Safety, immunogenicity, and preliminary clinical efficacy of a vaccine against extraintestinal pathogenic *Escherichia coli* in women with a history of recurrent urinary tract infection: a randomised, single-blind, placebo-controlled phase 1b trial

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Summary

Background *Escherichia coli* infections are increasing worldwide in community and hospital settings. The *E coli* O-antigen is a promising vaccine target. We aimed to assess the safety and immunogenicity of a bioconjugate vaccine containing the O-antigens of four *E coli* serotypes (ExPEC4V).

Methods In this multicentre phase 1b, first-in-human, single-blind, placebo-controlled trial, we randomly assigned (1:1) healthy adult women with a history of recurrent urinary tract infection (UTI) to receive a single injection of either intramuscular ExPEC4V or placebo. The primary outcome was the incidence of adverse events among vaccine and placebo recipients throughout the study. Secondary outcomes included immunogenicity and antibody functionality, and the incidence of UTIs caused by *E coli* vaccine serotypes in each group. This study is registered with ClinicalTrials.gov, number NCT02289794.

Findings Between Jan 20, 2014, and Aug 27, 2014, 93 women received target-dose ExPEC4V and 95 received placebo. The vaccine was well tolerated: no vaccine-related serious adverse events occurred. Overall, 56 (60%) target-dose vaccines and 47 (49%) placebo recipients experienced at least one adverse event that was possibly, probably, or certainly related to injection. Vaccination induced significant IgG responses for all serotypes: at day 30 compared with baseline, O1A titres were 4·6 times higher, O2 titres were 9·4 times higher, O6A titres were 4·9 times higher, and O25B titres were 5·9 times higher (overall $p<0.0001$). Immune responses persisted at 270 days but were lower than those at 30 days. Opsonophagocytic killing activity showed antibody functionality. No reduction in the incidence of UTIs with 10^3 or more colony-forming units per mL of vaccine-serotype *E coli* was noted in the vaccine compared with the placebo group (0·149 mean episodes *vs* 0·146 mean episodes; $p=0.522$). In post-hoc exploratory analyses of UTIs with higher bacterial counts ($\geq 10^5$ colony-forming units per mL), the number of vaccine serotype UTIs did not differ significantly between groups (0·046 mean episodes in the vaccine group *vs* 0·110 mean episodes in the placebo group; $p=0.074$). However, significantly fewer UTIs caused by *E coli* of any serotype were noted in the vaccine group compared with the placebo group (0·207 mean episodes *vs* 0·463 mean episodes; $p=0.002$).

Interpretation This tetravalent *E coli* bioconjugate vaccine candidate was well tolerated and elicited functional antibody responses against all vaccine serotypes. Phase 2 studies have been initiated to confirm these findings.

Funding GlycoVaxyn, Janssen Vaccines.

Introduction

Extraintestinal pathogenic *Escherichia coli* is the most frequent cause of bloodstream and urinary tract infections (UTIs),[1,2] and a noteworthy cause of other community-associated and health-care-associated infections.[3] The worldwide emergence of multidrug-resistant extraintestinal pathogenic *E coli* has resulted in increased incidence of treatment failure, hospital admissions, and mortality, and intensified the economic burden on health-care systems and communities.[2,4] With an inadequate antibiotic pipeline[5] and annual costs associated with UTIs and septicaemia in the USA of more than US$2 billion and $20 billion, respectively, a

prophylactic vaccine targeting extraintestinal pathogenic *E coli* is urgently needed.[6]

The surface lipopolysaccharide-linked O-antigen contributes to *E coli* survival,[7] and the production of anti-O-antigen antibodies confers protective effects against *E coli* infections.[8] The clinical efficacy of multivalent glycoconjugate vaccines against *Haemophilus influenzae* type b, pneumococcal and meningococcal bacteraemia, and meningitis further supports the use of surface polysaccharides in conjugate vaccine development.[9–12] Although chemically conjugated *E coli* O-antigen vaccines seem to be safe and immunogenic in human beings,[13,14] production of multiple O-conjugates is technically

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Research in context

Evidence before this study

We did a literature review of the cause and epidemiology of urinary tract infections, current therapies, and vaccine candidates. We searched the MEDLINE databases with the terms "urinary tract infection", "cystitis", and "uropathogenic *Escherichia coli*" for articles published in English between Jan 1, 1980, and Jan 13, 2017 (the date of our final search). Search terms were subsequently combined with: "placebo-controlled clinical trial", "aetiology", "epidemiology", "prevalence", "pathogenesis", "therapy", or "vaccine". Some papers cited in the screened publications were also included, as were guidelines on urological infections, additional articles on antibiotic resistance and uropathogenic *E coli* pathogenicity, and information from company websites, government agencies, and the ClinicalTrials.gov registry.

Although we identified two vaccine candidates (targeting *E coli* and *Proteus mirabilis*, respectively) undergoing testing in murine models, we identified no other vaccine candidates in clinical development (human testing) targeting uropathogenic *E coli* or urinary tract infections in general. Additionally, we did an epidemiological study of urinary tract infections in community and hospital settings throughout Switzerland to assess the prevalence of *E coli* associated with urinary tract infections and to identify the O-serogroups of the predominant strains (unpublished). Pathogens associated with more than 3000 urinary tract infections were characterised and more than 1800 *E coli* strains isolated from urine were collected to identify the main O-serogroups.

Added value of this study

Our study shows a strong safety profile and high immunogenicity with durable and functional immune responses for the parenteral, multivalent *E coli* O-antigen bioconjugate vaccine. Additionally, this first-in human trial shows moderate protective efficacy against urinary tract infections with bacterial loads of 10^5 colony-forming units per mL or more. Collectively these findings support an early proof of concept for the development of *E coli* bioconjugate vaccines.

Implications of all the available evidence

Increases in antibiotic-resistant bacteria causing pyelonephritis and urosepsis call for further development of preventive approaches. Our study suggests that there is the potential for further investigation of a parenteral bioconjugate vaccine for prevention of extraintestinal *E coli* infections.

difficult.[15] Bioconjugation is an innovative technique allowing in-vivo synthesis and conjugation of polysaccharide structures to carrier proteins via appropriately engineered bacterial cells.[16–18] We previously used the protein glycosylation machinery of *E coli* to produce a conjugate vaccine incorporating a genetically detoxified form of exoprotein A from *Pseudomonas aeruginosa* linked to the four O-serotype surface polysaccharide antigens of *E coli* serotypes.[19]

We did a multicentre phase 1b first-in-human trial to assess the safety, immunogenicity, and preliminary clinical efficacy of the tetravalent bioconjugate candidate vaccine, ExPEC4V, in healthy women with a history of recurrent UTI.

See Online for appendix

Methods

Study design and participants

We did a phase 1, first-in-human, staggered, randomised, placebo-controlled single-blind trial in 13 medical centres in Switzerland. Eligible participants were healthy women aged 18 to 70 years with a self-reported clinical history of recurrent UTIs (ie, two infections in the past 6 months, or three in the past year) and at least one urine culture with documented *E coli* growth in the preceding 5 years, and were in general good health, without clinically significant medical history, physical examination findings, or clinical laboratory abnormalities as per clinical judgment of the investigator. Pregnant or lactating women; women with acute urinary tract disease or infection, HIV infection, or uncontrolled diabetes mellitus; and women who planned to use postcoital antibiotics as secondary prophylaxis or had previously used (ie, within the preceding 3 months) or planned to use immune stimulatory therapy (ie, Urovaxom, Strovac, or Urovac) for prevention of UTIs were ineligible for inclusion. Patients were either attending the health centre for reasons other than UTIs, or specifically attended because they saw flyers or advertisements for the study. The study and all protocol amendments were reviewed and approved by the independent ethics committees of each site and by the Swiss Agency for Therapeutic Products (Swissmedic). All participants provided written, informed consent before their inclusion. Additional exclusion criteria, ethics committee review, and consent procedures and the work of the safety monitoring board are detailed in the appendix.

Randomisation and masking

Participants were enrolled by study investigators. The randomisation sequence was computer-generated by Clinipace (Morrisville, NC, USA) with randomly permuted blocks of varying sizes. Once a participant was included, the study investigator at the study site signalled the inclusion electronically, and the system then revealed the allocation to that investigator immediately before injection (participants could not see the allocation on the screen). The first eight participants were randomly assigned in a 3:1 ratio to either the vaccine group or the placebo group. Another eight participants were then randomly assigned in the same ratio. Thereafter, participants were randomly allocated to both groups in a 1:1 ratio. Participants were

blinded to their treatment group throughout the study, but investigators, study site staff, and those who did the data analysis were unblinded. Vaccine and placebo filled syringes were indistinguishable and labelled in a coded manner.

Procedures

The ExPEC4V candidate vaccine consisted of four bioconjugates containing the O-antigens of serotypes O1A, O2, O6A, and O25B— the four extraintestinal pathogenic *E coli* serotypes most frequently noted in a pretrial epidemiological survey of Swiss urinary isolates (unpublished). These serotypes were also the most prevalent serotypes causing *E coli* bacteraemia (unpublished). Each O-antigen surface polysaccharide was conjugated to detoxified *Pseudomonas aeruginosa* exotoxin A carrier protein (EPA) as previously described.[18] The suspension for parenteral application was formulated in Tris-buffered saline, pH 7·4 (25 mmol Tris, 137 mmol NaCl, 2·7 mmol KCl). Placebo consisted of buffer only.

In a staggered safety approach, the first eight participants received a single 0·5 mL intradeltoid injection of either reduced-dose ExPEC4V (1 μg of each surface polysaccharide) or placebo. In the absence of safety signals, the next eight participants received either target-dose ExPEC4V (4 μg of each surface polysaccharide) or placebo;[20] thereafter, all remaining participants received either target-dose ExPEC4V or placebo. Participants were followed up for 9 months, with on-site visits for clinical and laboratory assessments on days 1 (ie, the day of injection), 7, 30, and 270 and telephone check-ups on days 2, 90, 150, and 210.

All adverse events in the 7 days after injection were recorded on diary cards. Solicited adverse events were fever and local pain, swelling, or erythema in the 7 days after injection. Unsolicited adverse events were recorded from days 1 to 30. Serious adverse events, medically attended adverse events, or adverse events deemed related to vaccination by site investigators were recorded from day 1 until study termination. Severity was assessed according to the Common Terminology Criteria for Adverse Events (version 4.0). Laboratory safety analyses included complete blood count, creatinine, and liver function tests.

Whole blood was collected on day 1 and on days 30 and 270. Serum concentrations of IgG antibodies to vaccine serotypes were quantified by ELISA, and an in-vitro opsonophagocytic killing (OPK) assay was used to estimate functionality of vaccine-induced antibodies (appendix). Participants were asked to attend their recruiting health-care centre if they developed UTI symptoms, and to provide a clean-catch, midstream urine specimen for culture. Isolates were tested for antibiotic resistance, and *E coli* isolates were O-serotyped (appendix). UTIs were defined by the presence of at least one specified symptom (dysuria, urgency, frequency, flank pain,

bladder tenderness, suprapubic pain, fever, or nausea and vomiting) and a midstream urine culture with bacterial counts of 10^3 colony-forming units (CFU) per mL or more.[21] Confirmed infections were treated with standard antibiotic therapy at investigators' discretion.

Outcomes

Our primary outcome was incidence of adverse events among vaccine and placebo recipients throughout the study. Secondary outcomes were immunogenicity as measured by IgG geometric mean titres and antibody functionality by OPK assay, the number and incidence of UTIs caused by *E coli* vaccine serotypes, and the intensity and duration of clinical symptoms. Exploratory outcomes were incidence of UTIs caused by any *E coli* serotype or any other pathogen.

Statistical analysis

Although the primary focus was the safety of ExPEC4V, we chose a sample size suitable to assess efficacy trends and thus provide a clinical proof of concept. Sample size was calculated to detect a 64% decrease in the incidence of vaccine-serotype-specific UTIs in the treatment groups compared with the placebo group, with a power of 80%, assuming the incidence of vaccine-serotype-specific infections would be 25% among placebo recipients. This estimate was based on local epidemiological data obtained in the pretrial surveillance study.

Safety, immunogenicity, and efficacy analyses were done in the modified intention-to-treat population, consisting of all participants who were randomly assigned and received an injection. Additional immunogenicity and efficacy analyses were done in the per-protocol population, comprising all randomly assigned and injected participants attending all clinical study visits who had no major protocol deviations that would be considered to have an impact on immunogenicity (ie, the immunogenicity population) or efficacy (ie, the efficacy population). Efficacy analyses included UTIs occurring between days 30 and 270 after vaccination in the per-protocol population and between days 1 and 270 in the intention-to-treat population. Safety results are reported for the modified intention-to-treat population, and efficacy and immunogenicity results for the per-protocol populations, unless otherwise stated.

Geometric mean titres of vaccine-specific serum antibodies and responder rates were summarised with descriptive statistics. We did two-sample t tests for continuous data and χ^2 tests for categorical data. All statistical tests had a significance level of 5%; no adjustments were made for multiple comparisons. For number of UTIs, vaccine efficacy was calculated as 1–(mean number of infections in vaccinees)/(mean infections in placebo group). We calculated 95% CIs and p values on the basis of a normal approximation by using a generalised linear model assuming a Poisson distribution for the number of infections with
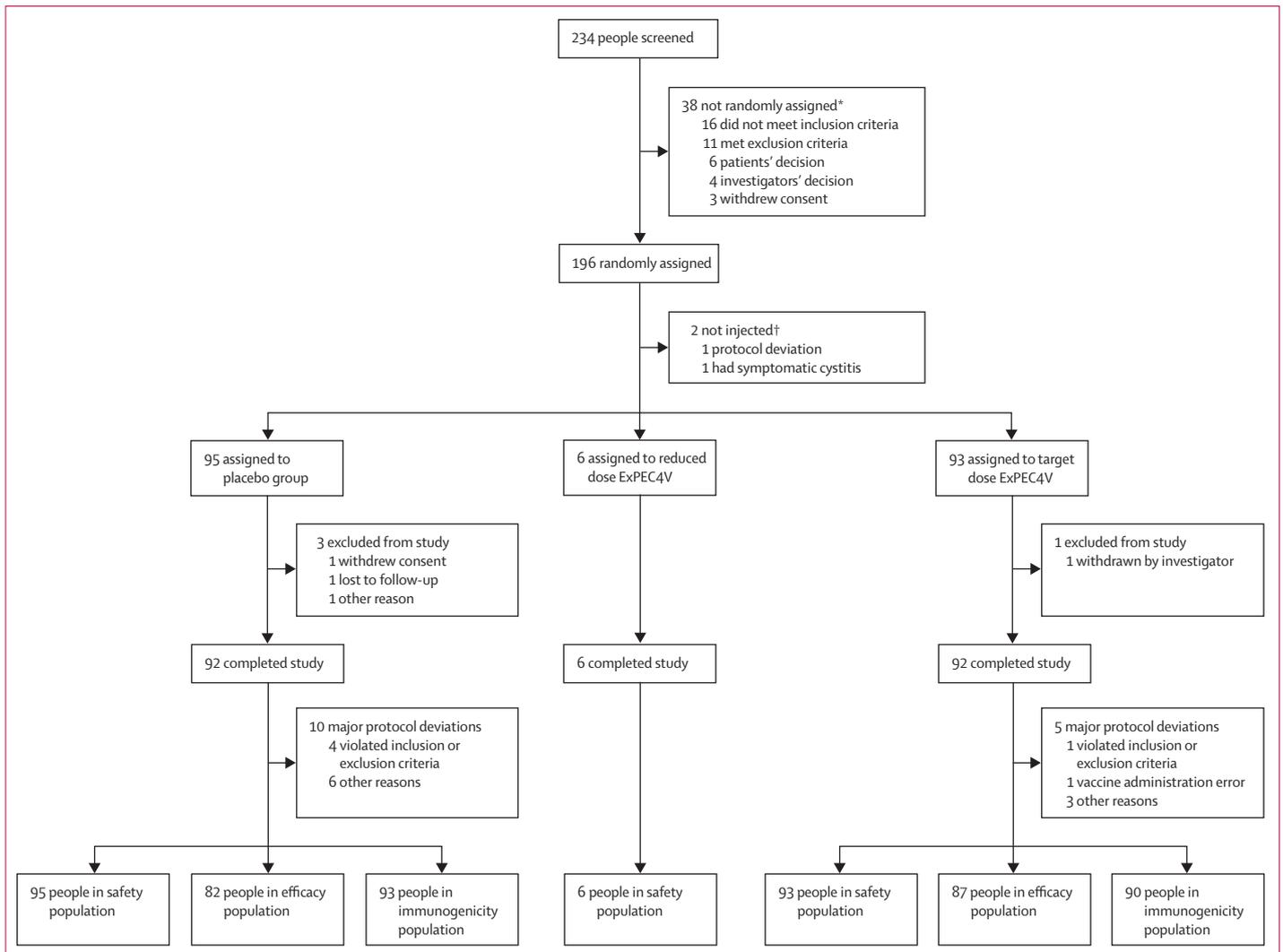
Figure 1: Trial profile
ExPEC4V is a tetravalent bioconjugate candidate vaccine against extraintestinal pathogenic *Escherichia coli*. *Some patients were not randomly assigned for more than one reason. †Both patients were assigned to target dose ExPEC4V.

an offset of log(days in study). The p value tested the null hypothesis—ie, that vaccine efficacy equals 0—against the alternative hypothesis: vaccine efficacy is greater than 0. Analogous efficacy analyses were done post hoc for UTIs with bacterial counts of 10^5 CFU/mL or more.

Role of the funding source
The funder of the study had no role in clinical data collection, data monitoring, safety monitoring, or data analysis. Serological endpoints were analysed by employees of the funder, who were blinded to allocation. Data monitoring was done by an independent monitor (Clinipace). Clinipace statisticians, who were funded by the study funder, did the statistical analysis. All authors

had access to all study data and the results of the analysis and agreed to submit the report in its present form. AHu and SH had final responsibility for the decision to submit for publication.

Results
We recruited participants between Jan 20, 2014, and Aug 27, 2014. 234 women were screened, 196 were included and randomly assigned, and 194 received either reduced-dose vaccine (n=6), target-dose vaccine (n=93), or placebo (n=95; figure 1). 190 participants completed the study (figure 1). Demographic characteristics in the two main treatment groups were similar (table 1). Mean age in both groups was roughly 42 years, women in both groups had a median of four UTIs in the year before

	Placebo (n=95)	ExPEC4V target dose (n=93)
Age, years		
Mean (SD)	41·7 (15·9)	41·6 (17·6)
Median (range)	42·1 (18–71)	38·3 (19–71)
Age category		
18–35 years	39 (41%)	43 (46%)
>35–55 years	34 (36%)	22 (24%)
>55 years	22 (23%)	28 (30 %)
Race		
White	91 (96%)	87 (94%)
Other	4 (4%)	6 (6%)
Body-mass index		
Mean (SD)	23·2 (4·1)	23·3 (3·8)
Median (range)	22·0 (17·8–33·7)	22·7 (17·6–34·3)
Fertility		
Premenopausal	64 (67%)	57 (61%)
Of childbearing potential	55 (86%)	54 (95%)
Not of childbearing potential	9 (14%)	3 (5%)
Postmenopausal	31 (33%)	36 (39%)
<2 years since menopause	5 (16%)	3 (8%)
≥2 years since menopause	26 (84%)	33 (92%)
Total number of UTIs in previous year		
n	95	93
Mean (SD)	4·2 (1·7)	4·2 (1·4)
Median (range)	4 (0–9)	4 (2–9)
Preventive treatment in past year		
Urovaxom	14 (15%)	17 (18%)
Urovac	0	0
Stovac	0	0
Postcoital preventive antibiotic	4 (4%)	5 (5%)
Oestrogen	10 (11%)	12 (13%)
Other	44 (46%)	45 (48%)

Data are n (%), unless otherwise specified. ExPEC4V is a tetravalent bioconjugate candidate vaccine against extraintestinal pathogenic *Escherichia coli*.

Table 1: Characteristics of participants (intention-to-treat population)

	Placebo (n=95)				ExPEC4V (n=93)			
	Any severity	Mild	Moderate	Severe	Any severity	Mild	Moderate	Severe
Any event	47 (49%)	43 (45%)	12 (13%)	..	56 (60%)	52 (56%)	18 (19%)	..
Injection-site erythema	20 (21%)	19 (20%)	1 (1%)	..	28 (30%)	21 (23%)	7 (8%)	..
Injection-site pain	17 (18%)	15 (16%)	2 (2%)	..	29 (31%)	20 (22%)	9 (10%)	..
Injection-site swelling	12 (13%)	9 (9%)	3 (3%)	..	18 (19%)	11 (12%)	7 (8%)	..
Headache	11 (12%)	7 (7%)	4 (4%)	..	16 (17%)	11 (12%)	5 (5%)	..
Nausea	3 (3%)	3 (3%)	..	..	4 (4%)	3 (3%)	1 (1%)	..
Dizziness	..	..	..	..	4 (4%)	3 (3%)	1 (1%)	..
Fever	..	..	..	..	3 (3%)	2 (2%)	1 (1%)	..
Chills	..	..	..	..	2 (2%)	2 (2%)	..	..
Diarrhoea	..	..	..	..	2 (2%)	1 (1%)	1 (1%)	..
Dysgeusia	..	..	..	..	2 (2%)	1 (1%)	1 (1%)	..
Extremity pain	..	..	..	..	2 (2%)	2 (2%)	..	..
Hyperhidrosis	..	..	..	..	2 (2%)	1 (1%)	1 (1%)	..
Injection-site warmth	..	..	..	..	2 (2%)	2 (2%)	..	..
Upper abdominal pain	..	..	..	..	2 (2%)	1 (1%)	1 (1%)	..

Data are n (%), unless otherwise specified. ExPEC4V is a tetravalent bioconjugate candidate vaccine against extraintestinal pathogenic *Escherichia coli*.

Table 2: Incidence of the most common adverse events (occurring in ≥2% of participants) thought to be related to study treatment

inclusion, and use of pretrial prophylaxis was broadly similar between groups (table 1).

The vaccine candidate was well tolerated: we noted no vaccine-related severe adverse events or no vaccine-related serious adverse events throughout the 9 month follow-up. Overall, 56 (60%) of 93 target-dose vaccines and 47 (49%) of 95 placebo recipients experienced at least one adverse event that was possibly, probably, or certainly related to injection (table 2; appendix). Solicited events occurred in 38 (41%) target-dose vaccinees and 32 (34%) placebo recipients (p=0·309; appendix). No significant differences were noted in the frequency of solicited adverse events (appendix). Solicited injection-site pain occurred more frequently in target-dose vaccines than in placebo recipients but this difference was not significant (26 [28%] of 93 vs 16 [17%] of 95; p=0·067; appendix). Local injection-site reactions were transient and generally

mild in nature (data not shown). We detected no clinically notable differences in haematological or biochemical parameters before injection and at days 7 and 30 in either group (data not shown).

At days 30 and 270, ExPEC4V induced robust antibody responses to all four serotypes compared with both placebo and baseline (figure 2). IgG geometric mean titres at day 30 were 9460 for O1A, 27 973 for O2, 4475 for O6A, and 2164 for O25B, compared with 1887, 3502, 953, and 282, respectively, in the placebo group (table 3). From baseline, median individual titres of O1A were 4·6-times increased, of O2 were 9·4-times increased, of O6A were 4·9-times increased, and of O25B were 5·9-times increased in the target-dose group (overall p<0·0001). At day 270, geometric mean titres in the ExPEC4V group had decreased slightly compared with titres on day 30, but remained significantly increased from baseline for all serotypes (p<0·0001; table 3; appendix).

The functionality of vaccine-induced antibodies as measured by OPK was robust: day 30 OPK geometric mean titres in the ExPEC4V group were significantly higher compared with baseline for all vaccine serotypes (p<0·0001; appendix). The OPK geometric mean titres at 30 days were 951 for O1A, 4132 for O2, 1542 for O6A, and 415 for O25B, corresponding to median individual titre increases of 3·5 times, 15 times, 1·4 times, and 2·5 times, respectively, from baseline (table 3; appendix).
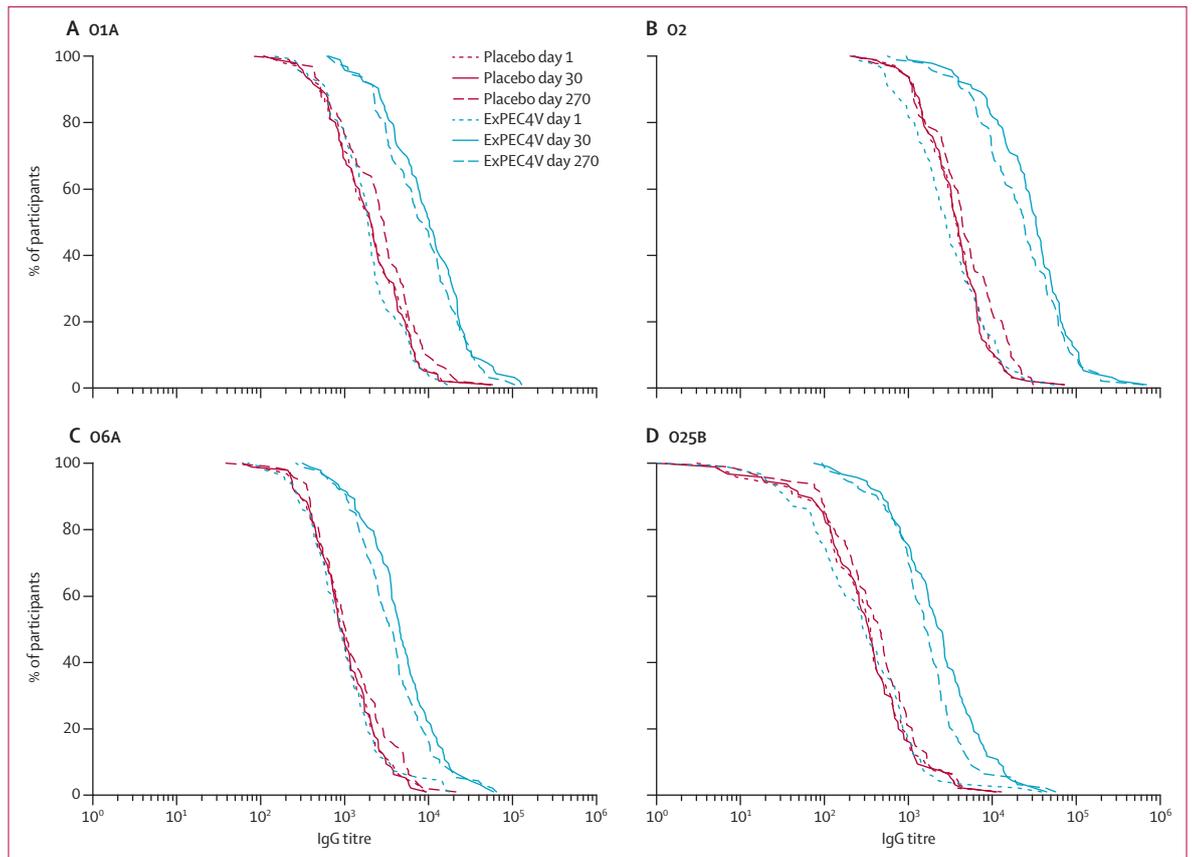
Figure 2: **Reverse cumulative distribution of IgG titres to O1A (A), O2 (B), O6A (C), and O25B (D) in the ExPEC4V target-dose group and placebo group at days 1, 30, and 270 after injection (intention-to-treat population)**
ExPEC4V is a tetravalent bioconjugate candidate vaccine against extraintestinal pathogenic *Escherichia coli*.

Day 30 serotype-specific IgG concentrations and OPK titres were strongly correlated for all serotypes (appendix). The correlation was strong for O1A and O2, moderate for O6A, and weak for O25B (all were significant; appendix).

Subgroup analyses by age (18–35 years, 36–55 years, >55 years), showed significant increases in vaccine-specific antibody titres for all vaccine serotypes in all age groups (appendix).

155 (71%) of the 218 reported suspected UTIs were confirmed by urine culture (table 4). The other 63 cases either showed no growth (n=36) or sample contamination (n=11), or no urine was obtained for culture (n=16). Of the microbiologically confirmed cultures, 129 (83%) were due to single-pathogen infections. *E coli* was the most commonly isolated uropathogen (present in 108 [70%] of 155 infections), followed by *Enterococcus faecalis*, *Streptococcus agalactiae*, and *Klebsiella* species (appendix). Around a third of *E coli* isolates were resistant to antibiotics targeting UTIs (appendix). Among all serotyped *E coli* isolates (including polymicrobial infections) from placebo recipients, 15 of 74 were vaccine serotypes, providing a vaccine coverage of 20% (data not shown).

45 (52%) of 87 vaccinees and 34 (41%) of 82 placebo recipients had no confirmed UTIs during the study period (table 5). 31 (36%) target-dose vaccinees and 39 (48%) placebo recipients had at least one *E coli* infection during follow-up (table 5). Based on the protocol definition, 13 vaccine-specific serotype UTI events were recorded among target-dose vaccinees and 12 among placebo recipients (0·149 *vs* 0·146 mean UTIs per participant; p=0·522; figure 3A). Although vaccine efficacy was not noted for vaccine-specific serotype UTI events, vaccine efficacy reached 32% (lower 95% CI 2·7) for single-pathogen UTIs caused by *E coli* of any serotype (0·414 mean UTIs per vaccinee *vs* mean 0·610 UTIs per placebo recipient; p=0·038; figure 3A).

Symptom type, severity, and duration did not differ significantly between groups or between different bacterial loads for vaccine-serotype UTI (data not shown). Among UTIs caused by any *E coli* serotype, the duration of frequency was significantly shorter in the vaccine group than in the placebo group (mean 4·37 days *vs* 6·15 days, p=0·050).

Exploratory subgroup analyses of UTIs confirmed by a bacterial load of 10^5 CFU/mL or higher showed vaccine

	ELISA		OPK assay	
	ExPEC4V geometric mean (95% CI)	Placebo geometric mean (95% CI)	ExPEC4V geometric mean (95% CI)	Placebo geometric mean (95% CI)
O1A				
Day 1	1807 (1489–2191)	1895 (1515–2369)	192 (159–233)	156 (126–193)
Day 30	9460 (7511–11 916)	1887 (1517–2347)	951 (693–1304)	161 (131–198)
Day 270	7844 (6245–9853)	2474 (1958–3125)	549 (430–699)	183 (150–222)
O2				
Day 1	2855 (2279–3576)	3529 (2926–4257)	301 (238–382)	342 (275–425)
Day 30	27 973 (22 056–35 526)	3502 (2910–4214)	4132 (3036–5626)	342 (274–426)
Day 270	20 819 (16 102–26 918)	4352 (3508–5400)	1703 (1243–2332)	269 (219–331)
O6A				
Day 1	920 (743–1138)	943 (777–1145)	791 (677–924)	693 (589–814)
Day 30	4475 (3608–5549)	953 (789–1151)	1542 (1264–1882)	715 (614–832)
Day 270	3530 (2834–4397)	1130 (915–1397)	1435 (1190–1731)	992 (831–1183)
O25B				
Day 1	261 (188–363)	285 (211–384)	114 (75–174)	87 (58–128)
Day 30	2164 (1676–2794)	282 (209–381)	415 (273–629)	97 (54–147)
Day 270	1567 (1234–1990)	366 (273–489)	268 (180–398)	157 (97–254)

ExPEC4V is a tetravalent bioconjugate candidate vaccine against extraintestinal pathogenic *Escherichia coli*. OPK=opsonophagocytic killing.

Table 3: Geometric mean IgG titres to O1A, O2, O6A, and O25B measured by ELISA and OPK assay at days 1, 30, and 270 after injection

efficacy in target-dose vaccinees of 55·4% (lower 95% CI 28·5; p=0·002) and 58·1% (–12·7%; p=0·074) for UTIs caused by any *E coli* serotype and vaccine-specific serotypes, respectively (figure 3B). In the intention-to-treat population, vaccine efficacy was 30·5% (–1·2%; p=0·055) for UTIs caused by any *E coli* serotype and 62·9% (3·2%; p=0·045) for those caused by vaccine-specific serotypes.

The mean bacterial count was lower in the target-dose vaccine than in the placebo group for single-pathogen UTIs caused by both *E coli* of any serotype (mean 4·58 *vs* 5·06 log CFU/mL; p=0·012) and *E coli* vaccine-specific serotypes (mean 4·15 *vs* 4·92 log CFU/mL; p=0·023; appendix).

Discussion

Our findings suggest that the bioconjugate ExPEC4V candidate vaccine has an acceptable safety profile, consistent with previous studies of other conjugate vaccines,[13,18,22–24] and elicits a robust vaccine-specific immune response for all four serotypes in all age groups.

The vaccine was well tolerated; no vaccine-related severe or serious adverse events were noted throughout the study. There were no significant differences among vaccinees and placebo recipients in the frequency or severity of adverse events, and although transient injection-site pain was numerically more common among vaccinees than among those in the placebo group, this difference was not significant. No clinically notable differences in haematological or biochemical parameters were detected among groups.

As expected of a study population with a history of recurrent *E coli* UTIs and previous exposure to O-antigen, baseline IgG titres were heterogeneous.

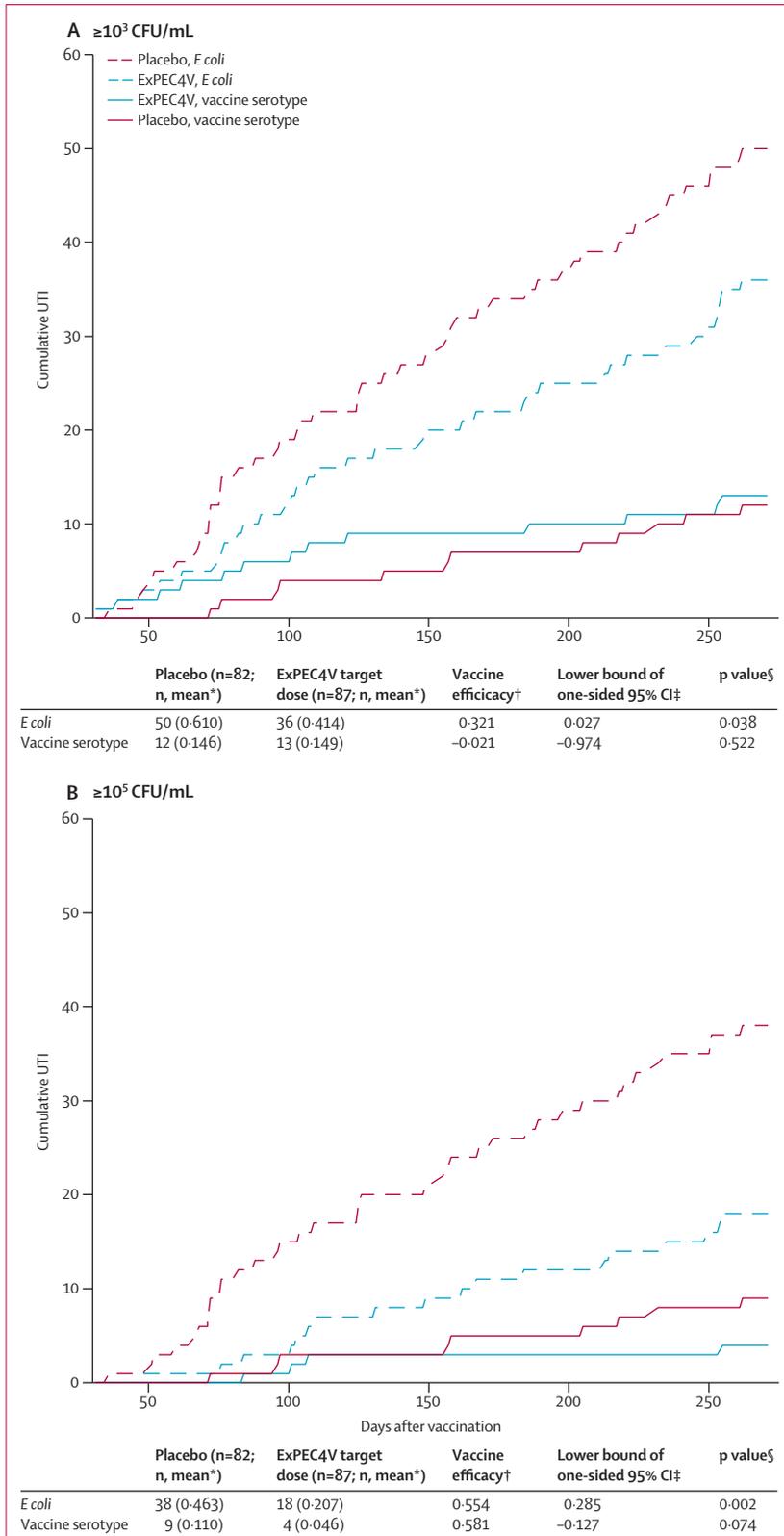|	Infections (n=218)
Microbiologically confirmed (n=155)	
Single pathogen	129 (83%)
E coli single pathogen	86 (67%)
Other single pathogen	43 (33%)
Polymicrobial	26 (17%)
E coli (polymicrobial)	22 (85%)
Other (polymicrobial, no *E coli*)	4 (15%)
Not microbiologically confirmed (n=63)	
No growth	36 (57%)
Contaminated	11 (17%)
No urine collected	16 (25%)

Data are n (%).

Table 4: Reported urinary tract infections in all study participants, by microbiological confirmation of *Escherichia coli*

	Placebo (n=82)	ExPEC4V (n=87)
No urinary tract infections	34 (41%)	45 (52%)
≥1 urinary tract infections	48 (59%)	42 (48%)
≥1 urinary tract infections, *E coli* (single pathogen and polymicrobial)	39 (48%)	31 (36%)
≥1 urinary tract infections, *E coli* (single pathogen)	36 (44%)	28 (32%)
≥1 urinary tract infections, *E coli* vaccine serotype (single pathogen)	9 (11%)	11 (13%)
≥1 urinary tract infections, other pathogen (single pathogen and polymicrobial)	17 (21%)	18 (21%)

Data are n (%). ExPEC4V is a tetravalent bioconjugate candidate vaccine against extraintestinal pathogenic *Escherichia coli*. Pathogens were identified from urine culture; their presence was confirmed if the concentration was 10³ colony-forming units per mL or greater.

Table 5: Incidence of microbiologically confirmed urinary tract infections, by study group

A ≥10³ CFU/mL

Legend:
- Placebo, *E coli*
- ExPEC4V, *E coli*
- ExPEC4V, vaccine serotype
- Placebo, vaccine serotype

	Placebo (n=82; n, mean*)	ExPEC4V target dose (n=87; n, mean*)	Vaccine efficacy†	Lower bound of one-sided 95% CI‡	p value§
E coli	50 (0·610)	36 (0·414)	0·321	0·027	0·038
Vaccine serotype	12 (0·146)	13 (0·149)	–0·021	–0·974	0·522

B ≥10⁵ CFU/mL

Days after vaccination

	Placebo (n=82; n, mean*)	ExPEC4V target dose (n=87; n, mean*)	Vaccine efficacy†	Lower bound of one-sided 95% CI‡	p value§
E coli	38 (0·463)	18 (0·207)	0·554	0·285	0·002
Vaccine serotype	9 (0·110)	4 (0·046)	0·581	–0·127	0·074

Figure 3: **Cumulative number of microbiologically confirmed UTIs caused by *Escherichia coli* (single-pathogen only) and of *E coli* vaccine-specific serotypes (ExPEC4V) with bacterial loads ≥10³ CFU/mL urine (A), and bacterial loads ≥10⁵ CFU/mL urine (B), in the per-protocol population**
ExPEC4V is a tetravalent bioconjugate candidate vaccine against extraintestinal pathogenic *E coli*. UTI=urinary tract infection. CFU=colony-forming units.
*Mean UTI is calculated separately for the vaccine and placebo groups as: (total number of UTIs during study period)/(total number of participants). †Vaccine efficacy is calculated as 1–(ExPEC4V target dose mean UTI /placebo mean UTI). ‡95% CI for the vaccine efficacy was calculated with a Poisson distribution for the number of UTIs with an offset of log(days on study). §The p value is testing the null hypothesis that vaccine efficacy is 0 against the alternative hypothesis that vaccine efficacy is greater than 0. The p value was calculated on the basis of a normal approximation with a generalised linear model assuming a Poisson distribution for the number of UTIs with an offset of log (days on study).

Nonetheless, each vaccine serotype elicited substantial increases in antibodies targeting its O-antigen irrespective of baseline IgG concentrations, suggesting a stronger immune response to the vaccine than to natural *E coli* exposure. UTIs that are limited to the lower urinary tract induce an immune response mainly via local innate defences; adaptive responses to these primarily mucosal infections are limited.[25] Antibody functionality, with improved clearance of the pathogen in the in-vitro OPK assay, increased significantly for all serotypes after vaccination compared with baseline. Vaccine-serotype-specific IgG antibody titres and OPK predict functional antibody activity and clinical efficacy for other licensed vaccines against bacterial infections.[26] The correlations shown between ELISA and OPK for the ExPEC4V vaccine are of the same order as those previously reported for pneumococcal conjugate vaccines.[27]

The low baseline titres of antibodies targeting O25B compared with the other antigens could indicate weak natural immunogenicity against O25 strains and could, in combination with the acquisition of antibiotic-resistance genes, explain the pandemic expansion of the multidrug-resistant clonal group O25:H4-ST131.[28] In this context, the elicitation of O25-specific antibody responses by the vaccine is encouraging.

The study population of female volunteers in good health but with a history of recurrent UTI was chosen to allow for a preliminary assessment of the vaccine's potential clinical efficacy. Despite a sample size informed by a prospective surveillance study and previously documented *E coli* UTI being a prerequisite for inclusion, fewer UTI events than expected occurred: 41% of placebo recipients did not experience any microbiologically confirmed UTI in the 9 months after injection, and 11% instead of the expected 25% had a UTI caused by a vaccine-specific *E coli* serotype. This low incidence among placebo recipients could be a result of scant laboratory confirmation of recurrent *E coli* UTI, a follow-up period of only 9 months, a placebo effect, or seasonal patterns (most follow-up excluded summer months[29]). Furthermore, only 20% of the serotyped *E coli* isolates from the placebo group

were vaccine serotypes, which is lower than the 30% vaccine coverage estimated on the basis of the pre-trial epidemiological survey.

Despite the reduced power of the study to demonstrate vaccine efficacy, when we analysed participants with UTIs caused by any *E coli* serotype, we noted a significant reduction in incidence in the vaccine compared with the placebo group. This observation could be explained by some cross-reactivity between serotypes, a general unspecific boosting of the immune response, and the increased sample size. The partial protective effect of ExPEC4V was further supported by the reduction in bacterial load noted for UTIs due to vaccine serotypes and *E coli* with any serotype compared with the placebo group (appendix).

Parenteral ExPEC4V seems potentially more effective in preventing UTIs with higher bacterial loads than preventing lower-count infections, which might require a stronger or different immune response. The reduction in high-count UTIs suggests that a parenteral *E coli* O-conjugate vaccine might be useful to prevent more invasive *E coli* disease, such as bloodstream infections. Parenteral pneumococcal conjugate vaccines had only intermediate efficacy against otitis media but high efficacy against invasive pneumococcal disease.[30] More data are needed to validate this hypothesis, however. Additional studies of the safety and immunogenicity of different doses of ExPEC4V in healthy adult participants are underway in Japan (NCT02748967) and the USA (NCT02748967).

In conclusion, the tetravalent ExPEC4V O-conjugate candidate vaccine was well tolerated and safe. In a proof-of-concept assessment, the candidate vaccine elicited strong, durable, and functional immune responses. Preliminary data demonstrate a reduction in UTIs with high bacterial counts, raising optimism that ExPEC conjugates might be able to prevent invasive disease such as bacteraemia. Further studies of higher doses and different formulations of the candidate vaccine are warranted.

Contributors

AHu, CH, GvdD, ST, CA, SH, JP, and VGF designed the study. SdV, CA, and VGF set up the study, AHo, PM, IvdN, and VGF coordinated the study, RF oversaw operational management, and SH oversaw academic coordination. AK, KK, SG, and DS recruited patients. DS did the clinical work and oversaw sample collection. CA coordinated clinical assays. AHu, CH, GvdD, DA, TD, KF, SdV, AK, EB, VV, TK, KK, GR, TH, SG, DS, SH, and JP gathered data; AHu, KK, GR, and SH analysed the data; RF and AMD reviewed the data; and CH, GvdD, DA, AMD, PM, TD, KF, EB, CA, JP, and VGF interpreted the data. AHu, CH, DA, TD, KF, ST, and JP wrote the Article, which was revised by AHu, GvdD, AHo, RF, AMD, PM, IvdN, SdV, AK, EB, VV, TK, KK, GR, TH, SG, DS, CA, SH, and VGF.

Declaration of interests

AHo, RF, AMD, PM, CA, and VGF are employees of LimmaTech (formerly Glycovaxyn), which developed the vaccine candidate. GvdD, DA, KF, IvdN, ST, and JP are employed by Janssen, which is now further developing the vaccine candidate. SH received consulting fees from GlaxoSmithKline, Abbott, Johnson & Johnson (the owners of Janssen), Novartis, and Bioaster. EB is an advisory board member of Gilead Sciences, MSD, AbbVie, Basilea, Janssen, ViiV Healthcare, Astella, and AstraZeneca. All other authors declare no competing interests.

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