Efficacy, immunogenicity, and safety of the live attenuated nasal pertussis vaccine, BPZE1, in the UK: a randomised, placebo-controlled, phase 2b trial using a controlled human infection model with virulent *Bordetella pertussis*

Diane Gbesemete, Maheshi N Ramasamy, Muktar Ibrahim, Alison R Hill, Lucy Raud, Daniela M Ferreira, Jonathan Guy, Adam P Dale, Jay R Laver, Tyween Coutinho, Saul N Faust, Thomas A N Reed, Gavin Babbage, Lisa Weissfeld, Wei Lang, Camille Locht, Vivek Samal, Peter Goldstein, Ken Solovay, Keith Rubin, Stephanie Noviello, Robert C Read

Summary

Background Pertussis is a severe respiratory disease caused by *Bordetella pertussis*. Although vaccines prevent disease for a limited duration, they do not prevent infection and transmission. We aimed to assess the safety and efficacy of BPZE1 at preventing or substantially reducing colonisation by virulent *B pertussis* using a robust controlled human infection model.

Methods This randomised, placebo-controlled, phase 2b trial was conducted at University Hospital Southampton and University of Oxford in the UK. Eligible participants were healthy adults aged 18–50 years, who complied with the protocol, refrained from smoking and nasal sprays, and were fully vaccinated against SARS-CoV-2. Exclusion criteria were pertussis vaccination or illness (<5 years), baseline anti-pertussis toxin serum IgG (>20 International Units [IU]/mL) or anti-pertactin serum IgG (>30 IU/mL) concentrations, and a positive SARS-CoV-2 test. Participants were randomly assigned (1:1), using permuted blocks with a block size of four, to receive an intranasal dose of 10^9 colony-forming units (CFU) of BPZE1 or placebo (lyophilised buffer) and were challenged 60–120 days later with 10^5 CFU virulent *B pertussis*. Masked staff administered the study vaccine. Nasal mucosal secretion and blood samples were collected. The primary outcome was negative *B pertussis* cultures of nasal washes at days 9, 11, and 14 after virulent challenge in the modified intention-to-treat (mITT; defined as all participants randomly assigned to treatment who were vaccinated, challenged, and had at least one culture result at day 9, 11, or 14 post-challenge) and per protocol adequate inoculum populations (defined as all participants in the mITT population who received a challenge inoculum equal to or higher than the target ($\geq 0{\cdot}5 \times 10^5$ CFU; sensitivity analysis). This trial is registered with ClinicalTrials.gov, NCT05461131.

Findings Between June 23, 2022, and Oct 26, 2023, 141 participants were assessed for eligibility, of whom 88 were ineligible and 53 were randomly assigned (26 to the BPZE1 group and 27 to the placebo group). 26 (49%) participants were male and 27 (51%) were female, with a mean age of 30·42 years (SD 8·49). Participants self-identified as White (42 [79%]), Black (six [11%]), or Asian (five [9%]). Five (9%) participants did not receive virulent challenge and two (4%) were lost to follow-up before virulent challenge. 46 (87%) participants received virulent challenge at 60–120 days (24 in the BPZE1 group *vs* 22 in the placebo group). One in the BPZE1 group withdrew consent and one in the placebo group was not evaluable due to COVID-19. 44 (83%) completed the challenge trial unit stay (23 [88%] *vs* 21 [78%]). 24 (92%) participants in the BPZE1 group and 21 (78%) in the placebo group were included in the mITT population. In the mITT population, the number of participants with no detectable colonisation on days 9, 11, and 14 post-challenge was higher in the BPZE1 group (14 [58%; 95% CI 39–76] of 24 *vs* seven [33%; 17–55] of 21 in the placebo group; p=0·091). Four (17%) in the BPZE1 group and five (24%) in the placebo group received a lower challenge dose than the target. In the per protocol adequate inoculum population, 12 (60%; 39–78) of 20 in the BPZE1 group and four (25%; 10–50) of 16 in the placebo group had no detectable colonisation by *B pertussis* B1917 on days 9, 11, and 14 post-challenge (p=0·033). Most participants reported at least one solicited adverse event during the 7 days after vaccination (22 [85%] of 26 in the BPZE1 group *vs* 22 [81%] of 27 in the placebo group), which were mostly mild (grade 1) in severity. Unsolicited adverse events were reported in similar frequency in the BPZE1 and placebo groups during the 28 days after vaccination (seven [27%] of 26 *vs* nine [33%] of 27). No serious adverse events or discontinuations due to adverse events were reported during the trial.

Interpretation Intranasal BPZE1 vaccination prevented or substantially reduced infection after challenge with virulent *B pertussis* and is an attractive vaccine candidate. Given the favourable safety profile, large phase 3 trials are warranted to confirm these initial findings.

Funding ILiAD Biotechnologies.

Lancet Microbe 2025; 6: 101211

Published Online December 1, 2025
https://doi.org/10.1016/j.lanmic.2025.101211

NIHR Southampton Clinical Research Facility and Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, Southampton, UK (D Gbesemete PhD, M Ibrahim PhD, A R Hill PhD, L Raud BSc, J Guy BSc, A P Dale PhD, J R Laver PhD, T Coutinho MSc, S N Faust PhD, T A N Reed MD, G Babbage MPhil, R C Read MD); **Controlled Human Infection Group, School of Clinical & Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK** (D Gbesemete, M Ibrahim, A R Hill, L Raud, J Guy, A P Dale, J R Laver, T Coutinho, R C Read); **Oxford Vaccine Group, NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford University Hospitals NHS Foundation Trust, Oxford, UK** (M N Ramasamy PhD, D M Ferreira PhD); **Department of Infection, University Hospital Southampton NHS Foundation Trust, Southampton, UK** (M Ibrahim); **Faculty of Medicine and Institute for Life Science, University of Southampton, Southampton, UK** (S N Faust); **WCG Clinical, Princeton, NJ, USA** (L Weissfeld PhD, W Lang PhD); **Institut Pasteur de Lille, Inserm U1019, CNRS UMR9017, Centre for Infection and Immunity of Lille, CHU Lille, Inserm, University of Lille, Lille, France** (C Locht PhD); **ILiAD Biotechnologies, Weston, FL, USA** (V Samal MD, P Goldstein MS, K Solovay MS*, K Rubin MD, S Noviello MD MPH)

*Mr Solovay died in December, 2024

Correspondence to: Dr Camille Locht, Institut Pasteur de Lille, Inserm U1019, CNRS UMR9017, Centre for Infection and Immunity of Lille, CHU Lille, Inserm, University of Lille, F-59019 Lille, France
camille.locht@pasteur-lille.fr

Introduction

Pertussis or whooping cough, caused by *Bordetella pertussis*, is a highly contagious respiratory disease affecting all age groups,[1] but is particularly severe in the first year of life.[2] Resurgence of pertussis in the past 15 years has occurred in several European countries, China, and the USA,[3] despite high vaccination coverage, which might be due to rapid waning of vaccine-induced immunity, strain adaptation, and ineffective vaccines to prevent nasopharyngeal infection and transmission.[4,5] A mathematical modelling study suggests that asymptomatic transmission of *B pertussis* is the most likely explanation for the resurgence of this disease.[6] Since the infection rate of pertussis is second only to that of measles,[7] effective control will ultimately rely on vaccines that can prevent the disease and nasopharyngeal infection.[8]

BPZE1 is a live intranasal pertussis vaccine candidate, attenuated by genetic detoxification of pertussis toxin and removal of dermonecrotic toxin and tracheal cytotoxin.[9] In animal models BPZE1 protects against the disease and infection.[10,11] Several randomised controlled trials (collectively including more than 350 vaccine recipients)[12–15] have indicated that BPZE1 is safe in adults, inducing *B pertussis*-specific serum and mucosal antibody responses. Additionally, BPZE1 prevented nasal colonisation after a subsequent challenge with the *B pertussis* vaccine strain, whereas the tetanus–diphtheria–acellular pertussis vaccine did not prevent nasal colonisation.[15] We aimed to assess the safety and efficacy of BPZE1 at preventing or substantially reducing colonisation by virulent *B pertussis* in a controlled human infection model that induces asymptomatic respiratory tract infection with a virulent strain of *B pertussis*.[16]

Methods

Study design

This randomised, placebo-controlled, phase 2b trial was conducted at University Hospital Southampton (Southampton, UK) and University of Oxford (Oxford, UK). This study adhered to the 2013 Helsinki Declaration and ethical approval was given by the London Central Research Ethics Committee (22/FT/006). Written informed consent was obtained from all participants. The full trial protocol and statistical analysis

Research in context

Evidence before this study

Despite high global vaccination coverage with whole-cell vaccines and with acellular vaccines in high-income countries, pertussis or whooping cough is not effectively controlled in any country. In the past 15 years, a striking resurgence has been seen in several European countries, China, and the USA, particularly in those using acellular pertussis vaccines. The underlying causes of this resurgence include unsuccessful acellular vaccines to prevent *Bordetella pertussis* infection and transmission and short vaccine-induced protection from the disease. Prevention of infection and transmission likely relies on mucosal immunity in the nasal cavity, as *B pertussis* is strictly a mucosal pathogen. To identify vaccine candidates that induce mucosal immunity, we searched PubMed for articles published between database inception and Aug 1, 2024, using the terms "pertussis" OR "whooping cough" AND "mucosal vaccine", "intranasal vaccine" OR "live attenuated vaccine", with no language restrictions. We also searched ClinicalTrial.gov for novel pertussis vaccines in clinical development. This search yielded 319 references. The corresponding articles describe several mucosal vaccine candidates, none of which have entered clinical development, with the exception of the live attenuated nasal vaccines GamLPV and BPZE1. BPZE1 is the only novel vaccine for which human data are available showing safety, transient colonisation with the vaccine strain, systemic, and mucosal antibody responses, and prevention of colonisation from a second BPZE1 dose used as an attenuated challenge.

Added value of this study

To our knowledge, this trial is the first to investigate a novel pertussis vaccine candidate in a controlled human infection model using virulent *B pertussis*. In 14 (58%) of 24 participants in the BPZE1 group (modified intention-to-treat population), there was no detectable colonisation in nasal wash samples on days 9, 11, and 14 after challenge compared with seven (33%) of 21 in the placebo group. However, some participants received a challenge dose lower than the target ($<0{\cdot}5 \times 10^5$ colony forming units). In the per protocol adequate inoculum population, 12 (60%) of 20 in the BPZE1 group and four (25%) of 16 in the placebo group had no detectable colonisation. In a post-hoc analysis, BPZE1 vaccination also reduced the bacterial burden in the nasal cavity by more than 97% after challenge with the virulent *B pertussis* strain compared with the placebo group. The study also confirmed a robust mucosal and systemic antibody response induced by BPZE1 and a favourable safety profile.

Implications of all the available evidence

We show that a single nasal administration of the live attenuated vaccine BPZE1 can prevent infection by virulent *Bordetella pertussis* and substantially reduce bacterial load in the human respiratory tract. We confirm the ability of the vaccine to induce a robust systemic and mucosal antibody response, which, together with the favourable safety profile, supports BPZE1 as a safe and efficacious next-generation pertussis vaccine.

plan are shown in the appendix (pp 14–138, 139–177). This trial is registered with ClinicalTrials.gov, NCT05461131 (completed).

Participants

Eligible participants were healthy adults aged 18–50 years, who complied with the protocol, refrained from smoking and nasal sprays, and were fully vaccinated against SARS-CoV-2 (>14 days before study vaccination). Exclusion criteria were recent pertussis vaccination or illness (<5 years), baseline anti-pertussis toxin serum IgG concentrations of more than 20 International Units (IU)/mL or anti-pertactin serum IgG concentrations of more than 30 IU/mL, and a positive SARS-CoV-2 test (by a lateral flow test or PCR) within 3 days of vaccination or entry into the challenge trial unit. The full inclusion and exclusion criteria are shown in the appendix (pp 51–55).

Randomisation and masking

Participants were randomly assigned (1:1) to receive an intranasal dose of 10^9 colony-forming units (CFU) of BPZE1 or placebo (lyophilised buffer). Permuted block randomisation was generated using a block size of four without stratification. Unmasked clinical staff managed vaccine logistics, assignment per the supplied randomisation list, and preparation along with masking of the administration device using opaque tape but were not involved in study-related assessments or with participants for data collection. Masked staff administered the study vaccine. Participants and masked investigators and site staff did not know the assigned study vaccine.

Procedures

We evaluated BPZE1-mediated protection against colonisation by virulent *B pertussis* in a controlled human infection model.[16] BPZE1 and placebo were reconstituted in 1·0 mL sterile water and administered as 0·4 mL per nostril by the intranasal mucosal atomisation device (MAD Nasal; Teleflex, Morrisville, NC, USA). At 60–120 days post-vaccination, after clinical examination and a negative *B pertussis* culture of nasal wash samples taken 7 days before, participants were challenged with 10^5 CFU *B pertussis* strain B1917,[17] as droplets into each nostril with a Gilson pipette.[16] This dose was selected as it previously resulted in colonisation of approximately 80% of participants who were not vaccinated with any pertussis vaccine in the previous 5 years.[16] The 60–120 day period after vaccination was selected to improve compliance for participants to schedule the 17-day isolation period and provide flexibility to the site because of low bed capacity. Due to the durability of response anticipated from the study vaccine, the variable period was not expected to have an effect on the results. After challenge, participants stayed in a challenge trial unit with monitoring twice per day. Azithromycin 500 mg was administered for 3 days from day 14 post-challenge, with discharge on day 16. Positive *B pertussis* cultures on day 16 post-challenge necessitated additional azithromycin 500 mg for 3 days.

See Online for appendix

Quantitative culture of nasal washes on charcoal *B pertussis* selective agar (Oxoid, Basingstoke, UK) incubated in a humid aerobic incubator at 37°C for 7 days was done before challenge and on days 9, 11, 14, 16, and 28 post-challenge (detection limits from 4 to 123 600 CFU).[16] Nasal mucosal secretion and blood samples were collected at baseline, day 28 post-vaccination, during the week before the challenge, day 28 post-challenge, and at the end of the study to measure nasal secretory IgA, serum IgA, and serum IgG against whole-cell extracts, pertactin, filamentous haemagglutinin, serotypes 2 and 3 fimbriae (Fim2/3), and pertussis toxin. For nasal mucosal secretion collection, Nasosorption FX·i (Mucosal Diagnostics, Midhurst, UK) was used. Antigen-specific secretory IgA, serum IgA, and serum IgG were measured by electrochemiluminescence (Meso Scale Diagnostics, Rockville, MD, USA). A human IgA kit (Meso Scale Diagnostics) was used to measure total secretory IgA, which was used to normalise antigen-specific secretory IgA. All results were standardised against the WHO International Standard Human Pertussis Antiserum 06/140. A high-throughput serum bactericidal activity assay was developed and qualified (Nexelis, Laval, QC, Canada), modifying the method used previously[15] to a 96-well plate format using agar-overlay and automated microscopy.

For safety assessments, immediate nasal, respiratory, and systemic solicited adverse events, and vital signs were assessed 30 min post-vaccination. Participants recorded solicited adverse events in a paper diary once per day for 7 days post-vaccination, which was reviewed by clinical staff at the visit 7 days post-vaccination. Modified US Food and Drug Administration toxicity grading was used for assessment (appendix pp 95–99). Participants were advised to contact the site and seek medical attention within 24 h for severe grade 3 solicited adverse events. Unsolicited adverse events were collected from vaccination until 28 days post-vaccination and from challenge to 28 days post-challenge. Treatment-related adverse events were documented from vaccination to challenge and up to 3 months thereafter. Adverse events of special interest (COVID-19-related adverse events) and serious adverse events were reported throughout the study. Investigators assessed whether the cause of a reported unsolicited adverse event was linked to the vaccine, the atomisation device, or challenge with virulent *B pertussis*. Safety follow-up occurred for at least 6 months after vaccination and at least 3 months after challenge (appendix p 2).

During the challenge period, participants were reviewed at least twice per day, including measuring vital signs and symptoms of early pertussis disease (including rhinorrhoea, nasal congestion, epistaxis, sneezing, ear pain, eye pain, sore throat, cough, dyspnoea, feeling generally unwell, tiredness, and headache). In the event of pertussis disease onset, azithromycin treatment commenced, with observation for 3 days before discharge.

Outcomes

The primary outcome was to show that previous immunisation with BPZE1 protects against colonisation as evidenced by a negative *B pertussis* culture at days 9, 11, and 14 after virulent *B pertussis* challenge 60–120 days post-vaccination. Colonisation at each post-challenge day was also described.

Secondary outcomes were the evaluation of BPZE1 induction of anti-*B pertussis* mucosal secretory IgA, serum IgA, and serum IgG against whole-cell extract, pertactin, filamentous haemagglutinin, Fim2/3, and pertussis toxin as geometric mean fold rise from baseline to day 28 after vaccination; and safety including reactogenicity from vaccination up to 7 days post-vaccination, unsolicited treatment-emergent adverse events until 28 days post-vaccination and post-challenge, unsolicited treatment-emergent adverse events related to vaccination from study vaccination until challenge and related to challenge from challenge to 3 months post-challenge, and adverse events of special interest and serious adverse events throughout the study.

Prespecified exploratory efficacy outcomes included absolute colony counts; functional antibody responses measured using serum bactericidal activity assay; and geometric mean fold rises in secretory IgA, serum IgA, and serum IgG against whole-cell extract, pertactin, filamentous haemagglutinin, Fim2/3, and pertussis toxin throughout the study (including before challenge, 28 days post-challenge, and at the end of the study). A post-hoc exploratory analysis of absolute colony counts using the geometric mean area under the curve (AUC) of bacterial density of days 9, 11, and 14 post-challenge was also conducted.

Statistical analysis

For the challenge period, a sample size of 20 participants per group results in 90% power to detect a between-group difference of at least 50% in colonisation rate, with a placebo rate of at least 60% (appendix pp 139–77), based on the study by de Graaf and colleagues.[16] Evaluable participants were prespecified as those with nasal wash culture results during the challenge phase (days 9, 11, or 14 post-challenge). The likelihood ratio was used to test the null hypothesis of no difference in colonisation rate after virulent challenge between the two groups. A two-sided significance level of $p<0{\cdot}05$ was used for all analyses.

Primary efficacy analysis was done in the modified intention-to-treat (mITT) population (defined as all participants randomly assigned to treatment who were vaccinated, challenged, and had at least one culture result at day 9, 11, or 14 post-challenge). Prespecified sensitivity analyses included primary endpoint evaluation in the per protocol adequate inoculum and per protocol populations. The per protocol population excluded those with major protocol deviations from the mITT population. No major protocol deviations occurred; therefore, the per protocol population was the same as the mITT population. Nine participants were administered inocula in the first challenge cohorts of the study below the $0{\cdot}5\times10^5$ CFU target. The prospectively defined per protocol adequate inoculum population was defined as all participants in the mITT population who received a challenge inoculum equal to or higher than the target. CFU counts of inocula were quantified for 7 days post-inoculation by culture of residual material in inoculation vials; therefore, participants who received lower challenge doses than the target could be identified only after being in the challenge trial unit for 7 days.

Participants were classified as colonised at a given day if the culture result for that day was positive. For cases in which all three nasal wash samples on days 9, 11, and 14 post-challenge could not be provided, if any day had a positive result the participant was classified as positive; single imputation of negative results (no colonisation) was done when day 9 post-challenge was negative and day 11 or 14 (or both) post-challenge was negative or missing. The number and proportion of participants colonised or not colonised on any of the post-challenge days was reported by each study group with two-sided 95% CIs computed using the Agresti–Coull method. Two-sided 95% CI for the difference in colonisation rates between study groups was computed using the Agresti–Caffo method, with placebo group as a reference. A χ^2 test was used to compare study groups for the proportion of participants not colonised at all of the post-challenge days. If at least one of the cells had a count less than 5, the Fisher's exact test was used. Logistic regression was conducted as a sensitivity analysis for the primary outcome. The same methods were used to analyse colonisation at each day post-challenge.

Secondary and exploratory colonisation, immunogenicity, and serum bactericidal activity outcomes were analysed using descriptive statistics (geometric means with 95% CI computed using natural log transformation, minimum, maximum, quartiles, and the proportion of participants with at least a two-fold rise in serum bactericidal activity) in the ITT, mITT, per protocol, and per protocol adequate inoculum populations. The ITT population was defined as all participants randomly assigned to treatment who were vaccinated. The mITT population was defined as all participants randomly assigned to treatment who were vaccinated, challenged, and had at least one culture result at days 9, 11, or 14 post-challenge. The per protocol population was the same as the mITT population. The per protocol adequate inoculum population was defined as all participants in the mITT population who received a challenge inoculum of at least $0{\cdot}5\times10^5$ CFU. Differences in the immunogenicity outcomes between treatment groups were assessed by computing geometric mean ratios and corresponding 95% CI, with the placebo group as a reference. As sensitivity analyses, ANCOVA models were fitted with log change from baseline (ie, geometric mean fold rise in natural log scale) as response, treatment group as factor, and inoculum count and log of baseline value as covariates. A two-sample *t* test on log-transformed data was used to compare treatment groups, and a paired *t* test on

log-transformed data was used to evaluate change from baseline within each treatment group. Secondary safety analyses were done in the safety analysis population (defined as participants who were randomly assigned and vaccinated).

In post-hoc exploratory analyses of absolute colony counts, bacterial load density was estimated by AUC and calculated by the trapezoidal rule based on absolute count (at post-challenge days 9, 11, and 14) and the number of days, with 0 CFU/mL imputed for participants not colonised at each given day. For participants not colonised at all three post-challenge days, AUC was imputed to 1. Percentage reduction in bacterial load density was calculated relative to the placebo, with 95% CI computed on log-transformed data using Student's *t* distribution.

All statistical analyses were performed using SAS (version 9.4.1). An independent data safety monitoring board was established.

Role of the funding source

The funder of the study had a role in study design, data analysis, data interpretation, and writing of the report, but had no role in data collection.

Results

Between June 23, 2022, and Oct 26, 2023, 141 participants were assessed for eligibility, of whom 88 were ineligible (mostly due to serum concentration of anti-pertussis toxin IgG >20 IU/mL or anti-pertactin IgG >30 IU/mL [or both]; figure 1). 53 participants were randomly assigned from July 18, 2022, to April 20, 2023 (26 to the BPZE1 group and 27 to the placebo group; ITT population). Five (9%) participants did not receive virulent challenge and two (4%) were lost to follow-up before receiving the virulent challenge. 46 (87%) participants received virulent challenge at 60–120 days (24 [92%] in the BPZE1 group *vs* 22 [81%] in the placebo group) and 44 (83%) completed the challenge trial unit stay (23 [88%] *vs* 21 [78%]). Of the two participants who did not complete the challenge trial unit stay, one in the BPZE1 group withdrew consent but was evaluable due to the availability of a nasal wash culture on day 9 post-challenge and one in the placebo group was not evaluable due to COVID-19 necessitating early azithromycin treatment and withdrawal from the challenge trial unit.

24 (92%) participants in the BPZE1 group and 21 (78%) in the placebo group received virulent challenge and were evaluable (mITT population). Among those, four (17%) in the BPZE1 group versus five (24%) in the placebo group received a challenge dose of around 10^4 CFU, lower than the target, whereas 20 (83%) versus 16 (76%) received the per protocol adequate inoculum of at least $0{\cdot}5 \times 10^5$ CFU *B pertussis* strain B1917.

Demographic and baseline characteristics in the ITT population were similar between the BPZE1 and placebo groups (table 1) and similar to the modified ITT and per protocol adequate inoculum populations. 26 (49%) participants were male and 27 (51%) were female, with a mean age of 30·42 years (SD 8·49). Participants self-identified as White (42 [79%]), Black (six [11%]), or Asian (five [9%]).

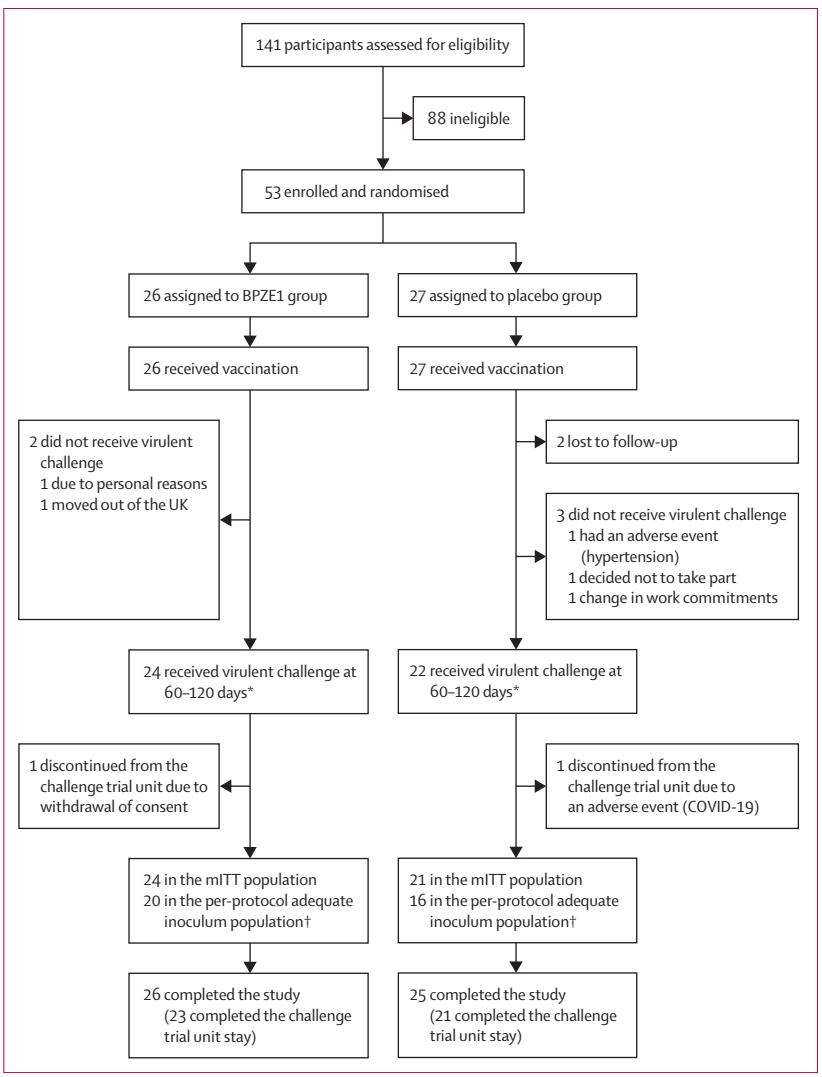

Figure 1: **Trial profile**
CFU=colony-forming unit. mITT=modified intention-to-treat. *Participants received the virulent challenge of 10^5 CFU *Bordetella pertussis* B1917 within a mean of 70·92 days (range 61–103) after vaccination in the BPZE1 group and 77·76 days (61–112) after vaccination in the placebo group. †Four participants in the BPZE1 group and five in the placebo group received a low dose of challenge inoculum of less than $0{\cdot}5 \times 10^5$ CFU and were excluded from the per protocol adequate inoculum population.

Among participants in the mITT population who entered the challenge trial phase, the mean number of days from vaccination to challenge was 70·92 days (range 61–103) for the BPZE1 group and 77·76 days (61–112) for the placebo group.

At the visit before challenge (60–120 days after study vaccination), all participants had a nasal wash culture negative for *B pertussis*, indicating clearance of BPZE1 before challenge, as expected. In the mITT population, the number of participants with no detectable colonisation on days 9, 11, and 14 post-challenge was higher in the BPZE1 group (14 [58%; 95% CI 39–76] of 24 *vs* seven [33%; 17–55]

	BPZE1 group (n=26)	Placebo group (n=27)
Age, years	29·35 (7·77)	31·44 (9·16)
Sex		
Male	12 (46%)	14 (52%)
Female	14 (54%)	13 (48%)
Race		
White	22 (85%)	20 (74%)
Black	3 (12%)	3 (11%)
Asian	1 (4%)	4 (15%)
Bodyweight, kg	74·87 (17·27)	74·51 (12·24)
BMI, kg/m^2	25·28 (4·51)	25·54 (3·82)

Data are mean (SD) or n (%). The ITT population was defined as all participants randomly assigned to treatment who were vaccinated. ITT=intention-to-treat.

Table 1: **Demographic and baseline characteristics of 10^9 colony forming units vaccinated groups (ITT population)**

of 21 in the placebo group; p=0·091; table 2; appendix p 3). In the per protocol adequate inoculum population, 12 (60%; 39–78) of 20 in the BPZE1 group and four (25%; 10–50) of 16 in the placebo group showed a statistically significant difference in those with no detectable colonisation by *B pertussis* B1917 on days 9, 11, and 14 post-challenge (p=0·033).

When colonisation was compared on a day-by-day basis before azithromycin administration, a higher proportion of participants in the BPZE1 group had no detectable colonisation than did those in the placebo group on each day post-challenge (table 2). On day 14 post-challenge, 16 (70% [95% CI 49 to 85]) participants in the BPZE1 group versus seven (33% [17 to 55]) in the placebo group had no detectable colonisation in the mITT population, whereas 14 (70% [48 to 86]) versus four (25% [10 to 50]) had no detectable colonisation in the per protocol adequate inoculum population. Two days after starting azithromycin treatment, only two (9%) of 23 in the BPZE1 group participants still had measurable bacterial counts versus six (29%) of 21 in the placebo group, necessitating additional antibiotic therapy. By day 28 post-challenge, all participants had cleared the infection.

In the prespecified exploratory efficacy analysis, lower absolute bacterial counts were found in the BPZE1 group than in the placebo group on days 9, 11, and 14 post-challenge, in both the mITT and per protocol adequate inoculum populations (table 2). In a post-hoc exploratory analysis, the geometric mean AUC of bacterial density on days 9, 11, and 14 post-challenge was reduced by 97% (95% CI 59–100) in the BPZE1 group compared with the placebo group in the mITT population and reduced by 99% (80–100) in the per protocol adequate inoculum population.

B pertussis-specific mucosal secretory IgA, serum IgA, and serum IgG concentrations were measured. In the BPZE1 group, the geometric mean fold rise from baseline to day 28 after vaccination was 3·0 (p<0·0001) for anti-whole-cell extract, 5·3 (p<0·0001) for anti-pertactin, 5·0 (p<0·0001) for anti-filamentous haemagglutinin, 9·0 (p<0·0001) for anti-Fim2/3, and 1·7 (p=0·011) for anti-pertussis toxin secretory IgA, whereas these antibody responses did not significantly change in the placebo group (figure 2; appendix p 5). Normalised secretory IgA responses on day 28 post-vaccination between the two groups showed statistically significant differences in geometric mean ratios for anti-whole-cell extract (3·0; p=0·0002), anti-pertactin (5·0; p<0·0001), anti-filamentous haemagglutinin (4·1; p<0·0001), and anti-Fim2/3 (7·9; p<0·0001) secretory IgA, but not for anti-pertussis toxin secretory IgA (1·3; p=0·32). Similar results were observed using the ANCOVA model. In the BPZE1 group, these secretory IgA responses decreased before challenge but increased again on challenge day 28 and remained stable until the end of the study. In the placebo group, secretory IgA responses increased on challenge day 28 and remained stable until the end of the study; however, these responses remained lower than those in the BPZE1 group after challenge (figure 2).

Participants in the BPZE1 group also showed significant increases in serum IgA against all tested antigens on day 28 after vaccination compared with baseline (appendix pp 8–10). The geometric mean fold rises were 2·6 (p<0·0001) for anti-whole-cell extract, 3·9 (p<0·0001) for anti-pertactin, 3·0 (p<0·0001) for anti-filamentous haemagglutinin, 5·2 (p<0·0001) for anti-Fim2/3, and 1·5 (p=0·0079) for anti-pertussis toxin serum IgA in the BPZE1 group; whereas in the placebo group, the serum IgA responses did not significantly change from baseline to day 28. A comparison of the serum IgA responses on day 28 showed statistically significant differences in geometric mean ratios between the two groups for all antigens (whole-cell extract 2·2 [p<0·0001], pertactin 3·7 [p<0·0001], filamentous haemagglutinin 2·8 [p<0·0001], and Fim2/3 5·2 [p<0·0001]), except for pertussis toxin (1·4 [p=0·076]) with similar results observed using the ANCOVA model. In the BPZE1 group, the serum IgA concentrations remained elevated throughout the challenge period before modestly decreasing by the end of the study. In the placebo group, virulent *B pertussis* challenge resulted in an increase of antigen-specific serum IgA to concentrations similar to those seen in the BPZE1 group from challenge day 28 until the end of the study.

Participants in the BPZE1 group showed a significant increase in serum IgG responses to all tested antigens on day 28 after vaccination compared with the placebo group (figure 3; appendix pp 11–13). In the BPZE1 group, the geometric mean fold rises from baseline to day 28 were 1·5 (p<0·0001) for anti-whole-cell extract, 3·4 (p<0·0001) for anti-pertactin, 1·7 (p=0·0015) for anti-filamentous haemagglutinin, 3·0 (p<0·0001) for anti-Fim2/3, and 2·2 (p<0·0004) for anti-pertussis toxin serum IgG, which resulted in statistically significant geometric mean ratios between BPZE1 and placebo groups for anti-whole-cell extract (1·5; p=0·0001), anti-pertactin (3·1; p=0·0004), anti-filamentous haemagglutinin (1·8; p=0·0029), anti-Fim2/3 (3·6; p<0·0001), and anti-pertussis toxin (2·4; p=0·0013) serum IgG, with similar results observed using the ANCOVA model. In the BPZE1 group, anti-pertactin,

	mITT population			Per-protocol adequate inoculum population		
	BPZE1 group (n=24)	Placebo group (n=21)	Percentage point difference (95% CI); p value	BPZE1 group (n=20)	Placebo group (n=16)	Percentage point difference (95% CI); p value
Primary analysis						
No colonisation on days 9, 11, and 14 post-challenge	14 (58% [39 to 76])	7 (33% [17 to 55])	25% (−4 to 50); p=0·091	12 (60% [39 to 78])	4 (25% [10 to 50])	35% (2 to 61); p=0·033
Colonisation by day						
No colonisation on day 9 post-challenge	15 (63% [43 to 79])	7 (33% [17 to 55])	29% (0 to 54); p=0·049	13 (65% [43 to 82])	4 (25% [10 to 50])	40% (7 to 65); p=0·015
No colonisation on day 11 post-challenge	15 (65% [45 to 81])	7 (33% [17 to 55])	32% (2 to 56); p=0·033	13 (65% [43 to 82])	4 (25% [10 to 50])	40% (7 to 65); p=0·015
No colonisation on day 14 post-challenge	16 (70% [49 to 85])	7 (33% [17 to 55])	36% (7 to 60); p=0·015	14 (70% [48 to 86])	4 (25% [10 to 50])	45% (12 to 69); p=0·0062
Prespecified exploratory efficacy analysis						
Absolute bacterial counts for colonised participants on day 9 post-challenge, CFU/mL	106 (10 to 1102)	611 (165 to 2262)	..	33 (5 to 215)	448 (103 to 1942)	..
Absolute bacterial counts for colonised participants on day 11 post-challenge, CFU/mL	95 (16 to 561)	1914 (349 to 10 490)	..	49 (18 to 136)	1180 (187 to 7438)	..
Absolute bacterial counts for colonised participants on day 14 post-challenge, CFU/mL	515 (43 to 6215)	4055 (1042 to 15 778)	..	342 (20 to 5838)	2319 (635 to 8477)	..
Post-hoc exploratory analysis						
Bacterial density (days 9, 11, and 14 post-challenge), CFU/mL × days	18 (4 to 87)	611 (61 to 6105)	97% (59 to 100)	13 (3 to 63)	946 (84 to 10 654)	99% (80 to 100)

Data are n (%; 95% CI) or geometric mean (95% CI), unless specified otherwise. The mITT population was defined as all participants randomly assigned to treatment who were vaccinated, challenged, and had at least one culture result at days 9, 11, or 14 post-challenge. The per protocol adequate inoculum population was defined as all participants in the mITT population who received a challenge inoculum of at least $0{\cdot}5 \times 10^5$ CFU. CFU=colony forming units. mITT=modified intention-to-treat.

Table 2: **Colonisation by virulent *Bordetella pertussis* and bacterial density outcomes**

anti-filamentous haemagglutinin, anti-Fim2/3, and anti-pertussis toxin serum IgG had further increased 7 days before challenge and remained stable after challenge until the end of the study, whereas anti-whole-cell extract serum IgG remained stable after day 28 until the end of the study. In the placebo group, IgG responses increased after virulent challenge and remained stable until the end of the study.

BPZE1 vaccination induced serum bactericidal activity against pertactin-positive and pertactin-negative *B pertussis* in the mITT population (figure 3). The geometric mean fold rise of bactericidal activity against pertactin-positive *B pertussis* from baseline to day 28 after vaccination was higher in the BPZE1 group than in the placebo group (3·9 [95% CI 1·52–9·88] *vs* 0·9 [0·69–1·26]), and nine (69%) of 13 participants in the BPZE1 group had at least a two-fold increase in bactericidal activity from baseline to day 28, compared with one (6%) of 16 in the placebo group (p=0·0010). The geometric mean fold rise of bactericidal activity against pertactin-negative *B pertussis* from baseline to day 28 after vaccination was also higher in the BPZE1 group than in the placebo group (4·3 [2·75–6·68] *vs* 1·0 [0·86–1·17]), and 19 (79%) of 24 participants in the BPZE1 group had at least a two-fold increase in serum bactericidal activity titres against pertactin-negative *B pertussis*, compared with zero of 19 in the placebo group (p<0·0001). Similar serum bactericidal activity against pertactin-positive and pertactin-negative *B pertussis* was observed between day 28 after vaccination in the BPZE1 group and challenge day 28 in the placebo group (figure 3).

The study showed a favourable safety profile for the BPZE1 investigational vaccine. There were no deaths, serious adverse events, or adverse events leading to study discontinuation (table 3). Most participants in the safety population (22 [85%] of 26 in the BPZE1 group *vs* 22 [81%] of 27 in the placebo group) reported at least one solicited adverse event during the 7 days after vaccination, which were mostly mild (grade 1) in severity. Eight (31%) of 26 in the BPZE1 group and three (11%) of 27 in the placebo group reported a moderate (grade 2) solicited adverse event, and no severe (grade 3) solicited adverse events were reported (appendix p 4). The most common reported solicited adverse events during the 7 days after vaccination were stuffy nose or congestion (11 [42%] in the BPZE1 group *vs* 12 [44%] in the placebo group), runny nose (ten [38%] *vs* eight [30%]), sore or irritated throat (ten [38%] *vs* five [18%]), sneezing (ten [38%] *vs* six [22%]), headache (15 [58%] *vs* nine [33%]), and fatigue (ten [38%] *vs* 12 [44%]). The mean duration of these solicited adverse events was between 2·1 days (SD 121) and 3·7 days (2·70). One participant in the BPZE1 group had a transient moderate fever of 38·6°C 1 day after vaccination.

At 28 days after vaccination, a similar proportion of participants reported unsolicited treatment-emergent adverse events in the BPZE1 versus placebo groups (seven [27%] of 26 *vs* nine [33%] of 27), and more unsolicited adverse events

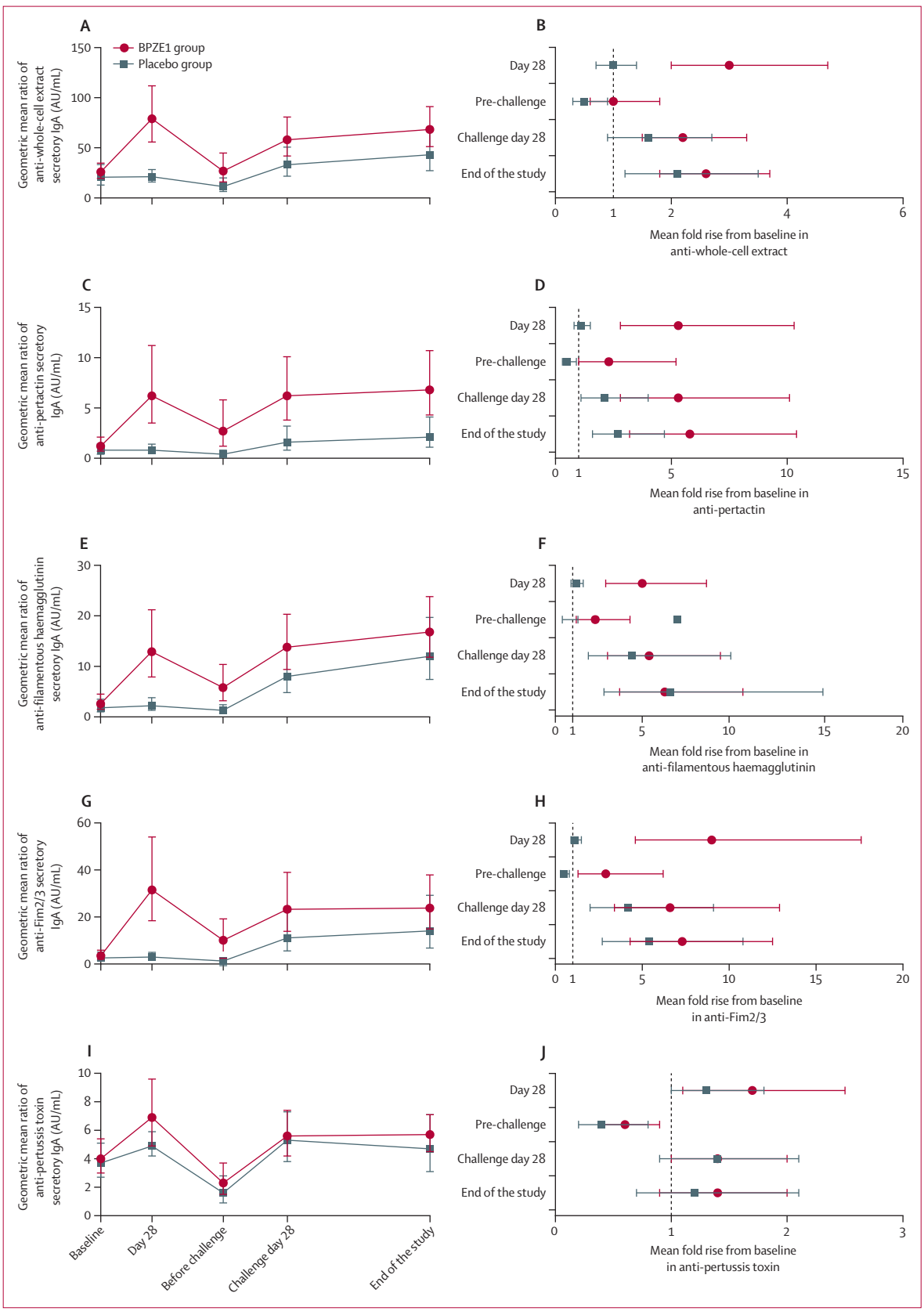

Figure 2: **Nasal secretory IgA responses to *Bordetella pertussis* antigens in the modified intention-to-treat population**

Geometric mean ratios of secretory IgA to whole-cell extracts, pertactin, filamentous haemagglutinin, Fim2/3, and pertussis toxin normalised by total secretory IgA (mg/mL) and geometric mean fold rise from baseline at indicated timepoints in BPZE1 and placebo groups. Bars indicate 95% CIs. AU=arbitrary unit. FIM2/3=serotype 2 and 3 fimbriae.

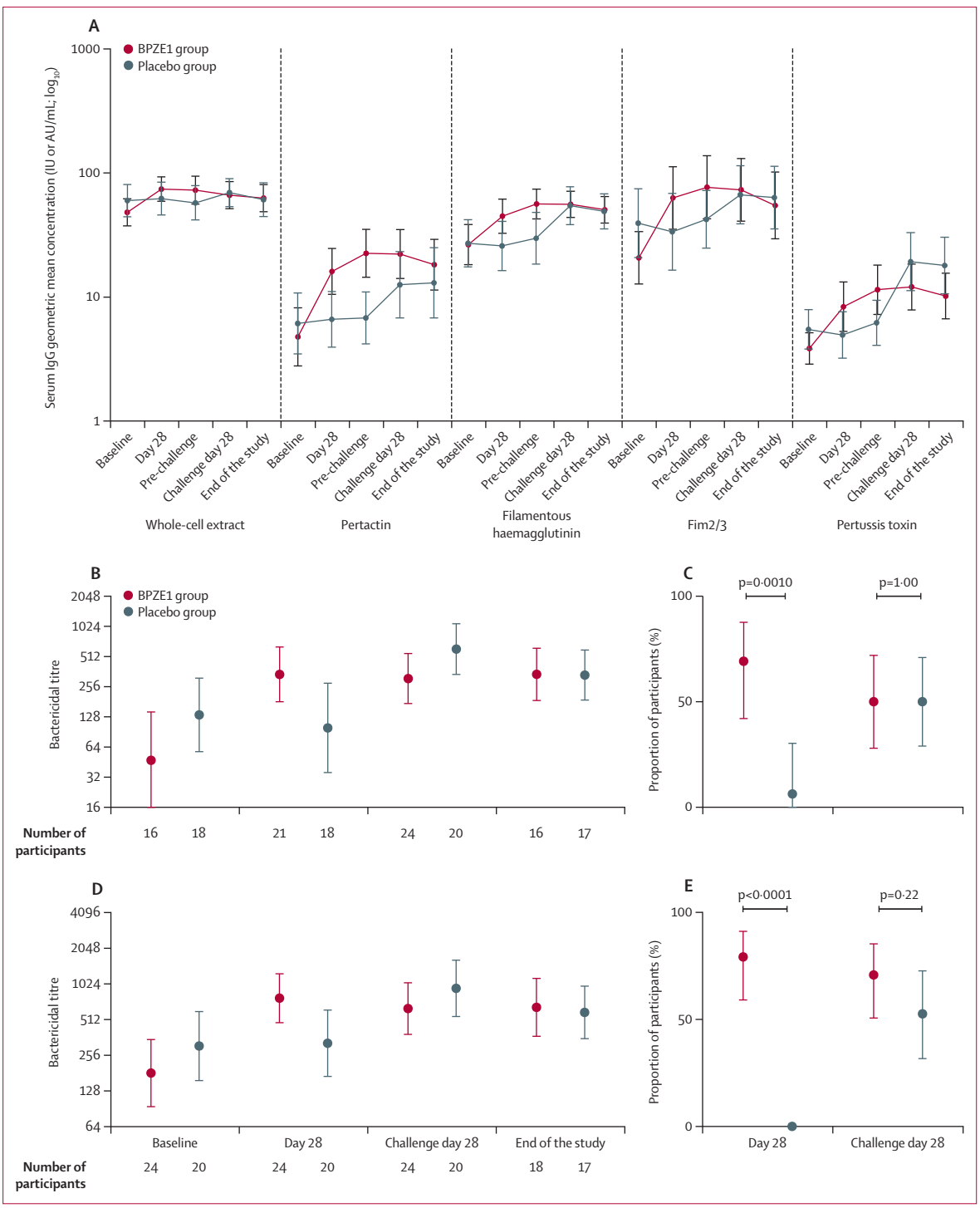

Figure 3: **Serum IgG responses to *Bordetella pertussis* antigens and serum bactericidal activity in the modified intention-to-treat population**
(A) Serum IgG geometric mean concentrations expressed as IUs for pertactin, filamentous haemagglutinin, Fim2/3, and pertussis toxin and as AUs for whole-cell extract and comparing the BPZE1 with placebo groups. Serum bactericidal activity against pertactin-positive *B pertussis* (B) and pertactin-negative *B pertussis* (D). Proportion of participants with at least a two-fold increase in serum bactericidal titres against pertactin-positive *B pertussis* (C) and pertactin-negative *B pertussis* (E) at indicated timepoints in BPZE1 and placebo groups. Bars indicate 95% CIs. AU=arbitrary unit. FIM2/3=serotype 2 and 3 fimbriae. IU=international unit.

	Vaccine phase		Virulent challenge phase	
	BPZE1 group (n=26)	Placebo group (n=27)	BPZE1 group (n=26)	Placebo group (n=27)
Any treatment-emergent adverse events*	7 (27%)	9 (33%)	20 (77%)	16 (59%)
Mild (grade 1)	3 (12%)	8 (30%)	13 (50%)	12 (44%)
Moderate (grade 2)	4 (15%)	1 (4%)	7 (27%)	4 (15%)
Severe (grade 3)	0	0	0	0
Treatment-emergent adverse events related to vaccination or mucosal atomisation device	2* (8%)	1 (4%)	0	0
Treatment-emergent adverse events related to challenge	NA	NA	3* (12%)	7 (26%)
Serious adverse events†	0	0	0	0
Adverse events leading to study discontinuation	0	0	0	0
Death	0	0	0	0

Data are n (%). The safety population was defined as all participants who were vaccinated. Vaccine phase is defined as time from study vaccination to virulent challenge administration 60–120 days after study vaccination. Virulent challenge phase is defined as time from virulent challenge administration to the end of the study (two participants in the BPZE1 group and five in the placebo group did not receive the virulent challenge administration). NA=not applicable. *One participant reported treatment-emergent adverse events related to vaccination and challenge. †Includes treatment-emergent adverse events collected for 28 days after vaccination and virulent challenge, and serious adverse events collected throughout the study.

Table 3: **Summary of adverse events (safety population; n=53)**

were reported after virulent challenge (20 [77%] *vs* 16 [59%]; table 3). More unsolicited treatment-emergent adverse events related to challenge were reported in the placebo group (seven [26%]) than the BPZE1 group (three [12%]). No serious adverse events or discontinuations due to adverse events were reported during the trial.

Laboratory test results and vital sign measurements were similar between treatment groups. The few abnormal laboratory values were mainly mild (grade 1) with the exception of a participant with moderate-to-low platelet counts and a participant with high (grade 3) urea concentrations including at baseline due to high dietary protein intake.

Discussion

This randomised, placebo-controlled trial shows that a single intranasal administration of the live attenuated pertussis vaccine BPZE1 can prevent infection by virulent *B pertussis* in a controlled human infection model. In the BPZE1 group, ten (42%) of 24 participants in the mITT population and eight (40%) of 20 in the per protocol adequate inoculum population had detectable challenge bacteria on days 9, 11, or 14 post-challenge, whereas in the placebo group, bacteria could be detected in 14 (67%) of 21 in the mITT population and 12 (75%) of 16 in the per protocol adequate inoculum population. The colonisation rate in the placebo group was similar to that in the earlier study.[16]

In a post-hoc analysis, the post-challenge bacterial burden measured at days 9, 11, and 14 post-challenge was reduced by more than 97% in the BPZE1 group compared with the placebo group. Furthermore, on day 14 post-challenge, 16 (70%) participants in the BPZE1 group versus seven (33%) in the placebo group had cleared the challenge bacteria in the mITT population and 14 (70%) versus four (25%) in the per protocol adequate inoculum population, indicating faster clearance in the BPZE1 group. Although this study was designed to evaluate protection by BPZE1 against *B pertussis* infection, rather than against pertussis disease, the substantial reduction in bacterial burden by BPZE1 vaccination suggests that it would greatly reduce disease severity and provide clinical benefit. Studies have previously shown that pertussis severity is strongly correlated with bacterial load;[18,19] therefore, reduction in bacterial burden can be considered a valid surrogate outcome for protection against disease.

BPZE1 vaccination induced *B pertussis*-specific secretory IgA in nasal secretions, especially evident against filamentous haemagglutinin, pertactin, and Fim2/3. Murine studies have shown the importance of BPZE1-induced nasal secretory IgA responses in the prevention of infection.[11] Filamentous haemagglutinin and fimbriae are involved in bacterial adherence to the respiratory epithelium;[20] therefore, anti-filamentous haemagglutinin and anti-fimbriae secretory IgA might prevent attachment of the bacteria to the epithelium. IgA is also able to opsonise *B pertussis*, resulting in phagocytosis and polymorphonuclear leukocyte activation via the Fcα receptor, leading to bacterial killing.[21]

BPZE1-induced secretory IgA responses showed similar patterns to serum IgA responses, which were also strongest against filamentous haemagglutinin, pertactin, and Fim2/3, although responses remained low against pertussis toxin, as shown previously.[22] However, BPZE1 vaccination resulted in increased serum anti-pertussis toxin IgG concentrations. Although no correlate of protection against pertussis has yet been established, pertussis toxin plays a key role in the pathogenesis of pertussis,[23] and serum IgG that neutralise pertussis toxin prevents severe disease in non-human primates.[24]

Anti-filamentous haemagglutinin, anti-pertactin, and anti-Fim2/3 serum IgG were also induced by BPZE1 vaccination. The role of anti-filamentous haemagglutinin and anti-fimbriae serum IgG in protection against pertussis is still unknown, although high anti-fimbriae serum IgG concentrations have been associated with clinical

protection against pertussis disease.[25] Whereas anti-pertactin serum IgG has shown complement-dependent bactericidal properties against *B pertussis*, and pertactin is the only antigen in acellular pertussis vaccines that induces bactericidal antibodies.[26] Approximately two-thirds of participants in the BPZE1 group showed at least a two-fold increase in serum bactericidal activity against pertactin-positive and pertactin-negative *B pertussis*. Notably, pertactin-deficient strains have been isolated with increasing frequencies in countries using acellular pertussis vaccines.[27] Although the role of serum bactericidal antibodies in protection against infection by *B pertussis*, a strictly mucosal pathogen, has not been established yet, bactericidal IgG and complement have each been shown to penetrate the mucosa of the upper respiratory tract.[28]

The vaccine was well tolerated. Nasal and respiratory and systemic solicited adverse events, including congestion, sneezing, headache, and fatigue were all mild to moderate, and their frequency was similar between groups. No serious adverse events or discontinuations due to adverse events occurred during the study. Overall, the study showed a favourable safety profile for BPZE1.

Some limitations of the study include the small numbers of participants who underwent vaccination and challenge. Challenge with the appropriate challenge dose of at least $0{\cdot}5 \times 10^5$ CFU included 20 participants in the BPZE1 group and 16 in the placebo group, whereas nine participants inadvertently received a challenge dose lower than the target. However, the statistical analysis plan included a prespecified sensitivity analysis of the primary outcome in those who did receive an adequate inoculum of the challenge dose. Given the nature of the study, with participants randomly assigned to treatment groups for vaccination and called back later for challenge, the challenge group became a subgroup of the trial and was exposed to the biases present in subgroup analysis. Despite the reduced sample size, the study was still powered for the primary analysis using the per protocol adequate inoculum population; therefore, even with this potential selection bias and the small sample size, this study provides the first proof of concept that a single nasal vaccination with BPZE1 can prevent colonisation by virulent *B pertussis* and substantially reduces bacterial burden. We also confirmed the inoculum dose to establish consistent infection of at least 75% of participants who have less than 20 IU/mL anti-pertussis toxin antibodies.[16]

Consistent with the model and to ensure robustness of the study, we excluded participants with high baseline anti-pertussis toxin and anti-pertactin antibody concentrations, as they are suggestive of recent (within 1–3 years) infection by *B pertussis*, likely to result in immunity against *B pertussis*, thereby limiting virulent challenge take in the placebo group. Furthermore, the study was conducted in non-smoking healthy adults who were not using nasal sprays; therefore, the findings might not be generally applicable to all populations. If licensed, BPZE1 would be used for all comers, regardless of health status and baseline antibody concentrations. Whereas in this latter population, including those with high baseline antibody concentrations, clinical benefit of BPZE1 vaccination might be more modest, the most susceptible population (ie, those who are not protected by natural infection) might greatly benefit from BPZE1 vaccination. Post-marketing studies will establish the general population representativeness, since they are likely to include young children and individuals with specific health conditions.

In this study, participants were challenged with a single *B pertussis* strain and vaccine efficacy might vary according to the challenge strain; however, strain B1917 was selected as the most representative strain circulating in Europe in the first decades of the 21th century, and animal studies have shown that BPZE1 is highly protective against other *Bordetella* strains as well, including *Bordetella parapertussis* and *Bordetella bronchiseptica*.[9–11] Additional field studies will be able to show vaccine efficacy against all circulating strains.

A further limitation of the study was that it was performed with healthy adults, most of whom have likely been primed with whole-cell pertussis vaccines in infancy. Although adults represent an important source of asymptomatic *B pertussis* transmission to other susceptible populations, pertussis is most severe in younger age groups, including infants younger than 1 year. A clinical trial is ongoing (NCT05116241), which involves school-aged children (6–17 years) primed with the acellular pertussis vaccine.

Finally, only antibody responses are reported. Since protection against pertussis relies on antibodies and involves cell-mediated immunity, including resident memory T cells,[29] we will analyse cellular immunity in a separate analysis.

In conclusion, a controlled human infection study showed that a single intranasal administration of the live attenuated BPZE1 vaccine prevents infection with virulent *B pertussis* in a substantial number of participants and decreases the bacterial burden after challenge compared with the placebo group. BPZE1 induced mucosal secretory IgA responses in the nasal cavity and serum antibodies against *B pertussis* antigens, which display bactericidal activity against pertactin-positive and pertactin-negative strains. BPZE1 is an attractive vaccine candidate for the prevention of *B pertussis* infection and likely disease. Given the favourable safety profile of the BPZE1 vaccine and encouraging protective results, large phase 3 trials are warranted to confirm these initial findings.

Contributors

DG, KS, KR, SN, and RCR contributed to the design and development of the trial protocol. DG, MNR, MI, ARH, LR, JG, APD, JRL, DMF, TC, SNF, TANR, GB, and RCR provided all participant and laboratory data. DG, MNR, MI, ARH, VS, SN, and RCR accessed and verified the data. DG, LW, WL, VS, PG, KS, SN, and RCR analysed the data. CL and SN wrote the initial draft of the manuscript. All authors read, provided input, and approved the final manuscript and had final responsibility for the decision to submit for publication. All authors had access to the summarised data but not patient-specific data to maintain the integrity of the trial.

Declaration of interests
VS, PG, KS, KR, and SN are employees and equity holders of ILiAD Biotechnologies. CL holds patents on the BPZE1 vaccine, which is licensed to ILiAD Biotechnologies, and reports consulting fees from and is an equity holder of ILiAD Biotechnologies. All other authors declare no competing interests.

Data sharing
All data requests should be submitted to the corresponding author for consideration. Access to anonymised data might be granted after review.

Acknowledgments
We thank trial participants and trial site personnel, nursing, medical, project management, and laboratory team members; members of the independent data safety monitoring board for their contributions; BioFabri and BioLyo for vaccine production; Pu Huang, Xianyi Kong, and Svetlana Rayner for help with the statistical analyses; Sue Charlton, Mary Matheson, Hannah Cuthbertson, and the Clinical Evaluation Team (Immunoassay Group) for the immunoassays at the screening visits; and André M Campos and Theressa Creighton for the operational management of the study.

References
1 Hewlett EL, Edwards KM. Clinical practice. Pertussis—not just for kids. *N Engl J Med* 2005; **352:** 1215–22.
2 Yeung KHT, Duclos P, Nelson EAS, Hutubessy RCW. An update of the global burden of pertussis in children younger than 5 years: a modelling study. *Lancet Infect Dis* 2017; **17:** 974–80.
3 Cherry JD. Epidemic pertussis in 2012—the resurgence of a vaccine-preventable disease. *N Engl J Med* 2012; **367:** 785–87.
4 Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. *Proc Natl Acad Sci U S A* 2014; **111:** 787–92.
5 Locht C. Will we have new pertussis vaccines? *Vaccine* 2018; **36:** 5460–69.
6 Althouse BM, Scarpino SV. Asymptomatic transmission and the resurgence of *Bordetella pertussis*. *BMC Med* 2015; **13:** 146.
7 Anderson RM, May RM. Directly transmitted infections diseases: control by vaccination. *Science* 1982; **215:** 1053–60.
8 Merkel TJ. Toward a controlled human infection model of pertussis. *Clin Infect Dis* 2020; **71:** 412–14.
9 Mielcarek N, Debrie AS, Raze D, et al. Live attenuated *B. pertussis* as a single-dose nasal vaccine against whooping cough. *PLoS Pathog* 2006; **2:** e65.
10 Solans L, Debrie AS, Borkner L, et al. IL-17-dependent SIgA-mediated protection against nasal *Bordetella pertussis* infection by live attenuated BPZE1 vaccine. *Mucosal Immunol* 2018; **11:** 1753–62.
11 Locht C, Papin JF, Lecher S, et al. Live attenuated pertussis vaccine BPZE1 protects baboons against *Bordetella pertussis* disease and infection. *J Infect Dis* 2017; **216:** 117–24.
12 Thorstensson R, Trollfors B, Al-Tawil N, et al. A phase I clinical study of a live attenuated *Bordetella pertussis* vaccine – BPZE1; a single centre, double-blind, placebo-controlled, dose-escalating study of BPZE1 given intranasally to healthy adult male volunteers. *PLoS One* 2014; **9:** e83449.
13 Jahnmatz M, Richert L, Al-Tawil N, et al. Safety and immunogenicity of the live attenuated intranasal pertussis vaccine BPZE1: a phase 1b, double-blind, randomised, placebo-controlled dose-escalation study. *Lancet Infect Dis* 2020; **20:** 1290–301.
14 Buddy Creech C, Jimenez-Truque N, Kown N, et al. Safety and immunogenicity of live, attenuated intranasal *Bordetella pertussis* vaccine (BPZE1) in healthy adults. *Vaccine* 2022; **40:** 6740–46.
15 Keech C, Miller VE, Rizzardi B, et al. Immunogenicity and safety of BPZE1, an intranasal live attenuated pertussis vaccine, versus tetanus-diphtheria-acellular pertussis vaccine: a randomised, double-blind, phase 2b trial. *Lancet* 2023; **401:** 843–55.
16 de Graaf H, Ibrahim M, Hill AR, et al. Controlled human infection with *Bordetella pertussis* induces asymptomatic, immunizing colonization. *Clin Infect Dis* 2020; **71:** 403–11.
17 Bart MJ, Zeddeman A, van der Heide HGJ, Heuvelman K, van Gent M, Mooi FR. Complete genome sequences of *Bordetella pertussis* isolates B1917 and B1920, representing two predominant global lineages. *Genome Announc* 2014; **2:** e01301–14.
18 Bolotin S, Deeks SL, Marchand-Austin A, et al. Correlation of real time PCR cycle threshold cut-off with *Bordetella pertussis* clinical severity. *PLoS One* 2015; **10:** e0133209.
19 Thuy Nga DT, Thi Bich Thuy P, Ainai A, et al. Association between real-time polymerase chain reaction cycle threshold value and clinical severity in neonates and infants infected with *Bordetella pertussis*. *Pediatr Infect Dis J* 2022; **41:** 388–93.
20 Rodríguez ME, Hellwig SMM, Pérez Vidakovics MLA, Berbers GAM, van de Winkel JGJ. *Bordetella pertussis* attachment to respiratory epithelial cells can be impaired by fimbriae-specific antibodies. *FEMS Immunol Med Microbiol* 2006; **46:** 39–47.
21 Rodriguez ME, Hellwig SMM, Hozbor DF, Leusen J, van der Pol WL, van de Winkel JGJ. Fc receptor-mediated immunity against *Bordetella pertussis*. *J Immunol* 2001; **167:** 6545–51.
22 Lin A, Apostolovic D, Jahnmatz M, et al. Live attenuated pertussis vaccine BPZE1 induces a broad antibody response in humans. *J Clin Invest* 2020; **130:** 2332–46.
23 Locht C, Antoine R. The history of pertussis toxin. *Toxins* 2021; **13:** 623.
24 Nguyen AW, DiVenere AM, Papin JF, Connelly S, Kaleko M, Maynard JA. Neutralization of pertussis toxin by a single antibody prevents clinical pertussis in neonatal baboons. *Sci Adv* 2020; **6:** eaay9258.
25 Storsaeter J, Hallander HO, Gustafsson L, Olin P. Levels of anti-pertussis antibodies related to protection after household exposure to *Bordetella pertussis*. *Vaccine* 1998; **16:** 1907–16.
26 Lesne E, Cavell BE, Freire-Martin I, et al. Acellular pertussis vaccines induce anti-pertactin bactericidal antibodies which drives the emergence of pertactin-negative strains. *Front Microbiol* 2020; **11:** 2108.
27 Barkoff AM, Mertsola J, Pierard D, et al. Pertactin-deficient *Bordetella pertussis* isolates: evidence of increased circulation in Europe, 1998 to 2015. *Euro Surveill* 2019; **24:** 1700832.
28 Persson C. Humoral first-line mucosal innate defence in vivo. *J Innate Immun* 2020; **12:** 373–86.
29 Chasaide CN, Mills KHG. Next-generation pertussis vaccines based on the induction of protective T cells in the respiratory tract. *Vaccines* 2020; **8:** 621.