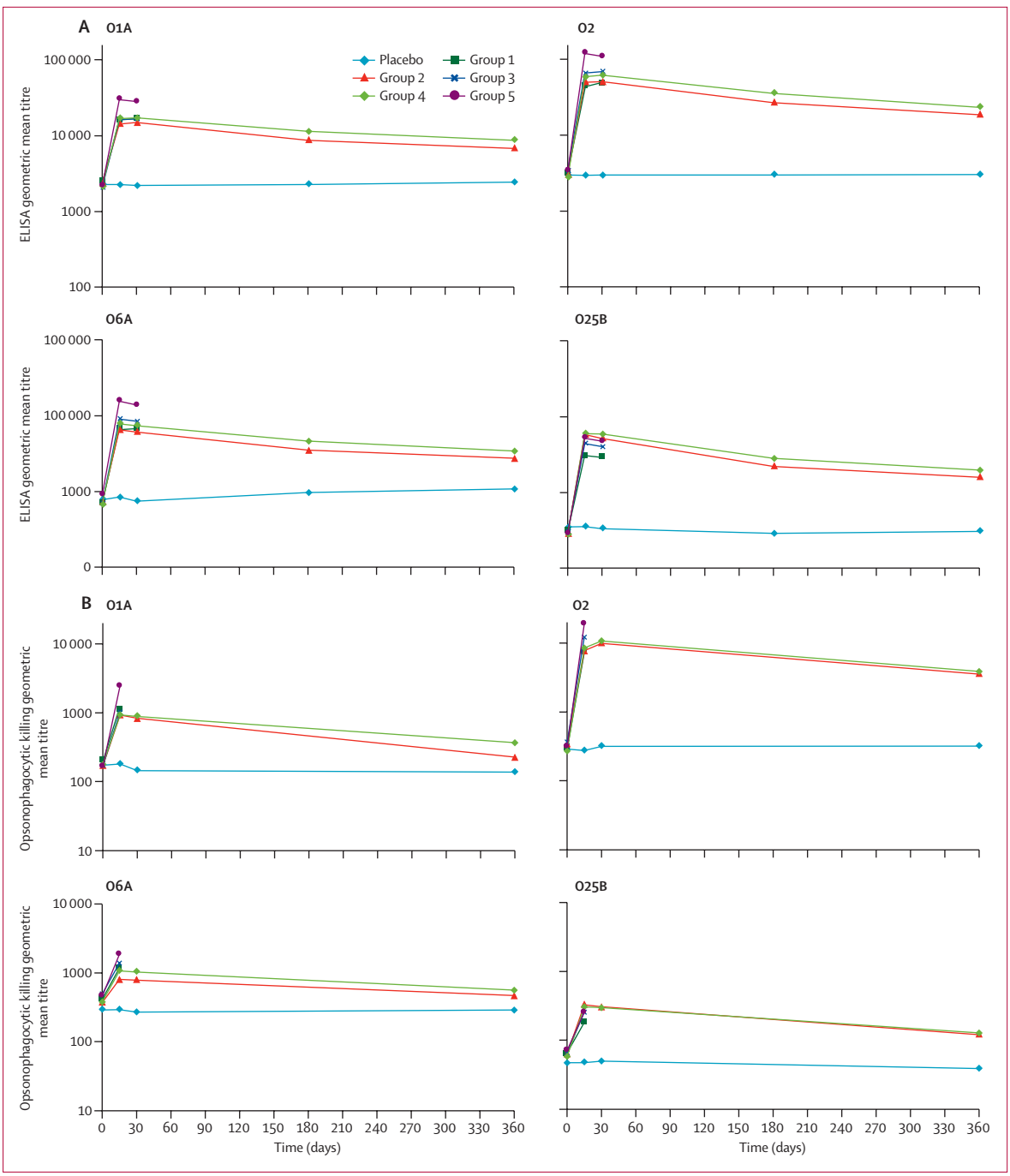

Figure 2: **Vaccine-induced antibody titres in participants receiving ExPECV4, by serotype**
(A) Vaccine-induced IgG antibody titres. (B) Vaccine-induced opsonophagocytic killing assay (functional) antibody titres.

those receiving the placebo (11 [13%] of 86; table 2). A similar pattern was noted for the frequency of solicited systemic adverse events (table 2). Solicited local and systemic adverse events were reported most frequently by group 5 (highest antigen dose).

The most frequently reported solicited local adverse event in the combined ExPEC4V groups versus placebo was pain or tenderness (205 [27%] of 757 *vs* 10 [12%] of 86; table 2). The most frequent solicited systemic adverse events were fatigue (209 [28%] of 757 *vs* 13 [15%] of 86), headache (181 [24%] of 757 *vs* 16 [19%] of 86), and myalgia (155 [20%] of 757 *vs* ten [12%] of 86; table 2). Solicited adverse events were primarily grade 1 or 2 in severity and only 13 (2%) of 843 participants had a grade 3 solicited

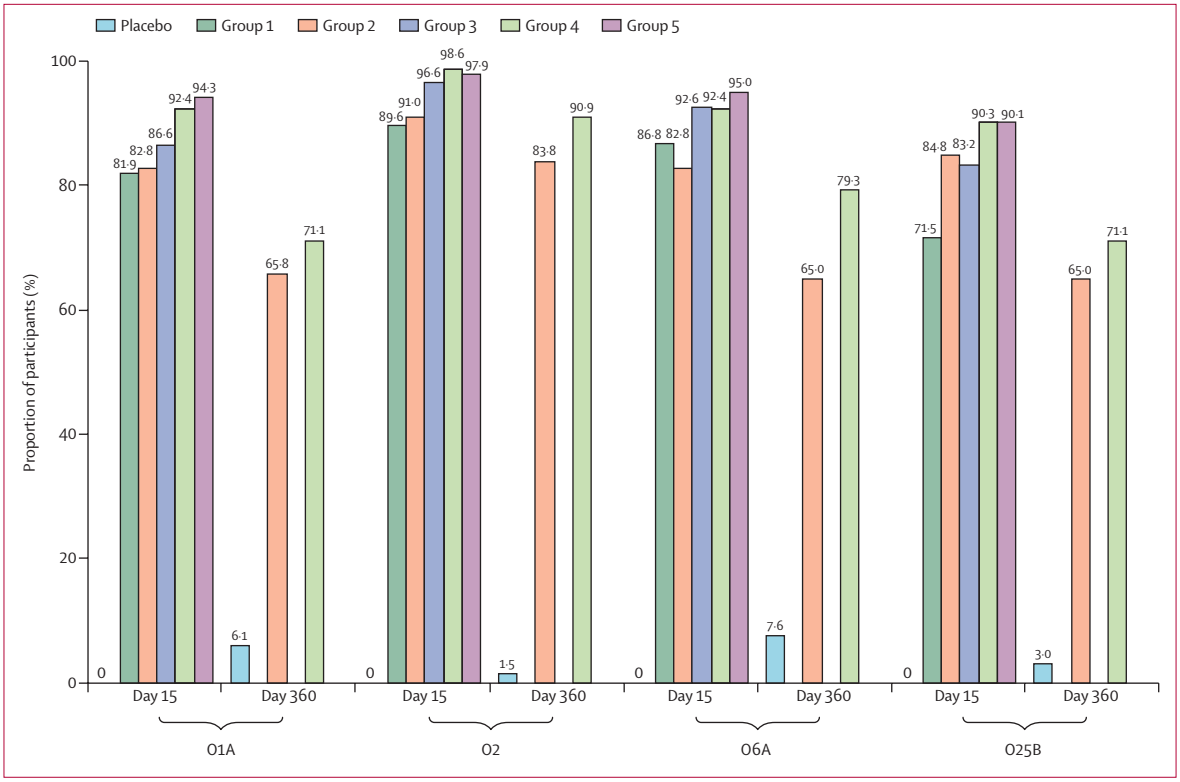

Figure 3: **Percentage of participants with a two times increase in ELISA IgG at days 15 and 360**

adverse event (appendix). No participants had grade 3 fever (≥38°C).

Unsolicited adverse events were reported in 241 (29%) participants overall, ranging from 40 of (26%) 152 (group 2) to 48 (32%) of 51 (group 3) compared with 23 (27%) of 86 in the placebo group. The most common unsolicited adverse events in the combined ExPEC4V groups versus placebo were upper respiratory tract infection (15 [2%] of 757 *vs* two [2%] of 86), diarrhoea (13 [2%] of 757 *vs* one [1%] of 86), neutropenia (12 [2%] of 757 *vs* one [1%] of 86), and anaemia (ten [1%] 757 *vs* one [1%] of 86).

One serious adverse event, trigeminal neuralgia, was reported in a participant in group 5 who had a history of shingles and a family history of trigeminal neuralgia, and had severe right-sided facial pain 87 days after vaccination. Because of the prolonged time-to-onset and the absence of a biologically plausible mechanism for the vaccine to cause trigeminal nerve compression, the event was considered unrelated to the vaccine (appendix). Three participants died during the double-blind phase of the study; none were considered related to the study vaccine.

Incidences and types of adverse events were similar in the 18–49 years and 50 years or older age cohorts (appendix).

Robust vaccine-induced ELISA IgG antibody responses were observed for all vaccine serotypes (figure 2A). Maximum titres were generally observed by day 15, with little or no further increase by day 30.

At day 15, a two times or greater increase in IgG antibodies was achieved in more than 80% of participants in all groups and for all serotypes, with the exception of O25B at the lowest dose (group 1, 103 [72%] of 144; appendix). Day 15 geometric mean titres were highest in group 5 for three out of four serotypes (O1A, O2, and O6A). Geometric mean titres for serotype O25B were highest in groups 2 and 4, but only slightly lower in groups 3 and 5 (figure 2A; appendix).

Although the immunogenicity dose-selection algorithm consistently selected the 16:16:16:16 μg dose (group 5) as the most immunogenic dose, 4:4:4:8 μg (group 2) and 8:8:8:16 μg (group 4) with selective increases in the O25B dose were chosen for further clinical evaluation, because of the potential immune interference for O25B (lower O25B titre in group 5) and a suggested dose-related increase in reactogenicity.

Durable serotype-specific antibody responses were observed in groups 2 and 4 up to day 360, with 65% or more in group 2 and 71% or more in group 4 maintaining a two times or greater increase in IgG from baseline (figure 3).

In this study at least 25% of participants had OPK titres of 100 or more at baseline, with the lowest initial titres observed for serotype O25B (figure 4). All ExPEC4V vaccines induced increases in functional antibodies to each serotype (figure 2B). At day 15, at least 70% of recipients of the ExPEC4V vaccine had OPK titres of 100 or more for each serotype. There was no consistent

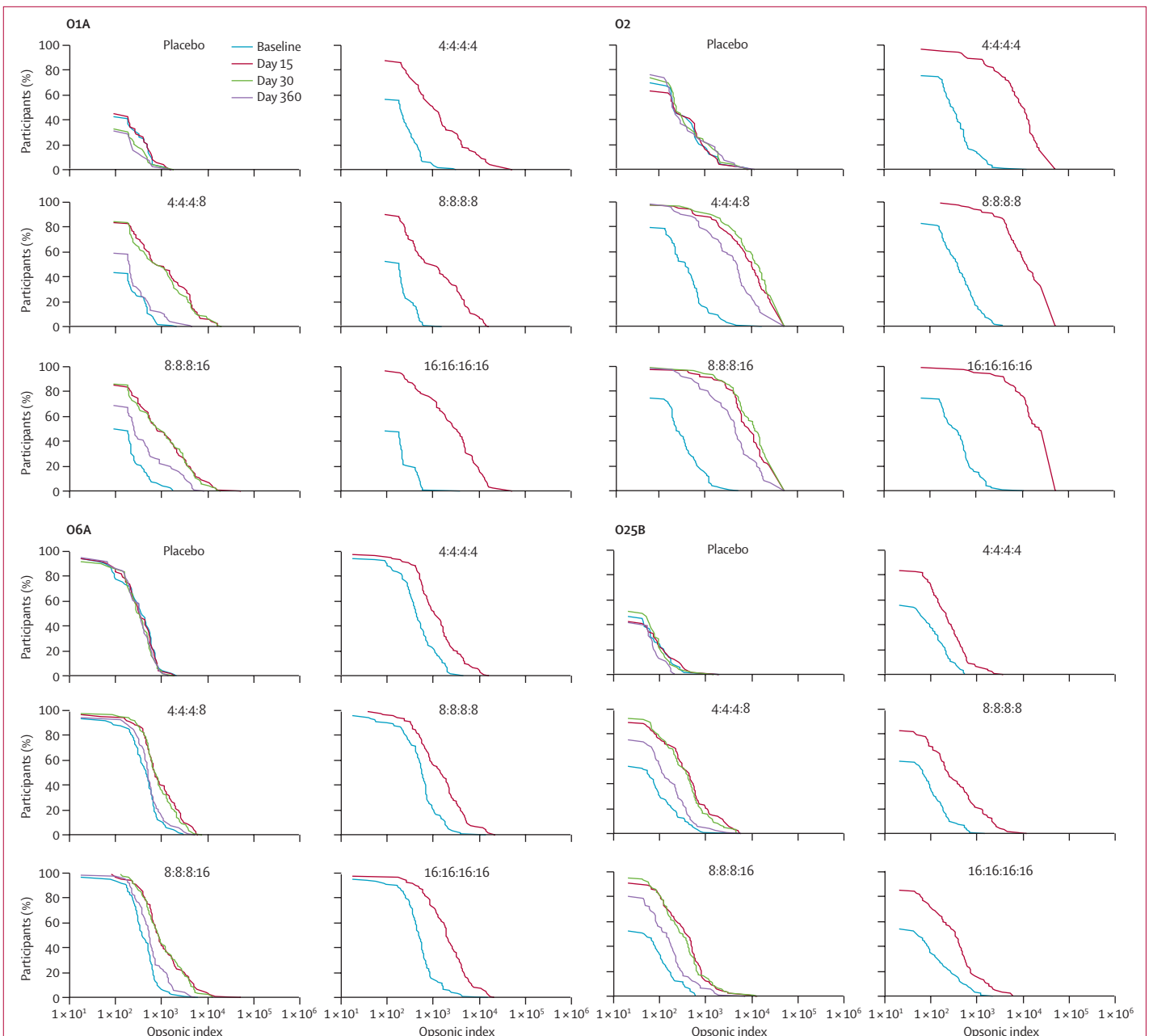

Figure 4: **Reverse cumulative distribution curves for opsonophagocytic killing assays by serotype and study vaccine group**

dose-related effect on OPK geometric mean titres (appendix).

Per serotype geometric mean increases from baseline in groups 2 and 4 ranged from 2·1 times to 26·6 times at day 15, and from 1·2 times to 12·7 times at day 360 (data no shown). At least 55% of participants maintained OPK titres of 100 or more until day 360 (figure 4).

Correlations between IgG antibody titres (ELISA) and OPK titres on day 15 were strong for serotypes O1A and O2 ($r \geq 0{\cdot}75$), and moderate for O6A and O25B ($r = 0{\cdot}61$–$0{\cdot}68$; appendix). Similar trends were observed at day 360 ($r = 0{\cdot}48$–$0{\cdot}90$).

Analysis of stool samples from vaccine or placebo recipients by qPCR for changes in the relative abundance of *E coli* serotypes O1A, O2, O6A, or O25B at days 1 (before vaccination), 30, and 180 showed that no ExPEC4V serotype was differentially abundant in samples from day 1 compared with day 30, or day 30 compared with day 180 in either the vaccine or placebo group (appendix). Evaluation of stools for metagenomic

changes to other (non-*E coli*) bacteria was done using 16S RNA analysis. No differences in microbiome structure or composition, including pathogenic bacteria such as *C difficile*, were detected in participants during different visits in either treatment group (data not shown). Thus, no changes in stool metagenomic composition for *E coli* serotypes or other species were detected over 180 days in response to vaccination with ExPEC4V.

Discussion

The increasing global incidence and infectious disease burden associated with ExPEC, coupled with multidrug resistance, makes the need for an effective vaccine against ExPEC infections urgent. No commercially available ExPEC vaccines exist. Building on a phase 1 study that showed that ExPEC4V was immunogenic and had a clinically acceptable safety profile in healthy women, this study explored the dose needed to maximise immunogenicity up to 1 year after vaccination.

All doses of the ExPEC4V vaccine were well tolerated, although a dose-related trend in the incidence of local and systemic symptoms occurred, with the highest incidences observed in the highest dose group (group 5). Solicited adverse events were generally mild-to-moderate in severity, with the most commonly reported solicited adverse events being fatigue and injection site pain or tenderness.

All doses of ExPEC4V elicited robust increases in IgG and functional antibodies across all four serotypes, with more than 80% of participants overall showing a two times or greater increase in vaccine-mediated ELISA IgG titres by day 15 (with the exception of the lowest dose with serotype O25B; 72%). The 4:4:4:8 µg (group 2) and 8:8:8:16 µg (group 4) doses were evaluated up to day 360 and showed durable IgG titres and functional antibody responses up to this timepoint, the last one evaluated to date.

Development of a multivalent ExPEC conjugate vaccine shares some of the same challenges addressed during the development of pneumococcal vaccines, including identification and measurement of a protective immune responses and the absence of a serological correlate of protection.[11] A two times or more increase in antibody titre 2–3 weeks after vaccination is a relevant measure for pneumococcal polysaccharide vaccines and was observed in 80% of young vaccinated adults, with vaccine effectiveness in case-control studies including older adults ranging from 56% to 81%.[12] This finding implies that a two times or greater increase in antibody titre in 80% of individuals could be a reasonable measure of immunogenicity for ExPEC vaccines. We observed serotype-specific two times or more increases in antibody responses in more than 80% of ExPEC4V recipients in all groups except at the lowest dosage, although whether this threshold will translate into clinical effectiveness is still to be determined.

Pre-existing IgG antibodies to *E coli* serotypes are not unexpected given that humans are actively colonised with these serotypes. The high percentage of participants with pre-existing IgG and functional antibodies in the USA, particularly to serotype O6A, was observed in the phase 1 study involving Swiss participants,[8] possibly reflecting higher carriage or exposure to this serotype. Nevertheless, vaccination induced robust increases in functional antibodies for all serotypes, with at least 70% reaching the conservative OPK threshold of 100, a threshold likely to be sufficient for protection based on previous experience with meningococcal and pneumococcal conjugate vaccines. Of note, antibody titres and functional antibody responses decreased until 1 year after vaccination but remained well above titres before vaccination. The threshold antibody response needed for protection against ExPEC bacteraemia is not known. Drawing on experience with pneumococcal conjugate vaccines, OPK responses might correlate more closely to clinical protection than ELISA antibody titres.[13,14] In the Community Acquired Pneumonia immunization Trial in Adults study of 13-valent pneumococcal conjugate vaccine in older adults,[15] about 70–80% of participants achieved OPK titres of 100 or more after vaccination, decreasing over time to as low as 10% by year 1 for some serotypes. Despite decreasing OPK titres, vaccine efficacy against community-acquired pneumococcal pneumonia due to vaccine serotypes was sustained throughout 4 years of follow-up.[16] Robust and persistent functional antibody responses in a similar percentage of participants in our study are promising, potentially implying good efficacy against ExPEC bacteraemia that could be sustained over several years. The four serotypes in ExPEC4V were selected on the basis of their prevalence of O-serotypes from bacteraemic isolates (Poolman J, Janssen Vaccines & Prevention, Leiden, Netherlands, personal communication). The degree to which these serotypes will provide cross-protection is not yet known, but it seems likely that vaccines containing more serotypes would be required for broader coverage. Available serotype data indicate that a vaccine targeting ten to 12 O serotypes would account for more than 60% of bacteraemia isolates and about 90% of meningitis isolates.[8]

The limitations of the study relate to the absence of available immunogenicity data on which to base definitions of immunogenicity threshold for protection. Although ESTELLA is a phase 2 study, it was done using a large sample size covering a broad age range, with a long follow-up period, which will continue up to 3 years after vaccination.

Strengths of the study included exploration of multiple vaccination dosages, including formulations with enhanced concentrations of O25B polysaccharide that were eventually selected for long-term follow-up. Additional O25B polysaccharide was included in some dose formulations because of the clinical importance and the prevalence of antibiotic resistance in this serotype.

All doses of the candidate ExPEC4V bioconjugated vaccine were well tolerated and elicited robust and functional antibody responses against each of the four vaccine serotypes. Two dosages (4:4:4:8 μg and 8:8:8:16 μg) showed durability of the immune response for 1 year.

Contributors

DA, JP, ST, and PIdP participated in study conception and design. RWF Jr, DA, OG, WH, and PIdP participated in acquisition of data. RWF Jr, DA, BS, OG, WH, ST, and PIdP did the data analysis. All authors participated in interpretation of data. All authors participated in development of manuscript.

Declaration of interests

DA, BS, OG, WH, GvdD, JP, ST, and PIdP are employees of Janssen, Pharmaceutical Companies of Johnson & Johnson, and may be Johnson & Johnson stockholders.

Data sharing

The data sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available on their Clinical Trial Data Transparency website. As noted on this site, requests for access to the study data can be submitted through Yale Open Data Access Project site.

For **Janssen's Clinical Trial Data Transparency website** see https://www.janssen.com/clinical-trials/transparency

For **Yale Open Data Access Project** see http://yoda.yale.edu

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