Immunogenicity and safety of BPZE1, an intranasal live attenuated pertussis vaccine, versus tetanus–diphtheria–acellular pertussis vaccine: a randomised, double-blind, phase 2b trial

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Summary

Background ***Bordetella pertussis*** **epidemics persist as transmission remains unabated despite high acellular pertussis vaccination rates. BPZE1, a live attenuated intranasal pertussis vaccine, was designed to prevent *B pertussis* infection and disease. We aimed to assess the immunogenicity and safety of BPZE1 compared with the tetanus–diphtheria–acellular pertussis vaccine (Tdap).**

Methods In this double-blind, phase 2b trial at three research centres in the USA, healthy adults aged 18–50 years were randomly assigned (2:2:1:1) via a permuted block randomisation schedule to receive BPZE1 vaccination followed by BPZE1 attenuated challenge, BPZE1 vaccination followed by placebo challenge, Tdap followed by BPZE1 attenuated challenge, or Tdap followed by placebo challenge. On day 1, lyophilised BPZE1 was reconstituted with sterile water and given intranasally (0·4 mL delivered to each nostril), whereas Tdap was given intramuscularly. To maintain masking, participants in the BPZE1 groups received an intramuscular saline injection, and those in the Tdap groups received intranasal lyophilised placebo buffer. The attenuated challenge took place on day 85. The primary immunogenicity endpoint was the proportion of participants achieving nasal secretory IgA seroconversion against at least one *B pertussis* antigen on day 29 or day 113. Reactogenicity was assessed up to 7 days after vaccination and challenge, and adverse events were recorded for 28 days after vaccination and challenge. Serious adverse events were monitored throughout the study. This trial is registered with ClinicalTrials.gov, NCT03942406.

Findings Between June 17 and Oct 3, 2019, 458 participants were screened and 280 were randomly assigned to the main cohort: 92 to the BPZE1–BPZE1 group, 92 to the BPZE1–placebo group, 46 to the Tdap–BPZE1 group, and 50 to the Tdap–placebo group. Seroconversion of at least one *B pertussis*-specific nasal secretory IgA was recorded in 79 (94% [95% CI 87–98]) of 84 participants in the BPZE1–BPZE1 group, 89 (95% [88–98]) of 94 in the BPZE1–placebo group, 38 (90% [77–97]) of 42 in the Tdap–BPZE1 group, and 42 (93% [82–99]) of 45 in the Tdap–placebo group. BPZE1 induced broad and consistent *B pertussis*-specific mucosal secretory IgA responses, whereas Tdap did not induce consistent mucosal secretory IgA responses. Both vaccines were well tolerated, with mild reactogenicity and no serious adverse events related to study vaccination.

Interpretation BPZE1 induced nasal mucosal immunity and produced functional serum responses. BPZE1 has the potential to avert *B pertussis* infections, which ultimately could lead to reduced transmission and diminished epidemic cycles. These results should be confirmed in large phase 3 trials.

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Introduction

Pertussis or whooping cough is a highly contagious severe respiratory disease caused by *Bordetella pertussis*. Despite high global vaccination coverage,[1] pertussis remains a serious worldwide health problem, with periodic community epidemics and mortality occurring in young infants.[2] Even vaccinated adolescents and adults can easily be infected with *B pertussis* and experience prolonged symptoms; such people can serve as major bacterial reservoirs.[3] Because *B pertussis* is highly infectious,[4] pertussis vaccines should ideally prevent not only pertussis disease, but also *B pertussis* colonising infection and transmission.[5]

Two types of pertussis vaccines are currently in use: whole-cell vaccines and, more recently, acellular pertussis vaccines (aPVs).[6] Many countries began to exclusively use aPVs in the 1990s or early 2000s, and subsequently pertussis infections increased in frequency. Several factors might account for this resurgence,[7] but two major contributors are the short duration of aPV-induced immunity[8,9] and the fact that, similar to whole-cell pertussis vaccines, aPVs do not prevent infection with, or

Research in context

Evidence before this study

Most high-income countries now use acellular pertussis vaccines (aPVs) for the prevention of whooping cough or pertussis. However, pertussis outbreaks in highly vaccinated populations have shown the limitations of these vaccines. Rapid waning of vaccine-induced immunity and the failure of aPVs to prevent *Bordetella pertussis* infection and transmission are prevailing hypotheses for the resurgence of pertussis. *B pertussis* is a strictly mucosal pathogen, but—unlike natural infection—aPVs do not induce mucosal immunity. Vaccines that mimic natural infection could potentially limit acquisition and accelerate clearance of colonising *B pertussis*. We searched PubMed with the terms "pertussis", "whooping cough" AND "mucosal vaccine", "intranasal", "live attenuated" for studies of mucosal pertussis vaccination up to July 25, 2022, with no language restriction and in various combinations. Our search yielded 276 references, several of which describe novel mucosal anti-pertussis vaccines. However, none of these novel vaccines have reached clinical development, except for the live attenuated vaccine BPZE1. Two articles detailed safety, colonisation, and serum IgG responses after a nasal administration of a single dose of BPZE1 (containing up to 10^9 colony-forming units) in phase 1 trials.

Added value of this study

This is the first study of the effect of intranasal BPZE1 vaccination compared with the tetanus–diphtheria–acellular pertussis vaccine (Tdap) vaccine in terms of the induction of nasal secretory IgA response. BPZE1 had an acceptable safety profile and was well tolerated. A single BPZE1 dose induced potent nasal secretory IgA against *B pertussis* antigens. By contrast, Tdap did not consistently induce secretory IgA responses.

Implications of all the available evidence

This study provides the first proof of concept in humans that a single nasal administration of the live attenuated pertussis vaccine BPZE1 can produce a secretory IgA response against *B pertussis*. Our findings support further development of BPZE1 as a safe next-generation pertussis vaccine that could potentially reduce *B pertussis* infection and transmission.

human-to-human transmission of, *B pertussis*.[10] In a non-human primate challenge model, use of an aPV did not protect against colonising infection or transmission of *B pertussis*.[11] Animal studies suggest that immunisation with aPVs might prolong nasal carriage of *B pertussis*[11,12] and therefore increase the bacterial reservoir. By contrast, recovery from *B pertussis* infection protected primates against subsequent infection and disease when exposed to a second *B pertussis* challenge.[11]

An improved vaccine that safely mimics natural infection could ultimately control the spread of pertussis by limiting infection and transmission. The intranasal live attenuated pertussis vaccine BPZE1, which has been developed by genetic inactivation or removal of three major *B pertussis* toxins, was designed for such a purpose.[13] Studies in mice and non-human primates have shown that, in contrast to aPVs, a single BPZE1 dose provides effective protection against both nasal colonising infection and pertussis disease.[14,15] Furthermore, whereas aPV-induced protective immunity starts to wane 6 months after vaccination in mice, no sign of waning immunity was noted for up to 12 months after vaccination with BPZE1.[16] Phase 1 clinical studies[17–19] have shown that doses of 10^3–10^9 colony-forming units (CFU) of BPZE1 are well tolerated and induce antibody responses to a broad range of *B pertussis* antigens and Th1-type cellular responses.

In this phase 2b trial we assessed the safety and induction of mucosal and systemic immunity of BPZE1 compared with the tetanus–diphtheria–acellular pertussis vaccine (Tdap) in healthy adults. We also assessed protection against a subsequent re-administration of BPZE1 as an attenuated challenge.

See Online for appendix

Methods

Study design and participants

We did a randomised, double-blind, phase 2b study at three research centres in the USA. We recruited healthy people aged 18–50 years. Participants with childbearing potential could not be pregnant or lactating and had to use an appropriate contraceptive method. Major exclusion criteria included pertussis vaccination in the past 5 years, diagnosis of pertussis in the past 10 years, immunosuppression, acute or chronic pulmonary disease, unwillingness to refrain from tobacco products, routine use of nasal steroids, sprays or neti pots, and recent nasal surgery. Further details of eligibility criteria are provided in the appendix (pp 55–59). The trial protocol was approved by a central institutional review board. The study proceeded in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. All participants provided written informed consent before enrolment. This trial is registered with ClinicalTrials.gov, NCT03942406.

Randomisation and masking

Eligible participants were randomly assigned (2:2:1:1) to receive the BPZE1 vaccine (day 1) followed by an intranasal attenuated challenge with BPZE1 (day 85), BPZE1 (day 1) followed by placebo challenge (day 85), the Tdap vaccine (day 1) followed by BPZE1 attenuated challenge (day 85), or Tdap (day 1) followed by placebo challenge (day 85; appendix p 1). Participants were recruited and enrolled by trial investigators and their staff. A dedicated randomisation team (from a clinical research organisation) generated a permuted block randomisation schedule in SAS (version 9.4) using interactive response technology to link sequential

participant randomisation numbers to treatment codes. The project team was masked to the randomisation schedule, except for unmasked pharmacy staff, who managed vaccine logistics, reconstitution, administration, product accountability, and destruction. The pharmacy staff were not involved in study-related assessments and had no participant contact after vaccine administration. Participants and the project team overseeing study assessments remained masked until after database lock. Because the study vaccines are administered via different routes (BPZE1 is intranasal, whereas Tdap is intramuscular), to maintain masking all participants allocated to BPZE1 groups received a concomitant intramuscular placebo vaccine on day 1, and all those in Tdap groups received a concomitant intranasal placebo vaccine on day 1. The first 48 participants were designated as the safety cohort and followed the randomisation scheme (ie, 2:2:1:1). For the first 24 participants randomly assigned in the safety cohort, the dose of BPZE1 given was 10^7 CFU. After medical review of reactogenicity data up to day 8, the dose was upped to 10^9 CFU for the next 24 participants. Participants in the safety cohort who were assigned to Tdap followed by placebo challenge received the same dose of Tdap as those in the main analysis cohort. The independent safety monitoring committee reviewed all safety data up to 7 days after vaccination at both dose levels and then approved randomisation of the remaining participants using the dose of 10^9 CFU of BPZE1.

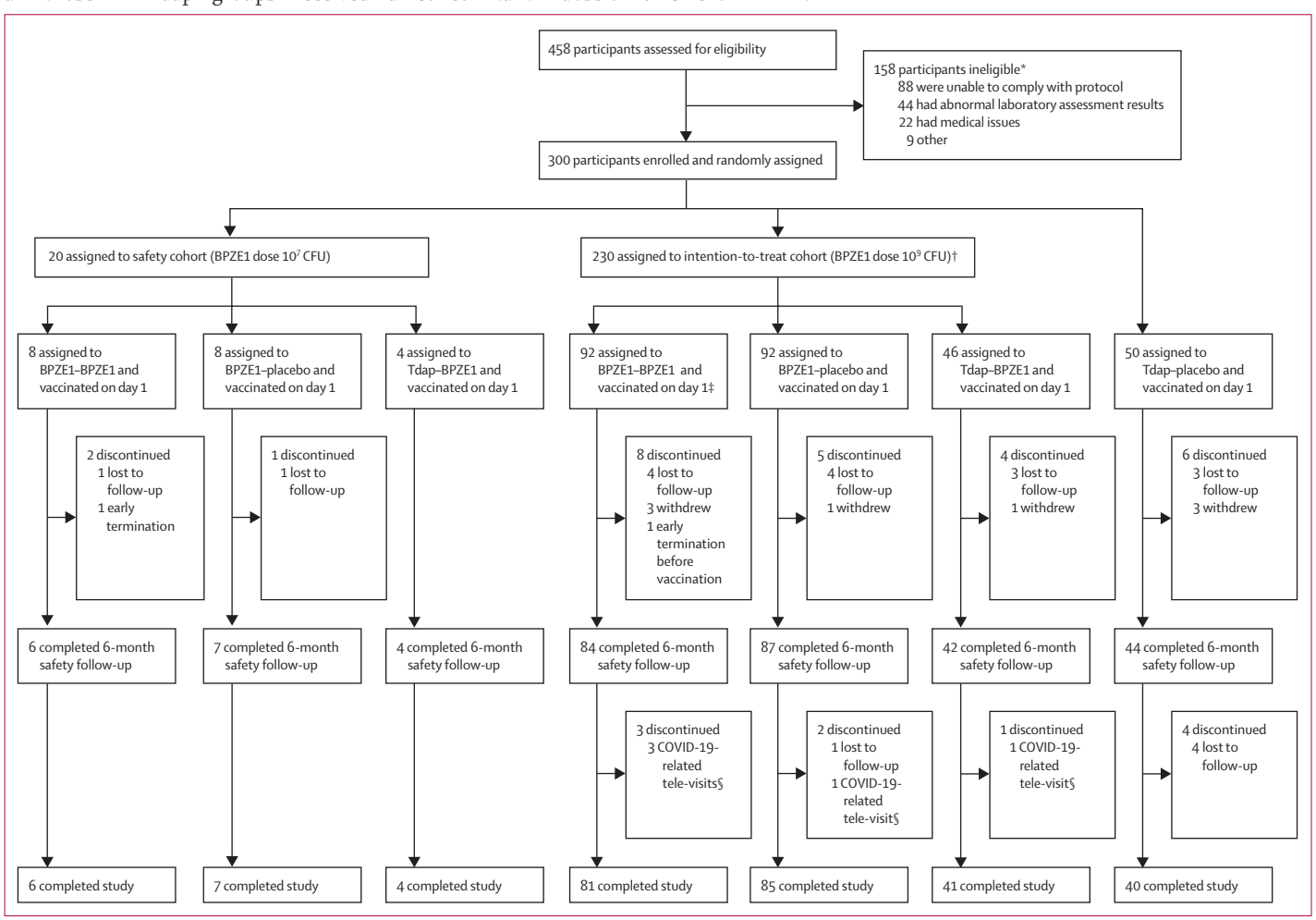

Figure 1: **Trial profile**
In the safety cohort, six participants in the BPZE1–BPZE1 group, seven in the BPZE1–placebo group, and four in the Tdap–BPZE1 group underwent challenge. In the intention-to-treat cohort, 82 participants in the BPZE1–BPZE1 group, 85 in the BPZE1–placebo group, 42 in the Tdap–BPZE1 group, and 43 in the Tdap–placebo group underwent challenge. The immunogenicity analysis set includes 85 participants in the BPZE1–BPZE1 group, 94 in the BPZE1–placebo group, 43 in the Tdap–BPZE1 group, and 45 in the Tdap–placebo group. Participants excluded from the immunogenicity analysis set included one participant who never received vaccination, two who received Tdap vaccines outside the study, and ten who had missing or no samples and discontinued early, with none undergoing challenge. The four patients assigned to Tdap–placebo in the safety cohort were analysed as part of the full cohort. CFU=colony-forming units. Tdap=tetanus–diphtheria–acellular pertussis vaccine. *Some participants were ineligible for more than one reason. †Includes members of their respective safety cohort who received at least one vaccine or challenge with 10^9 CFU BPZE1. ‡One participant assigned to the BPZE1–BPZE1 group (10^9 CFU dose) was not vaccinated. §In-person site visits were affected by the COVID-19 pandemic. After institutional review board approval, telemedicine visits for long-term safety follow-up were permitted; these participants provided safety information up to the end of the study but are reported as premature discontinuations per protocol.

Procedures

On day 1, participants received either BPZE1 intranasally or 0·5 mL Tdap (Boostrix, GlaxoSmithKline, London, UK) intramuscularly (appendix pp 62–63). Lyophilised BPZE1 from single-use vials was reconstituted with sterile water for intranasal administration, and 0·4 mL was delivered to each nostril with a VaxINator mucosal atomisation device (Teleflex Medical, Salt Lake City, UT, USA). Participants receiving BPZE1 on day 1 also received an intramuscular placebo injection of normal saline (Fresenius Kabi, Lake Zurich, IL, USA), whereas those in the Tdap group received lyophilised placebo buffer (BioLyo Technologies, Ghent, Belgium).

On day 85, participants underwent attenuated challenge with either BPZE1 or placebo delivered intranasally (0·4 mL per nostril). After receiving their doses of vaccine or placebo on days 1 and 85, participants remained in the clinic for 1 h to assess safety (solicited reactogenicity and vital signs). Participants received a diary and self-monitored reactogenicity daily for the next 7 days, including nasal or respiratory, local (only after the first vaccination), and systemic symptoms. US Food and Drug Administration (FDA) toxicity grading was applied.[20,21]

Nasal secretion samples (used to measure mucosal secretory IgA against *B pertussis* antigens) were obtained at baseline and on days 29, 78, 113, 169, and 254 from each nostril. Leukosorb absorption paper composed of a synthetic absorptive matrix (Accuwik Ultra; both manufactured by Pall Scientific, Port Washington, NY, USA) was used to collect these samples. Participants were instructed on inserting the Leukosorb paper strip while being observed by study staff. The paper was retained against the nasal mucosal surface using clips for 2 min, then removed via tweezers and placed into collection tubes.[22] Serum samples were taken to measure IgA and IgG concentrations targeted against *B pertussis* antigens. Nasal samples were taken approximately 6 days before intranasal vaccination and challenge, 28 days after vaccination and challenge, and 6 months and 9 months after randomisation. Serum samples were obtained on the day of vaccination and challenge (before administration), 28 days after vaccination and challenge, and 6 months and 9 months after randomisation. All samples were stored at –80°C until analysed.

Electrochemiluminescence immunoassays were done to detect antibodies against pertactin and whole-cell *B pertussis* extract (Meso Scale Diagnostics, Rockville, MD, USA). We used commercial enzyme-linked immunosorbent assay kits (EuroImmun, Lübeck, Germany) to measure antibodies against pertussis toxin and filamentous haemagglutinin. We used a human IgA kit (Meso Scale Diagnostics) to measure total secretory IgA concentrations, which were used as a basis to normalise *B pertussis*-specific secretory IgA responses. All assays were validated before final analyses and results were standardised against the WHO International Standard Human Pertussis Antiserum 06/140.

To assess colonisation before and after attenuated challenge (day 85), participants underwent nasal aspiration using a syringe aspiration NPak kit (M-PRO, Nisswa, MN, USA) on days 78, 92, 96, and 113. Nasal aspirates were stored in 70% glycerol at –80°C. The samples were cultured on charcoal agar medium and the presence of *B pertussis* was confirmed by matrix-assisted laser desorption ionisation–time of flight mass spectrometry (International Health Management Associates, Schaumburg, IL, USA). For all positive samples, a second aliquot was plated in serial dilutions on charcoal agar medium to assess colony counts. Participants in whom *B pertussis* colonies were present on day 113 were retested before the end of study to ensure clearance.

Exploratory immunogenicity analyses were done to establish whether participants who received aPVs in infancy (who we assumed were those aged 18–22 years) had a different response to BPZE1 vaccination or attenuated challenge and to investigate protection against colonisation after attenuated challenge with BPZE1 relative to the degree of mucosal or serum antibodies measured after the first study vaccination.

A retrospective convenience set of samples was identified to undergo functional antibody assay testing after the primary data analysis. For the two treatment groups selected (the BPZE1–BPZE1 group and the Tdap–placebo group), anti-pertussis toxin and anti-pertactin IgG responses were ranked by quartile. A randomised subset of participants in the highest and lowest quartiles was generated by the study sponsor. Samples from the first 30 of these participants were used to test functional antibody responses to serum bactericidal activity and pertussis toxin neutralising activity (PTNA) assays. Samples were selected on the basis of there being sufficient sample quantity for all 3 days of testing

	BPZE1 group		Tdap group	
	Attenuated challenge BPZE1 (n=92)	Attenuated challenge placebo (n=92)	Attenuated challenge BPZE1 (n=46)	Attenuated challenge placebo (n=50)
Age, years	35·6 (9·0)	35·2 (8·8)	34·6 (9·0)	34·2 (9·9)
Sex				
Male	36 (39%)	46 (50%)	20 (43%)	23 (46%)
Female	56 (61%)	46 (50%)	26 (57%)	27 (54%)
Race				
White	70 (76%)	65 (71%)	31 (67%)	41 (82%)
Black	19 (21%)	22 (24%)	9 (20%)	8 (16%)
Other	3 (3%)	5 (5%)	6 (13%)	1 (2%)
Hispanic ethnicity	15 (16%)	11 (12%)	8 (17%)	7 (14%)
Weight, kg	82·1 (18·7)	82·4 (19·0)	81·3 (16·6)	79·7 (16·7)
BMI, kg/m²	28·0 (5·4)	28·1 (5·6)	27·7 (5·0)	27·2 (5·0)

Data are mean (SD) or n (%). Tdap=tetanus–diphtheria–acellular pertussis vaccine.

Table 1: **Demographic and baseline characteristics (intention-to-treat population)**

(days 1, 29 and 113). Samples were then sent to the separate laboratories, masked to treatment assignment. The serum bactericidal activity assay was done with pertactin-positive *B pertussis* B1917 and pertactin-deficient *B pertussis* B1917, and interpolated bactericidal titres were measured.[23] The Chinese hamster ovary cell assay was used to measure PTNA; the last serum dilution of the titre without clusters was reported.[24]

Outcomes

The primary immunogenicity endpoint was the proportion of participants achieving nasal secretory IgA seroconversion against at least one *B pertussis* antigen on day 29 or day 113 (either absolute *B pertussis*-specific secretory IgA or normalised *B pertussis*-specific secretory IgA based on total secretory IgA). The primary safety endpoints were reactogenicity (in all participants) and serum chemistry panel (see appendix p 71 for details of the specific tests), haematology, and bleeding indices (in the safety cohort) according to FDA toxicity score 7 days after vaccination and challenge.[20]

Our secondary immunogenicity endpoints included *B pertussis*-specific nasal mucosal secretory IgA and serum IgA and IgG responses by group at baseline, on days 29, 78 (nasal only), 85 (serum only), 113, 169, and at the end of the study. Secondary endpoints for colonisation included protection against any acquisition of *B pertussis* after attenuated challenge with BPZE1 by culture and quantitation of bacterial burden by colony count (7, 11, and 28 days after challenge). The appendix (pp 45–48) includes a full list of all secondary immunogenicity and colonisation endpoints.

Secondary safety endpoints were all unsolicited adverse events during the 28 days after vaccination or attenuated challenge and any adverse events related to vaccination between day 1 and day 113. Adverse events for these endpoints were classified according to the Medical Dictionary for Regulatory Activities (version 22.0). Serious adverse events were monitored throughout the study. Vital sign measurements were graded according to the FDA severity scoring.[20]

Statistical analysis

We judged that a sample size of 300 participants would be sufficient for this trial based on clinical and safety considerations (the study did not test a formal null hypothesis). Continuous variables were summarised descriptively with the mean and SD. Categorical variables were presented as frequency counts, percentages, and two-sided 95% CIs for proportions computed using the Agresti-Coull method.

Descriptive statistics reported for antibody response parameters included geometric mean concentrations, geometric mean fold rise of post-vaccination levels over baseline levels, and seroconversion (a two-fold increase over the baseline value or a four-fold increase over the minimal detection limit of the assay). *B pertussis*-specific

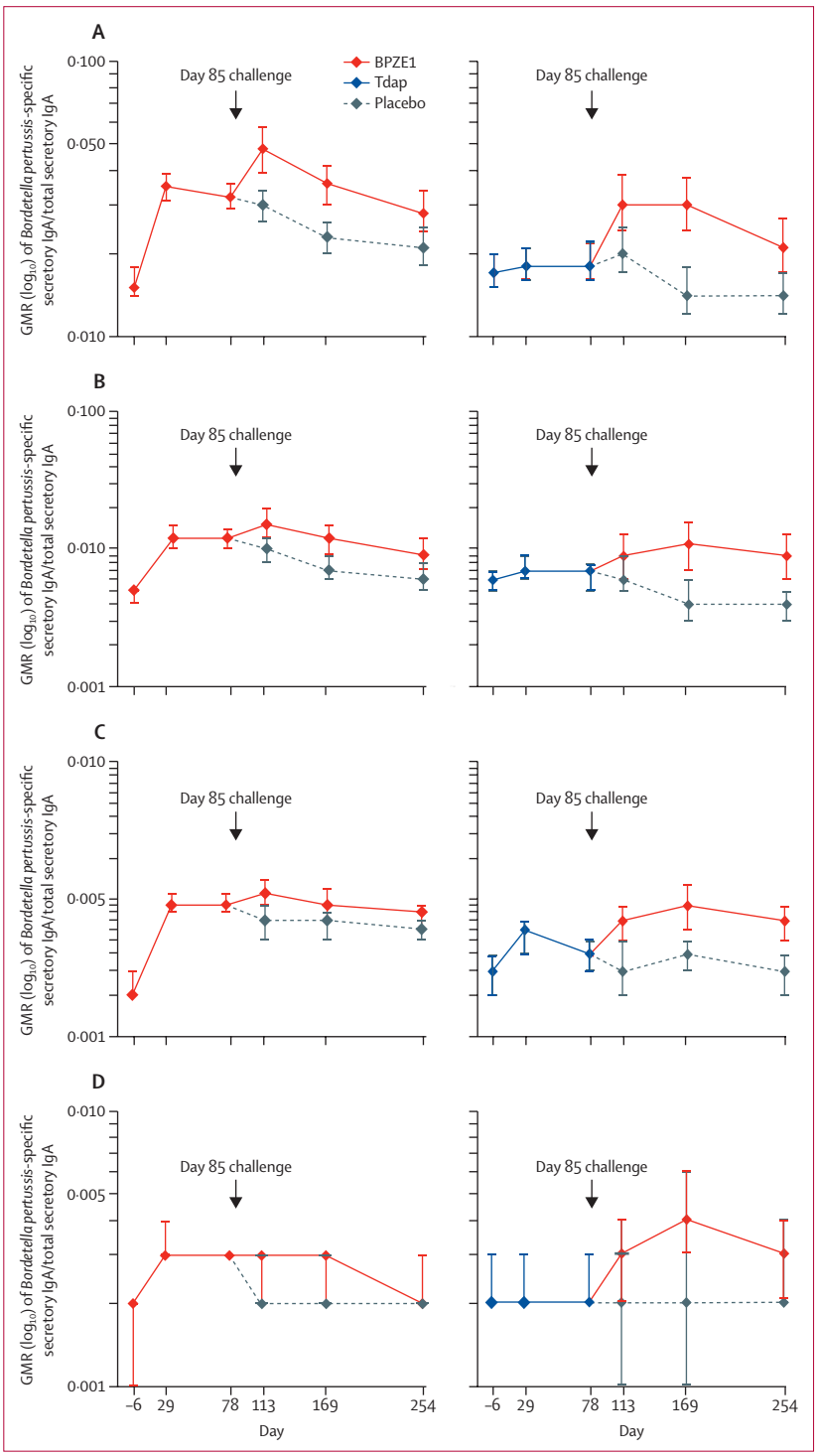

***Figure 2:* *Bordetella pertussis*-specific nasal secretory IgA responses to whole-cell extract (A), pertactin (B), filamentous haemagglutinin (C), and pertussis toxin (D) after BPZE1 or Tdap vaccination and attenuated challenge**

Vaccines were administered on day 1. Data were normalised relative to total secretory IgA concentrations measured over a nasal-collection period of 2 min. Error bars represent 95% CIs. GMR=geometric mean ratio. Tdap=tetanus–diphtheria–acellular pertussis vaccine.

secretory IgA measurements were calculated as absolute antigen-specific geometric mean concentrations and as geometric mean ratios after normalising responses relative to total nasal secretory IgA, and were expressed as AU/pg.

In the retrospective convenience set, paired or independent *t* tests on a logarithmic scale were used to assess functional antibody responses (geometric mean titres) within or between treatment groups. Geometric mean-fold rise (day 29 *vs* baseline) was calculated, and the proportion of participants with at least a two-fold increase of either serum bactericidal titre or pertussis toxin neutralising antibody titres was reported.

Participant outcomes and demographics were summarised in the intention-to-treat population, which included all randomly assigned participants except for the 20 participants in the safety cohort who received 10^7 CFU BPZE1. The safety analysis set consisted of all participants who received at least one dose of the study vaccine and for whom any safety data were available. A separate safety cohort comprised people who received 10^7 CFU BPZE1. The immunogenicity analysis set included all participants who received the first vaccination, contributed at least one valid immune sample both before and after the first vaccination and did not receive non-study Tdap during the study. Participants who were assigned to receive BPZE1 as challenge (day 85) but did not receive the challenge were placed into the placebo challenge arm according to the first vaccination received. Participants who discontinued the study before the attenuated challenge were analysed in their originally assigned group. All analyses were done in SAS (version 9.4).

Role of the funding source

The funder of this study was involved in the study design, data collection, data analysis, data interpretation, and writing of the report.

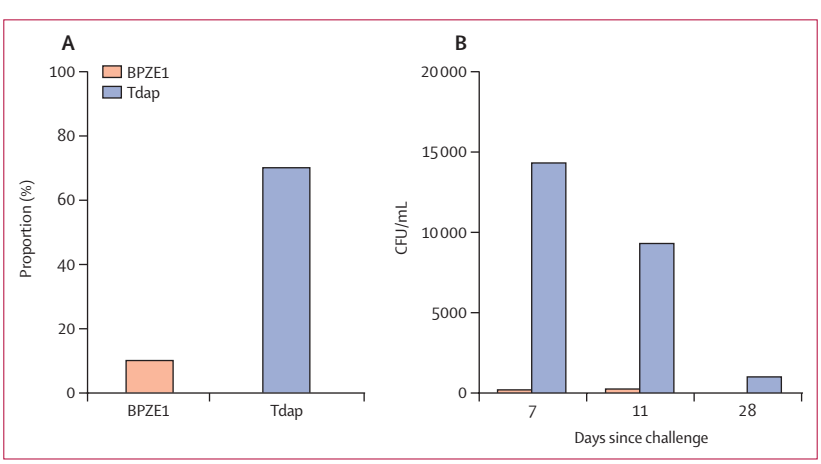

Figure 3: **Proportion of participants with nasopharyngeal *Bordetella pertussis* colonisation within 28 days of attenuated challenge with BPZE1 (A) and mean CFU/mL 7, 11, and 28 days after challenge in colonised participants (B)**

CFU=colony-forming units. Tdap=tetanus–diphtheria–acellular pertussis vaccine.

Results

Between June 17 and Oct 3, 2019, 458 people were screened and 300 were enrolled in the study and randomly assigned (figure 1). Follow-up observations were completed on June 24, 2020. Of the 300 participants, 20 (eight in the BPZE1–BPZE1 group, eight in the BPZE1–placebo group, and four in the Tdap–BPZE1 group) were part of the safety cohort that received 10^7 CFU BPZE1. Among the other 280 participants, one participant was unable to be vaccinated on day 1, leaving 279 participants. Demographics and other baseline characteristics were well balanced across groups (table 1).

The primary immunogenicity endpoint of seroconversion of at least one *B pertussis*-specific nasal secretory IgA on day 29 or day 113 was met in 79 (94% [95% CI 87–98]) of 84 participants in the BPZE1–BPZE1 group, 89 (95% [88–98]) of 94 in the BPZE1–placebo group, 38 (90% [77–97]) of 42 in the Tdap–BPZE1 group, and 42 (93% [82–99]) of 45 in the Tdap–placebo group. *B pertussis*-specific secretory IgA responses were higher in the BPZE1 than the Tdap groups when expressed as geometric mean ratios and geometric mean fold rises (figure 2; appendix p 2). One dose of BPZE1 induced an increase in *B pertussis*-specific secretory IgA concentrations by day 29 (geometric mean ratio for whole-cell extract 0·035, pertactin 0·012, filamentous haemagglutinin, 0·009, and pertussis toxin 0·003; corresponding geometric mean fold rises 2·2, 2·6, 4·1, and 2·0). By contrast, a dose of Tdap induced an increase in *B pertussis*-specific nasal secretory IgA responses only at day 29 against filamentous haemagglutinin (geometric mean ratio for whole-cell extract 0·018, pertactin 0·007, filamentous haemagglutinin 0·006, and pertussis toxin 0·002; corresponding geometric mean fold rises 1·1, 1·3, 2·1, and 1·1). The increase seen in anti-filamentous haemagglutinin antibody concentrations disappeared by day 78.

In participants in the BPZE1–BPZE1 group, additional increases in *B pertussis*-specific secretory IgA concentrations were noted after challenge, but geometric mean ratios at day 113 were less than double those at day 78 (figure 2; appendix p 2). Increases in *B pertussis*-specific nasal secretory IgA responses were sustained at the end of study in all participants vaccinated with BPZE1 (figure 2). In Tdap-vaccinated participants who underwent challenge with BPZE1, *B pertussis*-specific secretory IgA responses were also induced after challenge, with geometric mean ratios and fold rises at the end of study similar to those in the BPZE1–placebo group (figure 2).

Figure 3 details findings about colonisation with *B pertussis* after attenuated challenge with BPZE1. All participants were free of colonising infections before attenuated challenge (day 78). 72 (90%) of 80 BPZE1 vaccinees had undetectable BPZE1 organisms in their nasopharynx at any time after attenuated challenge (ie, on days 7, 11, or 28 after challenge). In the eight participants who were colonised, mean colony counts were low (≤260 CFU/mL). By contrast, 28 (70%) of

40 Tdap-vaccinated participants were colonised after challenge, with substantially higher mean colony counts recorded (≤14 325 CFU/mL). All participants who remained colonised 1 month after challenge (day 113) were negative upon follow-up nasal aspiration and none required antibiotic therapy for clearance.

BPZE1 induced systemic anti-pertussis toxin, anti-filamentous haemagglutinin, and anti-pertactin IgA and IgG responses, and anti-whole-cell extract IgG responses, with a balanced IgA to IgG response (table 2). Greater IgG responses were noted after Tdap vaccination, resulting in immuno-dominance of IgG over IgA. Antibody decay was faster after Tdap than after BPZE1 vaccination (table 2). Tdap-induced IgG responses were reduced by approximately a third on day 113 and by half at the end of the study compared with peak response on day 29 (table 2), resulting in serum *B pertussis*-specific antibody concentrations approaching those of participants in the BPZE1 groups by the end of the study.

Most participants in this study were older than 22 years and had received whole-cell pertussis vaccines in their infancy. However, 27 participants were aged 22 years or

	Baseline* GMC (95% CI)	Day 29		Day 113		Day 254 (end of study)	
		GMC (95% CI)	GMFR (95% CI)	GMC (95% CI)	GMFR (95% CI)	GMC (95% CI)	GMFR (95% CI)
***B pertussis*-specific IgA responses**							
Pertactin							
BPZE1–BPZE1	7·8 (5·5–11·2)	24·1 (16·6–35·1)	3·1 (2·4–4·0)	30·0 (20·9–43·1)	4·0 (3·1–5·1)	18·7 (12·6–27·7)	2·6 (2·0–3·3)
BPZE1–placebo	7·0 (5·0–9·9)	21·5 (15·1–30·4)	3·0 (2·4–3·8)	17·3 (12·3–24·2)	2·6 (2·0–3·2)	11·8 (8·3–16·8)	1·7 (1·4–2·1)
Tdap–BPZE1	9·5 (5·6–16·3)	42·0 (23·6–74·7)	4·4 (2·9–6·7)	34·7 (19·1–62·9)	3·8 (2·5–5·9)	25·5 (14·2–45·6)	2·5 (1·7–3·8)
Tdap–placebo	7·4 (4·3–12·6)	35·7 (21·7–58·6)	4·8 (3·1–7·5)	25·2 (15·0–42·1)	3·0 (2·0–4·5)	17·3 (10·5–28·6)	2·3 (1·6–3·4)
Filamentous haemagglutinin							
BPZE1–BPZE1	7·9 (5·7–11·0)	21·0 (16·2–27·1)	2·7 (2·1–3·3)	23·6 (17·3–32·3)	3·0 (2·4–3·7)	19·8 (14·3–27·3)	2·5 (2·1–3·1)
BPZE1–placebo	6·3 (4·8–8·3)	20·1 (15·8–25·7)	3·2 (2·6–3·9)	18·6 (14·5–23·7)	2·8 (2·2–3·5)	16·6 (13·2–21·0)	2·6 (2·1–3·2)
Tdap–BPZE1	7·3 (4·7–11·5)	50·6 (33·3–77·0)	6·9 (4·3–11·0)	36·6 (25·0–53·7)	4·8 (3·3–7·1)	33·0 (22·5–48·4)	4·2 (2·9–6·0)
Tdap–placebo	8·6 (5·5–13·6)	40·3 (25·8–62·8)	4·7 (3·2–6·9)	22·9 (14·6–36·1)	2·7 (1·7–4·3)	20·0 (11·7–34·3)	2·3 (1·5–3·7)
Pertussis toxin							
BPZE1–BPZE1	1·9 (1·5–2·4)	4·0 (3·2–5·0)	2·1 (1·7–2·6)	4·0 (3·2–5·2)	2·1 (1·7–2·6)	3·4 (2·6–4·4)	1·8 (1·4–2·2)
BPZE1–placebo	1·7 (1·3–2·1)	3·7 (2·9–4·7)	2·2 (1·8–2·7)	3·3 (2·6–4·1)	1·9 (1·7–2·3)	3·1 (2·5–3·9)	1·8 (1·5–2·1)
Tdap–BPZE1	2·0 (1·4–2·9)	7·1 (5·1–9·9)	3·6 (2·6–4·9)	5·7 (4·0–8·2)	3·0 (2·2–4·1)	4·2 (2·9–6·2)	2·3 (1·7–3·0)
Tdap–placebo	2·6 (1·8–3·7)	5·8 (4·0–8·2)	2·2 (1·7–2·9)	3·6 (2·3–5·5)	1·4 (1·0–1·9)	3·4 (2·3–5·1)	1·3 (0·9–1·9)
***B pertussis*-specific IgG responses**							
Whole-cell extract							
BPZE1–BPZE1	75·0 (59·2–95·0)	140·4 (117·7–167·4)	1·9 (1·6–2·2)	158·4 (132·2–189·8)	2·1 (1·8–2·5)	123·2 (103·0–147·4)	1·7 (1·4–2·0)
BPZE1–placebo	76·0 (62·3–92·7)	132·5 (111·7–157·2)	1·7 (1·5–2·0)	125·8 (105·6–149·9)	1·6 (1·4–1·9)	105·5 (88·5–125·7)	1·4 (1·3–1·6)
Tdap–BPZE1	74·3 (54·8–100·6)	230·0 (183·0–289·2)	3·1 (2·3–4·1)	190·7 (146·3–248·7)	2·7 (2·0–3·7)	146·6 (113·9–188·9)	2·0 (1·5–2·6)
Tdap–placebo	89·0 (65·6–120·7)	268·4 (214·1–336·4)	3·0 (2·5–3·7)	202·5 (163·9–250·2)	2·0 (1·6–2·6)	137·2 (108·1–174·2)	1·4 (1·1–1·7)
Pertactin							
BPZE1–BPZE1	38·5 (27·5–53·9)	65·6 (47·9–90·0)	1·7 (1·4–2·0)	72·7 (52·8–100·0)	2·0 (1·6–2·5)	54·5 (38·8–76·4)	1·5 (1·2–1·8)
BPZE1–placebo	39·8 (28·9–54·8)	68·9 (52·2–91·1)	1·7 (1·5–2·0)	65·0 (50·0–84·6)	1·6 (1·4–2·0)	53·3 (39·6–71·8)	1·3 (1·1–1·6)
Tdap–BPZE1	52·5 (31·7–87·0)	318·0 (222·5–454·6)	6·1 (3·8–9·5)	226·4 (155·4–329·9)	4·1 (2·7–6·4)	156·8 (106·2–231·5)	2·5 (1·7–3·7)
Tdap–placebo	41·8 (25·7–68·0)	330·8 (220·2–496·8)	7·9 (4·7–13·3)	244·0 (154·1–386·2)	5·1 (2·9–9·0)	178·7 (116·4–274·4)	3·8 (2·3–6·4)
Filamentous haemagglutinin							
BPZE1–BPZE1	39·0 (29·8–51·2)	77·7 (64·1–94·2)	2·0 (1·7–2·4)	79·9 (65·5–97· 6)	2·1 (1·7–2·6)	69·9 (56·4–86·5)	1·9 (1·6–2·3)
BPZE1–placebo	40·6 (32·3–51·1)	80·1 (65·7–97·7)	2·0 (1·7–2·3)	76·9 (64·5–91·7)	1·8 (1·6–2·2)	74·8 (62·4–89·7)	1·8 (1·5–2·2)
Tdap–BPZE1	44·5 (32·3–61·4)	292·0 (233·2–365·6)	6·6 (4·6–9·4)	198·2 (152·1–258·2)	4·3 (3·0–6·0)	141·4 (108·6–184·2)	2·9 (2·1–3·9)
Tdap–placebo	52·4 (36·0–76·3)	267·5 (207·0–345·7)	5·1 (3·5–7·4)	201·9 (161·8–251·9)	3·6 (2·4–5·2)	153·6 (124·3–189·9)	2·6 (1·9–3·6)
Pertussis toxin							
BPZE1–BPZE1	15·4 (12·0–19·8)	31·2 (25·4–38·3)	2·0 (1·6–2·6)	31·8 (26·1–38·8)	2·1 (1·7–2·6)	27·2 (22·2–33·3)	1·9 (1·6–2·2)
BPZE1–placebo	14·0 (11·2–17·4)	28·3 (23·6–34·0)	2·0 (1·8–2·4)	27·2 (22·7–32·4)	1·9 (1·6–2·2)	27·7 (23·3–33·1)	2·0 (1·7–2·3)
Tdap–BPZE1	10·9 (7·8–15·4)	68·9 (55·1–86·1)	6·3 (4·6–8·5)	44·7 (35·0–56·9)	4·2 (3·1–5·8)	36·5 (27·4–48·6)	3·2 (2·4–4·5)
Tdap–placebo	16·7 (11·6–23·9)	80·3 (61·5–104·8)	4·8 (3·5–6·6)	56·4 (42·6–74·7)	3·2 (2·5–4·2)	44·4 (33·9–58·1)	2·6 (2·0–3·4)

Data are for 85 people in the BPZE1–BPZE1 group, 94 in the BPZE1–placebo group, 43 in the Tdap–BPZE1 group, and 45 in the Tdap–placebo group. Units for IgG are AU/mL for whole-cell extract and IU/mL for pertactin, filamentous haemagglutinin, and pertussis toxin. GMC=geometric mean concentration. GMFR=geometric mean fold rise. Tdap=tetanus–diphtheria–acellular pertussis vaccine. *Defined as the last non-missing value before first vaccination on day 1.

Table 2: **Systemic *Bordetella pertussis*-specific IgA and IgG responses**

younger and had probably been primed with an aPV. The demographics of this group were similar to those of the overall population (appendix p 3). An exploratory analysis of the 24 aPV-primed participants (13 of whom received BPZE1 and 11 of whom received Tdap) for whom immunogenicity and post-challenge culture data were available suggested that the induction of *B pertussis*-specific secretory IgA responses and protection against challenge mirrored that in the overall population (appendix p 4). Six of seven BPZE1-vaccinated participants in this subgroup who underwent BPZE1 challenge were protected from bacterial colonisation, whereas all four Tdap-vaccinated participants who underwent BPZE1 challenge were colonised (appendix p 4).

Baseline demographics of colonised and non-colonised participants were similar in the BPZE1–BPZE1 group and the Tdap–BPZE1 group, but in the BPZE1–BPZE1 group colonised participants seemed to have lower mean BMIs than non-colonised participants (appendix p 5). Non-colonised and colonised participants vaccinated with BPZE1 had similar anti-whole-cell extract, anti-filamentous haemagglutinin, and anti-pertactin secretory IgA responses at day 29 (figure 3; table 3), which could be related to the low colony counts noted after challenge in those who were colonised. Participants in the Tdap–BPZE1 group who were not colonised after attenuated challenge had higher serum *B pertussis*-specific IgG geometric mean concentrations at baseline than those who were colonised (figure 3; table 3), but secretory IgA responses remained low.

Functional responses were assessed with serum bactericidal activity or PTNA assays in 13 patients from the BPZE1–BPZE1 group and 17 from the Tdap–placebo

	Baseline serum IgG GMC (95% CI)	*Bordetella pertussis*-specific secretory IgA		
		Baseline GMR	Day 29 GMR	Day 29 GMFR
Whole-cell extract				
BPZE1–BPZE1 group				
Colonised	80·3 (36·8–175·3)	0·020 (0·008–0·051)	0·036 (0·013–0·096)	1·8 (1·0–3·3)
Uncolonised	69·3 (54·6–87·8)	0·016 (0·013–0·020)	0·034 (0·028–0·042)	2·1 (1·7–2·7)
Tdap–BPZE1 group				
Colonised	50·9 (37·7–68·7)	0·016 (0·011–0·024)	0·017 (0·012–0·023)	1·0 (0·7–1·5)
Uncolonised	150·8 (75·8–299·9)	0·021 (0·015–0·030)	0·019 (0·013–0·028)	1·0 (0·8–1·2)
Pertactin				
BPZE1–BPZE1 group				
Colonised	23·1 (4·6–116·2)	0·004 (0·002–0·010)	0·008 (0·003–0·020)	2·0 (0·9–4·3)
Uncolonised	38·3 (26·7–54·9)	0·005 (0·004–0·006)	0·014 (0·011–0·019)	3·1 (2·4–4·1)
Tdap–BPZE1 group				
Colonised	34·8 (18·8–64·5)	0·005 (0·004–0·008)	0·006 (0·004–0·010)	1·1 (0·7–1·7)
Uncolonised	140·7 (61·3–322·9)	0·007 (0·004–0·012)	0·009 (0·005–0·016)	1·3 (0·9–1·7)
Filamentous haemagglutinin				
BPZE1–BPZE1 group				
Colonised	36·2 (12·5–104·4)	0·003 (0·001–0·010)	0·009 (0·002–0·035)	2·8 (1·3–5·8)
Uncolonised	37·2 (27·5–50·1)	0·002 (0·002–0·003)	0·010 (0·008–0·013)	4·5 (3·3–6·1)
Tdap–BPZE1 group				
Colonised	30·2 (21·5–42·4)	0·003 (0·002–0·004)	0·006 (0·004–0·009)	2·2 (1·4–3·6)
Uncolonised	119·2 (69·6–204·1)	0·004 (0·002–0·011)	0·006 (0·003–0·013)	2·0 (1·2–3·5)
Pertussis toxin				
BPZE1–BPZE1 group				
Colonised	14·5 (5·3–40·1)	0·002 (0·001–0·005)	0·003 (0·002–0·007)	1·5 (0·7–3·2)
Uncolonised	14·9 (11·3–19·6)	0·001 (0·001–0·002)	0·003 (0·002–0·004)	2·1 (1·6–2·7)
Tdap–BPZE1 group				
Colonised	7·5 (5·1–11·2)	0·002 (0·001–0·003)	0·002 (0·002–0·003)	1·1 (0·9–1· 5)
Uncolonised	23·8 (12·9–43·8)	0·003 (0·002–0·007)	0·003 (0·001–0·005)	1·1 (0·8–1·5)

In the BPZE1–BPZE1 group, data are presented for eight colonised patients and 72 uncolonised patients, whereas in the Tdap–BPZE1 group, data are presented for 28 colonised patients and 12 uncolonised patients. Secretory IgA data are AU/pg because data were normalised by total secretory IgA (pg/ml), measured over a 2-min nasal collection period. When analysed as absolute antigen-specific secretory IgA GMC, the conclusions remain the same. Units for IgG are AU/mL for whole-cell extract and IU/mL for pertactin, filamentous haemagglutinin, and pertussis toxin. GMC=geometric mean concentration. GMR=geometric mean ratio. GMFR=geometric mean fold rise. Tdap=tetanus–diphtheria–acellular pertussis vaccine.

***Table 3:* *Bordetella pertussis*-specific mucosal secretory IgA and serum IgG concentrations after attenuated challenge, by colonisation status**

group. These subsets were representative of anti-pertactin and anti-pertussis toxin IgG concentrations in their groups (appendix p 6). In this subset, BPZE1 and Tdap induced similar functional responses at day 29 in both the serum bactericidal activity against pertactin-postive *B pertussis* and PTNA assays (figure 4). Only BPZE1 induced serum bactericidal activity against pertactin-negative *B pertussis* (geometric mean titre 233 in the BPZE1 group *vs* 45 in the Tdap group; geometric mean fold rise 5·5 *vs* 0·9).

Overall, BPZE1 vaccinations were well tolerated. Reactogenicity was mainly mild or non-existent, with a duration of three days or less. It occurred with similar frequency in the BPZE1 and Tdap groups. The most frequent solicited grade 1 or worse nasal or respiratory adverse events within 7 days of vaccination were stuffy nose or congestion (71 [39%] of 183 in the BPZE1 groups *vs* 34 [35%] of 96 in the Tdap groups), runny nose (66 [36%] *vs* 33 [34%]), and sneezing (60 [33%] *vs* 28 [29%]; figure 5). After challenge on day 85, regardless of previous vaccination, nasal or respiratory reactogenicity events did not worsen in frequency or severity (figure 5). One participant in the BPZE1–BPZE1 group reported severe nasal and respiratory reactogenicity symptoms of sore throat and sinus pressure 5 days after challenge and was treated for non-serious streptococcal pharyngitis, which was considered to be unrelated to vaccination.

The most frequently reported grade 1 or worse systemic solicited adverse events within 7 days of vaccination were headache (68 [37%] of 183 in the BPZE1 groups *vs* 35 [36%] of 96 in the Tdap groups) and fatigue (62 [34%] *vs* 21 [22%]; figure 5). After challenge on day 85, irrespective of previous vaccination, systemic solicited adverse events did not worsen in frequency or severity. One participant in the BPZE1–placebo group reported a constellation of systemic grade 3 reactogenicity events of fatigue, fever, headache, malaise, myalgia, and rash or hypersensitivity 3 days after vaccination, coinciding with a serious adverse event of bacterial sepsis and left leg cellulitis, which were considered to be unrelated to vaccination. The participant was hospitalised, received antibiotics, and was discharged the following day. The most frequently reported solicited local adverse events after Tdap vaccination were tenderness, which occurred in 58 (60%) participants, and pain at the injection site, which occurred in 49 (51%) participants. These events were mild to moderate in severity.

There were no deaths or treatment-emergent adverse events leading to study discontinuation in the participants in the safety analysis set and those treated with BPZE1 10^7 CFU (table 4; appendix p 7). Treatment-emergent adverse events were similar across groups (appendix p 8). Severe treatment-emergent adverse events were infrequent, and none were judged to be related to vaccination. There were no serious adverse events related to vaccination (appendix p 8). There were no notable safety trends in vital sign measurements or physical examination findings. Safety laboratory assessments (serum chemistry, haematology, and bleeding indices) 7 days after vaccination and attenuated challenge in the safety cohort did not reveal any abnormalities. There were no safety pauses during the study.

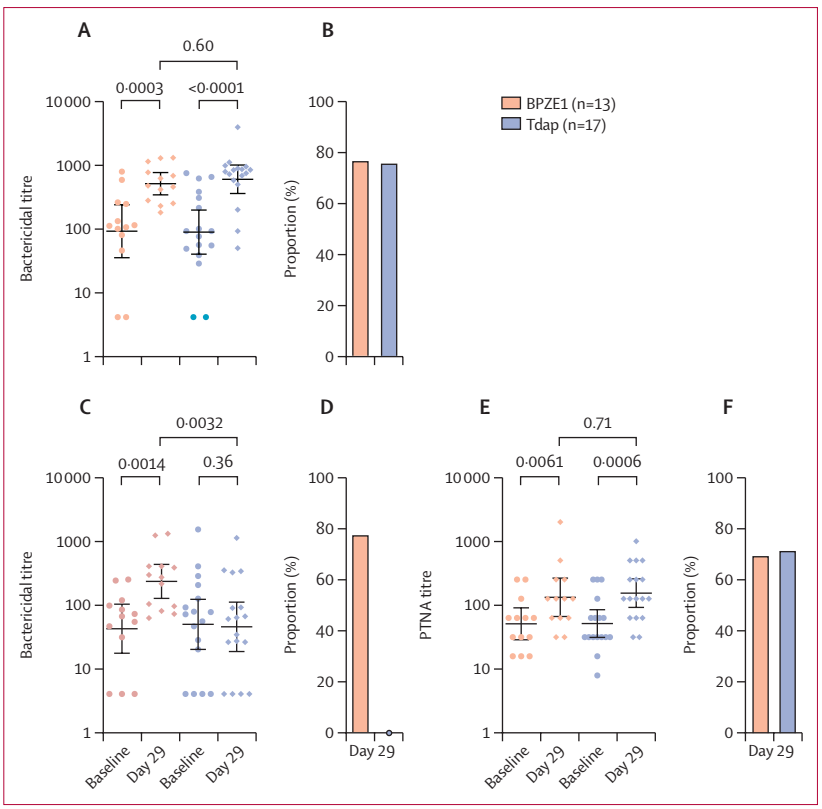

Figure 4: **Serum bactericidal activity and pertussis toxin neutralisation responses**
(A) Serum bactericidal titres at baseline and day 29 against pertactin-positive *Bordetella pertussis*. (B) Proportion of participants with at least a two-fold increase in serum bactericidal titres against pertactin-positive *B pertussis* at day 29. (C) Serum bactericidal titres at baseline and day 29 against pertactin-negative *B pertussis*. (D) Proportion of participants with at least a two-fold increase in serum bactericidal titres against pertactin-negative *B pertussis* at day 29. (E) Serum pertussis toxin neutralisation titres at baseline and day 29. (F) Proportion of participants with at least a two-fold increase in serum pertussis toxin neutralisation titres at day 29. In A, C, and E, geometric mean titres with 95% CIs are shown as horizontal lines; p values were calculated with paired or independent *t* tests on a logarithmic scale. Tdap=tetanus–diphtheria–acellular pertussis vaccine. PTNA=pertussis toxin neutralising activity.

Discussion

The findings of this randomised, double-blind phase 2b trial suggest that a single nasal administration of the live attenuated pertussis vaccine BPZE1 is safe and well tolerated and induces broad and sustained *B pertussis*-specific secretory IgA responses. The vaccine also seemed to protect against subsequent colonising infection after an attenuated BPZE1 challenge.

B pertussis is a strictly mucosal respiratory pathogen that primarily infects the upper airways. Mucosal immunity at the site of infection might thus be essential for the prevention of acquisition and subsequent transmission, as suggested in previous studies.[25]

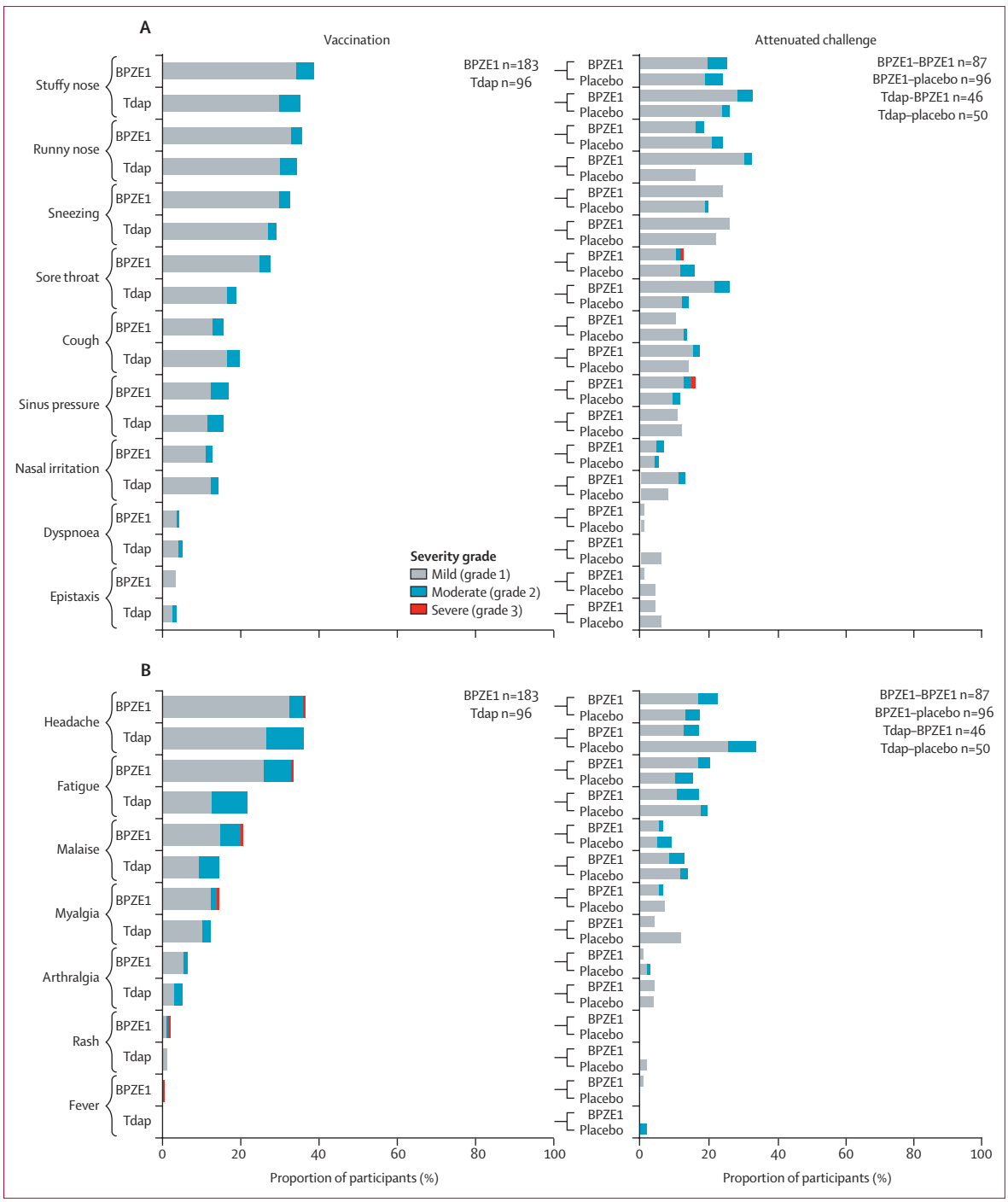

Figure 5: **Local respiratory reactogenicity (A) and systemic reactogenicity (B) solicited adverse events in the 7 days after vaccination and attenuated challenge**

Adverse events are broken down by US Food and Drug Administration severity grade (the highest severity of event is reported). Events are expressed relative to the most recent vaccine exposure. Tdap=tetanus–diphtheria–acellular pertussis vaccine.

Consistent with previous observations of aPVs, in this study the Tdap vaccine did not induce robust and sustained mucosal immunity, and most Tdap-vaccinated participants were colonised with *B pertussis* after attenuated challenge. Tdap-vaccinated participants who were not colonised after attenuated challenge had higher baseline serum *B pertussis*-specific antibody titres than those who were colonised. These higher baseline concentrations suggest recent undiagnosed exposure to *B pertussis*, which probably protected these participants from BPZE1 colonisation.

Although the primary aim of vaccination is protection against disease, especially severe disease, optimal control of respiratory pathogens requires control of person-to-person transmission. Therefore, vaccine-mediated prevention of airway infection is especially crucial for highly transmissible organisms, which has been well illustrated during the COVID-19 pandemic. Although several highly effective disease-preventing vaccines are now available, SARS-CoV-2 circulation has not been halted and waves of vaccine-escape variants continue to emerge.[26] Furthermore, the higher the transmissibility, as measured by the reproductive rate, the greater the importance of preventing initial infection, reducing infectious burden, and rapidly clearing the organism. Given that pertussis is a highly infectious respiratory disease,[4] an infection-blocking vaccine would optimally possess the ability to effectively control transmission and therefore reduce pertussis epidemics. Notably, the few BPZE1 recipients who were colonised after attenuated challenge had substantially lower nasopharyngeal bacterial counts and cleared infection faster than colonised Tdap recipients. This finding suggests that, even in colonised recipients BPZE1 could affect transmission dynamics and disease severity. Another study has shown a strong association between bacterial burden and clinical severity in infants infected with *B pertussis*.[27]

Intranasal BPZE1 vaccination also resulted in broad *B pertussis*-specific serum IgG and IgA responses. The concentrations gradually declined over 9 months but remained higher than baseline concentrations at the end of the study. Although, as expected, antibody concentrations after Tdap vaccination were higher than those after BPZE1 administration, the decline was faster in the Tdap group than in the BPZE1 group, and the concentrations for both groups approached convergence at the end of the study. This finding is consistent with those in previous studies showing that antibodies to chemically detoxified pertussis vaccines, such as those induced by Tdap, decay significantly faster than antibodies to genetically detoxified vaccines,[28] as is the case for BPZE1.

Despite an initial (day 29) difference in serum *B pertussis*-specific IgG responses between BPZE1 and Tdap recipients, functional antibody responses in a subset of participants measured by serum bactericidal activity against pertactin-positive *B pertussis* and PTNA assays showed similar responses between the two groups. PTNA titres in BPZE1-vaccinated participants were similar to those in Tdap recipients. Studies[29,30] have shown that pertussis toxin has a key role in the pathogenesis of severe pertussis, although the threshold titres of pertussis toxin-neutralising antibodies have yet to be identified. Bactericidal antibody titres against pertactin-positive *B pertussis* clinical isolates were also similar in both groups. Notably, only sera from BPZE1-vaccinated participants showed bactericidal activity against a pertactin-negative strain. A previous study[23] showed that, among aPV antigens, only pertactin induces bactericidal antibodies. Furthermore, since pertactin-containing aPVs, were introduced, vaccine-breakthrough pertactin-negative *B pertussis* isolates have been identified with increasing frequency due to vaccine-pressure-related strain adaptation.[31] The finding that BPZE1 has bactericidal activity against a pertactin-negative strain suggests potential broader induction of functional anti-*B pertussis* antibodies. Importantly, the generation and expansion of vaccine-escape mutants is much less likely when vaccines with high antigen diversity are deployed rather than vaccines with little antigen diversity.[23]

Intranasal administration of BPZE1 was well tolerated, with an acceptable safety profile. Nasal, respiratory and systemic symptoms were mainly mild and lasted for 3 days or less on average. A second dose of BPZE1 was also well tolerated. No serious adverse events related to vaccination occurred during the study.

Limitations of the study include that the population enrolled were healthy adults, most of whom had been primed in infancy with whole-cell pertussis vaccines. However, in a subgroup analysis of participants aged 18–22 years who had probably been primed with aPVs, safety and immunogenicity data were similar to those in the overall cohort. A study is underway to assess the immunogenicity and safety of BPZE1 in school-age children (NCT05116241). This study will provide additional information about BPZE1 performance in aPV-primed individuals. A second limitation of our trial was

	Vaccine (days 1–84)		Attenuated challenge (days 85–113)			
	BPZE1 (n=183)	Tdap (n=96)	BPZE1–BPZE1 (n=87)	BPZE1–placebo (n=96)	Tdap–BPZE1 (n=46)	Tdap–placebo (n=50)
Treatment-emergent adverse events						
Any*	54 (30)	30 (31)	13 (15)	22 (23)	9 (20)	15 (30)
Mild (grade 1)	38 (21)	20 (21)	11 (13)	16 (17)	7 (15)	14 (28)
Moderate (grade 2)	12 (7)	5 (5)	2 (2)	5 (5)	2 (4)	1 (2)
Severe (grade 3)	1 (1)	1 (1)	0	1 (1)	0	0
Related treatment-emergent adverse events	21 (11)	11 (11)	2 (2)	7 (7)	3 (7)	5 (10)
Serious adverse events						
Any†	3 (2)	0	0	1 (1)	0	0
Related serious adverse events	0	0	0	0	0	0
Adverse events leading to discontinuation	0	0	0	0	0	0
Death	0	0	0	0	0	0

Data are n or n (%). Treatment-emergent adverse events were collected for 28 days after each vaccination (see appendix p 8), and serious adverse events were collected from first vaccination to 6 months after the last vaccination. Tdap=tetanus–diphtheria–acellular pertussis vaccine. *One participant in the BPZE1–BPZE1 group became pregnant 11 weeks after the attenuated challenge; no pregnancy complications occurred and the baby was healthy. †Serious adverse events were bacterial sepsis (n=1), cellulitis (n=1), postprocedural haemorrhage (n=1), hyperglycaemia (n=1), and diabetic metabolic decompensation (n=1); and one additional serious adverse event (obesity) occurred after day 113. The appendix (p 9) contains further details of serious adverse events.

Table 4: **Summary of adverse events**

that serum bactericidal activity and PTNA assays were done retrospectively in only 30 participants who were selected by convenience sampling. Further testing to confirm these results is warranted. A third limitation is methodological, including limitations due to potential sampling and testing procedures (eg, potential sampling sensitivity for mucosal secretory IgA using a 2 min sample collection and assay sensitivity for pertussis toxin and filamentous haemagglutinin since commercial kits were used). However, in this study very little outcome data were missing, all assays were validated, and the gold standard of *B pertussis* culture was utilised. Finally, we used BPZE1 for the attenuated challenge, which is not representative of circulating virulent *B pertussis* strains. However, the evidence of BPZE1 offering protection against attenuated challenge in our study is supportive when placed in the context of induction of broad *B pertussis*-specific secretory IgA responses, coupled with data in non-human primates showing that BPZE1 reduced bacterial load by more than 99% after a challenge with a high-dose virulent clinical *B pertussis* isolate.[15] A controlled human infection model with virulent *B pertussis* that has been developed[32] uses the same strain as that used in the serum bactericidal activity assay. This finding, along with the attenuated challenge results, provides confidence in progressing to a virulent challenge study with BPZE1. If in future virulent challenge studies BPZE1 recapitulates the protection shown in this study, then the attenuated challenge model might allow for investigation of long-term durability and studies in broader populations in whom virulent challenge trials are not appropriate.

In conclusion, intranasal administration of live attenuated BPZE1 seems to be safe and well tolerated, and evokes broad, robust, and sustained mucosal and systemic immune responses. In view of the promising results of this study, BPZE1 holds the potential to achieve epidemic control of *B pertussis*. Large phase 3 trials are warranted to further explore these encouraging results.

Contributors

CK, KS, MT, and KR designed and developed the trial protocol. VEM, BR, CH, MJP, and JF provided all participant and laboratory data. CK, KS, SN, KR, CL, VEM, BR, CH, MJP, and JF accessed and verified the data. CK, SN, and CL wrote the initial draft of the Article, with assistance from PG, AG, BC, QH, A-MB, and KR. All authors read and approved the final version of the Article. All authors had access to the summarised data but not to patient-specific data to maintain the integrity of the trial. CK, SN, and PG had access to and verified all study data.

Declaration of interests

CK, KS, MT, PG, KR, and SN are equity holders in, and current or former employees of or consultants to, ILiAD Biotechnologies. VEM reports leadership of the Pearl institutional review. SN is a former employee of Novartis. AG has received honoraria from Sanofi Pasteur. CL holds patents on the BPZE1 vaccine (licensed to ILiAD Biotechnologies) and reports consulting fees from, and holds equity in, 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 requests have been reviewed.

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