



**HAL**  
open science

## Safety and immunogenicity of the live attenuated intranasal pertussis vaccine BPZE1: a phase 1b, double-blind, randomised, placebo-controlled dose-escalation study

Maja Jahnmatz, Laura Richert, Nabil Al-Tawil, Jann Storsaeter, Céline Colin, Claire Bauduin, Marcel Thalen, Ken Solovay, Keith Rubin, Nathalie Mielcarek, et al.

### ► To cite this version:

Maja Jahnmatz, Laura Richert, Nabil Al-Tawil, Jann Storsaeter, Céline Colin, et al.. Safety and immunogenicity of the live attenuated intranasal pertussis vaccine BPZE1: a phase 1b, double-blind, randomised, placebo-controlled dose-escalation study. *The Lancet Infectious Diseases*, 2020, 10.1016/s1473-3099(20)30274-7 . inserm-02904617

**HAL Id: inserm-02904617**

**<https://inserm.hal.science/inserm-02904617>**

Submitted on 24 Oct 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Safety and immunogenicity of the live attenuated intranasal pertussis vaccine BPZE1: a**  
2 **phase Ib double-blind randomized placebo-controlled dose-escalation study.**

3

4 Maja Jahnmatz, Laura Richert, Nabil al-Tawil, Jann Storsaeter, Céline Colin, Claire Bauduin,  
5 Marcel Thalen, Ken Solovay, Keith Rubin, Nathalie Mielcarek, Rigmor Thorstensson,  
6 Camille Locht\* and the BPZE1 study team.

7 **Public Health Agency of Sweden, SE-171 82 Solna, Sweden** (M. Jahnmatz PhD, J. Storsaeter MD  
8 PhD, R. Thorstensson PhD); **Univ. Bordeaux, Inserm, Bordeaux Population Health Research**  
9 **Center, UMR 1219; CHU Bordeaux; CIC 1401, EUCLID/F-CRIN Clinical Trials Platform, F-**  
10 **33000, Bordeaux, France** (L. Richert PhD, C. Colin MSc, C. Bauduin MSc); **Inria SISTM team, F-**  
11 **33405, Talence, France** (L. Richert PhD); **Karolinska Trial Alliance, Karolinska University**  
12 **Hospital, SE-141 86 Stockholm, Sweden** (N. al-Tawil MD); **ILiAD Biotechnologies, New York,**  
13 **NY 10003, USA** (M. Thalen PhD, K. Solovay MSc, K. Rubin MD); **Univ. Lille, CNRS, Inserm,**  
14 **CHU Lille, Institut Pasteur de Lille, U1019 – UMR8204 – CHIL – Center for Infection and**  
15 **Immunity of Lille, F-59000 Lille, France** (N. Mielcarek PhD, C. Locht PhD)

16

17 \*Corresponding author:

18 Camille Locht,

19 Center for Infection and Immunity of Lille, Institut Pasteur de Lille, 1, rue du Prof. Calmette, F-59019  
20 Lille Cedex, France.

21 e-mail: [Camille.locht@pasteur-lille.fr](mailto:Camille.locht@pasteur-lille.fr)

22 Tel : (+33) 3 20 87 11 51

23

24

25

26

27

28 **BPZE1 Study team**

29 *Karolinska Trial Alliance:*

30 Lena Dager, Nina Ekholm, Margareta Gustafsson, Åsa Linde, Cecilia Lång, Maria Nastase,

31 Inga-Lill Reinholdsson, Erla Sigurdardottir, Anneli Wahlberg, Izabella Zarea;

32 *Public Health Agency of Sweden:*

33 Teodora Aktas, Ingrid Andersson, Eva Hanson Pihlainen, Margaretha Ljungman, Maj

34 Ringman, Teghesti Tecleab, Lena Wehlin;

35 *Univ. Bordeaux, Inserm, Bordeaux Population Health Research Center, UMR 1219 ; CHU*

36 *Bordeaux ; CIC 1401, EUCLID/F-CRIN Clinical Trials Platform, Bordeaux, France:*

37 Florence Allais, Alex Assuied, Geneviève Chêne, Camille Gilbert, Delphine Jean, Fabien Le

38 Marec, Laetitia Moinot, Philippe Reboud, Emilie Rousseau, Céline Roy, Christine

39 Schwimmer, Ludivine Taïeb, Cédric Wallet;

40 *Inserm-PRC:*

41 Gabrielle Derocle, Sonia Gueguen, Claire Lévy-Marchal, Hélène Esperou;

42 *Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille:*

43 Anne-Sophie Debrie, Dominique Raze, Loïc Coutte;

44 *Inserm-ANRS pharmacovigilance:*

45 Alpha Diallo, Noémie Mercier

46

47 **Summary**

48 Background: Long-term protection and herd immunity induced by existing pertussis vaccines  
49 are imperfect and there is thus a need to develop novel pertussis vaccines. This study aimed at  
50 investigating safety, colonization and immunogenicity of the novel live attenuated pertussis  
51 vaccine BPZE1 given intranasally in humans.

52 Methods: We conducted a randomized, double-blind, placebo-controlled (3:1 within each  
53 group) phase Ib dose-escalation trial to evaluate the BPZE1 vaccine given to healthy adults  
54 aged 18 to 32 years at the Karolinska Trial Alliance in Stockholm, Sweden. Twelve  
55 participants per consecutive dose group received a single total dose of  $10^7$ ,  $10^8$  or  $10^9$  colony-  
56 forming units, administered as 0.4-ml droplets (containing half the dose) in each nostril. Four  
57 randomized controls per consecutive dose group received the diluent as placebo. The analysis  
58 was conducted by a modified intention to treat (mITT) approach, including all randomized  
59 participants who received a vaccine dose. Colonization with BPZE1 was determined by  
60 repeatedly culturing nasopharyngeal aspirates at 4, 7, 11, 14, 21 and 28 days after vaccination.  
61 Safety, measured as solicited and unsolicited adverse events, was the primary outcome.  
62 Immunogenicity, as serum IgG and IgA responses were assessed at days 0, 7, 14, 21, 28 and  
63 months 6 and 12 post-vaccination, as was the secondary outcome. This trial is registered at  
64 <https://www.clinicaltrials.gov> (NCT02453048).

65 Findings: The trial took place between September 1st 2015, and March 1st 2017 with 48  
66 participants. The tested doses of BPZE1/placebo were well tolerated, with no apparent  
67 difference in solicited or unsolicited adverse events following immunization, neither between  
68 the different active dose groups, nor between active vaccine and placebo. Colonization at least  
69 once post-vaccination was observed in 29/36 (81%: 95%CI 68-93) of vaccinated participants.  
70 The tested vaccine doses were immunogenic, with increases in serum antibody titers against  
71 *B. pertussis* from baseline to 12 months.

72 Interpretation: The tested vaccine was safe, induced a high colonization rate in an adult  
73 population and was immunogenic at all doses. This justifies further clinical development of  
74 BPZE1 to ultimately be used as a priming vaccine for neonates and/or a booster vaccine for  
75 adolescents and adults.

76 Funding: This study was funded by ILiAD Biotechnologies.

77

78 **Research in Context.**

79

80 - Evidence before this study

81 Pertussis is a severe and life-threatening respiratory disease mainly caused by *Bordetella*  
82 *pertussis*. Two types of vaccines are currently available, the first-generation, whole cell  
83 vaccines and the more recent, acellular vaccines. However, despite global vaccination  
84 coverage of more than 85%, according to the World Health Organization, the disease has not  
85 been eliminated in any part of the world. Instead, its incidence is on the rise in several  
86 countries, most likely due to rapid waning of vaccine-induced immunity and to the failure of  
87 current vaccines to prevent infection by and transmission of *B. pertussis*. Novel vaccines are  
88 therefore needed to both prevent disease and infection. Since *B. pertussis* is a strictly mucosal  
89 pathogen, mucosal vaccines may be more effective than the current injectable vaccines. We  
90 searched PubMed with the terms “pertussis”, “mucosal vaccine”, “intranasal”, “live  
91 attenuated”, “whooping cough” for articles in any language up to January 10, 2020. Several  
92 combinations of these terms yielded up to 220 references. Some of them relate to novel  
93 mucosal vaccines, but none of these vaccines has yet reached clinical development, except for  
94 a first-in-man trial of the live attenuated vaccine BPZE1. Intranasal administration to human  
95 volunteers of low doses of this vaccine was well tolerated and resulted in low frequencies of  
96 vaccine take and seroconversion. In non-human primates, a single administration of BPZE1  
97 protected non-human primates against both pertussis disease and infection by *B. pertussis*,  
98 Therefore, BPZE1 is a promising candidate for an effective control of pertussis.

99

100 - Added value of this study

101 This is the first clinical trial of a live attenuated pertussis vaccine delivered nasally to human  
102 adult volunteers at doses that result in seroconversion in 100% of vaccinees after a single

103 administration. BPZE1 was well tolerated at doses up to  $10^9$  colony-forming units.  
104 Frequencies and severity of adverse events following immunization were similar in the  
105 placebo and vaccine dosage groups. Transient colonization of the respiratory tract by BPZE1  
106 could be documented for more than 80% of the vaccine recipients. Serum antibody titers  
107 (both Immunoglobulin G and Immunoglobulin A) to the major protective *B. pertussis*  
108 antigens increased in the vaccinated subjects and remained high for at least up to 12 months  
109 after vaccination, when the study was terminated. At the highest dose tested, all subjects  
110 responded to at least one of the tested antigens.

111

112 - Implications of all the available evidence

113 This study defines the human dose for the nasal administration of the live attenuated pertussis  
114 vaccine BPZE1, which can be safely administered and results in sero-conversion of 100% of  
115 the study subjects. The safety and immunogenicity profile of BPZE1 supports the progression  
116 of this vaccine to larger efficacy trials. Together with preclinical studies showing that, unlike  
117 currently available vaccines, BPZE1 can induce sterilizing immunity, BPZE1 thus holds  
118 promise as an effective vaccine to protect both against pertussis disease and infection by *B.*  
119 *pertussis*.

120 **Introduction**

121 Pertussis or whooping cough is a highly contagious respiratory disease caused by *Bordetella*  
122 *pertussis*.<sup>1</sup> The disease affects all age groups but is particularly severe and life-threatening in  
123 young infants.<sup>2</sup> Vaccination campaigns starting in the 1950s have strongly reduced the  
124 incidence worldwide, but despite a >85% global vaccination coverage<sup>3</sup>, the disease is not  
125 under control in any part of the world. In fact, in recent years it has made an alarming rebound  
126 in several countries, especially those that have switched from the whole-cell vaccines to  
127 acellular vaccines.<sup>4</sup> In the US, epidemic peaks have reached incidences of close to  
128 16/100,000<sup>5</sup>, which has not been seen before the switch. However, the pertussis incidence is  
129 also increasing in countries in which whole-cell vaccines are still in use.<sup>6,7</sup>

130

131 Several reasons can account for this resurgence, including pathogen adaptation to escape  
132 vaccine-induced immunity, rapid waning of immunity, especially after vaccination with  
133 acellular vaccines, and failure of current vaccines to prevent infection by and transmission of  
134 *B. pertussis*.<sup>8</sup> Mathematical modelling studies suggest that the latter is the major driver of the  
135 current resurgence of pertussis in countries with high vaccination coverage.<sup>9</sup> Improved  
136 vaccines that protect against both pertussis disease and infection by *B. pertussis* are thus  
137 needed to ultimately control whooping cough.<sup>10</sup>

138

139 In contrast to current vaccines, infection by *B. pertussis* prevents subsequent colonization<sup>11</sup>,  
140 and immunity acquired by natural infection is longer lasting than that induced by  
141 vaccination.<sup>12</sup> Therefore, a live attenuated *B. pertussis* vaccine, named BPZE1, was developed  
142 to be delivered intranasally in order to mimic natural infection without causing disease. This  
143 vaccine strain was constructed by genetically removing dermonecrotic toxin, reducing  
144 tracheal cytotoxin to background levels, and by inactivating pertussis toxin (PT).<sup>13</sup> In a non-



145 human primate model, a single nasal administration of BPZE1 was found to provide strong  
146 protection against both pertussis disease and infection following challenge by a highly  
147 virulent recent clinical *B. pertussis* isolate.<sup>14</sup>

148

149 We previously reported the results of a first clinical evaluation in humans, where BPZE1 was  
150 found to be safe up to a dose of  $10^7$  colony-forming units (CFU), and able to transiently  
151 colonize the upper respiratory tract and to induce antibody responses in young male  
152 participants.<sup>15</sup> However, even at the highest dose tested in this initial study, only 5/12 of the  
153 participants were colonized and produced antibodies to *B. pertussis* antigens. This relatively  
154 low vaccine take may be due to several reasons. The dose or volume (100  $\mu$ l/nostril) used in  
155 the previous study might have been too low for optimal immune induction and/or pre-existing  
156 immunity might have hampered vaccine take.

157

158 Here we report findings on safety, colonization and serum antibody responses of a subsequent  
159 phase Ib study, in both females and males, in which both the volume and the dose of BPZE1  
160 were increased.

161

## 162 **Methods**

### 163 *Study design*

164 In this phase Ib, placebo-controlled, double-blind, dose-escalation, single-center trial  
165 performed at the phase I unit, Karolinska Trial Alliance, Karolinska University Hospital,  
166 Stockholm, Sweden, 48 individuals were recruited into three consecutive cohorts of 16  
167 participants. Within each group, participants were randomized 3:1 to receive vaccine at a  
168 given dose or placebo (12 receiving BPZE1 and 4 placebo). The vaccine doses for Groups 1  
169 (low dose), 2 (medium dose) and 3 (high dose) were  $10^7$ ,  $10^8$  and  $10^9$  CFU, respectively.

170

171 The clinical trial ([www.clinicaltrials.gov](http://www.clinicaltrials.gov) NCT02453048) was conducted in accordance with  
172 the study protocol, ICH Good Clinical Practices standards, Declaration of Helsinki and  
173 applicable regulatory requirements as well as any European and Swedish applicable laws and  
174 regulations relating. The Swedish Medical Product Agency (MPA) and the regional ethical  
175 review board in Stockholm approved the study protocol and later amendments.

176

### 177 *Study participants*

178 Healthy adults aged 18-32 years were screened and included sequentially, respecting pre-  
179 defined minimum time intervals between enrolment of individual participants as a safety  
180 principle (see Study protocol, page 31). The majority of the participants were born during the  
181 period 1979-1996 when pertussis vaccination was suspended in Sweden. Participants were  
182 eligible for enrolment if they had not experienced clinical or laboratory-verified pertussis  
183 during the preceding ten years and had serum anti-PT and anti-pertactin IgG levels below 20  
184 IU/ml. Detailed inclusion and exclusion criteria are shown in Method S1. Women of  
185 childbearing potential had to practice adequate contraception from two weeks pre-vaccination  
186 to one month post-vaccination and have a negative pregnancy test on the day of vaccination.

187

188 All participants signed the informed consent form after receiving written and oral information,  
189 given before and during the first visit, so that the participant had sufficient time to consider  
190 participation before vaccination. A new consent form was signed after protocol amendment to  
191 extend follow up from planned 6 months to 12 months.

192

### 193 *Experimental products*

194 A liquid formulation of the three different BPZE1 batches (low, medium and high dose) in  
195 phosphate-buffered saline containing 5% saccharose (manufactured by Q Biologicals, Gent,  
196 Belgium, for ILiAD Biotechnologies, LLC, in sequentially numbered vials) was used.  
197 Vaccine or placebo (phosphate-buffered saline containing 5% saccharose) was given as a  
198 single administration by nasal application of 0.4 ml (containing half the dose) in each nostril.  
199

#### 200 *Randomization and blinding*

201 Randomization was stratified on group (dose cohort) with a vaccine:placebo ratio of 3:1.  
202 Vials containing vaccine or placebo were indistinguishable when frozen and were coded by a  
203 number given by the manufacturer prior to shipment to the site. The randomization list,  
204 provided centrally by the academic clinical trials unit (EUCLID/F-CRIN Clinical Trials  
205 Platform, Bordeaux, France), established the order in which the coded vials were allocated to  
206 the participants. A participant was considered randomized when he/she was allocated a vial  
207 code on the day of vaccination (Day 0, visit 2). Because vaccine and placebo might be  
208 distinguishable when thawed, the vaccine preparation and administration was done by a study  
209 nurse not involved in any other trial procedures, so that observer-blinding was maintained.  
210 Throughout the trial, all personnel involved remained blinded to the treatment assignment.  
211 Since the BPZE1 cultivation results could have led to treatment unblinding, access to these  
212 results was strictly controlled within the cultivating laboratory. The only exception was a  
213 statistician performing the interim analysis and not being involved in any other study analysis.

214

#### 215 *Study procedures*

216 Participants were observed at the study site for at least six hours after vaccination. Then, on-  
217 site visits took place at days 4, 7, 11, 14, 21, 28 (Visits 3-8), at months 6 and 12 after  
218 vaccination (Visits 9 and 10), as detailed in Table S1. During Visits 3-10 the investigators did

219 physical examinations, including vital signs, sample collections and asked solicited questions  
220 concerning general and local adverse events (AE), and about other, unsolicited AE.

221 The participants were instructed to use a standardized paper diary with pre-written questions  
222 and space to record solicited and unsolicited AE between on-site visits up to Day 28 post-  
223 vaccination. The intensity of AE was recorded as maximum intensity observed and coded as  
224 mild and easily tolerated (grade 1), moderate and interfering with usual activity (grade 2) or  
225 severe with inability to do usual activity (grade 3), following FDA coding guidelines.<sup>16</sup>

226 Further details are described in Method S2.

227

228 Nasopharyngeal aspirates were collected at each of the Visits 3-8 to determine colonization  
229 by using a syringe aspiration kit (SAK-01; www.n-pak.com), as described in Method S3. The  
230 aspirates were grown on charcoal agar plates as described.<sup>15</sup> If a participant was culture-  
231 positive for BPZE1/*B. pertussis* at Visit 8 (Day 28), an additional visit was scheduled on Day  
232 45 to ensure clearing of the colonization. Given that the recovery and quality of  
233 nasopharyngeal aspirates differed greatly between participants and time points, thus raising  
234 concerns about the reproducibility of exact quantitative assessments, detection of bacteria was  
235 assessed qualitatively (detection of bacteria: yes vs. no).

236

237 Venous blood was collected at screening 1-6 weeks prior to vaccination, on the day of  
238 vaccination and at 1, 2, 3 and 4 weeks, and 6 and 12 months after vaccination (Table S1).

239 Serum IgG and IgA against PT, pertactin (PRN), filamentous hemagglutinin (FHA) and  
240 fimbriae sero-types 2 and 3 (Fim2/3) were analysed by a standardized ELISA, as described in  
241 Method S4.

242

243 *Endpoints*

244 As the trial included live attenuated *B. pertussis*, the main safety concern was potential  
245 symptoms of pertussis infection in the participant. Therefore, the primary safety endpoint was  
246 the number and proportion of participants with at least one of the following AE between days  
247 0 and 28: (i) cough or spasmodic cough of grade 2 or higher, (ii) other respiratory tract AE  
248 related or possibly related to vaccination of grade 3 or higher, (iii) any other AE related or  
249 possibly related to vaccination of grade 3 or higher. Secondary safety endpoints included  
250 additional AE in the respiratory tract, systemic AE and severe AE (SAE) as shown in Method  
251 S5.

252

253 Frequency and duration of nasal colonization, as well as serum IgG and IgA titres against PT,  
254 FHA, PRN, and Fim2/3 were further secondary endpoints. For each antigen, an antibody  
255 responder was defined as a participant with at least 100% increase of serum IgG or IgA levels  
256 from pre-vaccination to a given time point post-vaccination and at least four times minimum  
257 level of detection (Method S4), as defined previously.<sup>17</sup> Moreover, as a post-hoc exploratory  
258 endpoint, the positivity of the serological response evoked by BPZE1 in comparison to a  
259 natural *B. pertussis* infection was defined by using a pertussis case definition<sup>18</sup>, meaning an  
260 IgG or IgA level increase by a fold change of two or more between pre- and post-vaccination  
261 samples for either PT alone or for a combination of at least two other antigens (PRN+FHA;  
262 PRN+Fim2/3; FHA+Fim2/3).

263

264 *Statistical analyses*

265 *Sample size rationale*

266 Twelve participants receiving active vaccination per dose group constitute a trade-off between  
267 detectable event rate and power in the context of a phase I trial.<sup>19</sup> A sample size of 12  
268 participants allows to observe at least one primary safety endpoint event with 80% power if

269 the underlying event rate is at least 12.6%. If no primary safety endpoint event is observed  
270 among 12 participants, the upper bound of a two-sided 95% confidence interval for the event  
271 rate would be 26%. If no primary safety endpoint event is observed among 36 participants  
272 (pooled across groups 1, 2, 3), then the upper bound of the 95% confidence interval for the  
273 event rate would be 10%.

274

#### 275 *Statistical analysis methods*

276 The main analysis was conducted by a modified intention to treat (mITT) approach, including  
277 all randomized participants who received a vaccine dose in the analysis in the group to which  
278 they were initially randomized and using all their data regardless of protocol deviations  
279 during the trial. The only exception from the mITT was the exclusion of the M12  
280 immunogenicity data of one participant who had received acellular pertussis vaccine shortly  
281 after the 6 months follow up.

282 After assessment of baseline characteristics and antibody titers, not indicating any time trend  
283 in placebo observations between the consecutive dose groups, all placebo recipients from  
284 groups 1-3 were pooled for further analyses. Descriptive analyses were done using standard  
285 summary statistics for distributions per group. Antibody titers were described by their  
286 geometric mean and 95% confidence interval. Primary endpoint was described in terms of  
287 number, proportion and exact binomial confidence interval of proportion. Stratified  
288 descriptive analyses for colonized and non-colonized participants were pre-planned in the  
289 protocol. No statistical comparisons between groups were performed, as the trial was not  
290 designed for such comparisons.

291

#### 292 *Interim data reviews*

293 Interim safety data reviews by an independent Data Safety Monitoring Board (DSMB) were  
294 performed during the trial before enrolment of a higher-dose group. The DSMB decided to  
295 remain blinded (i.e. they never requested review of unblinded data while the trial was  
296 ongoing). All interim analyses results remained confidential and were not shared with any  
297 investigators until after the data was locked.

298

#### 299 *Role of the funding source*

300 The French public research institute Inserm was the legal sponsor and responsible for the  
301 oversight of the trial. In a public-private partnership, ILiAD Biotechnologies provided  
302 funding and experimental products. All trial-related activities, including protocol  
303 development, trial set-up and conduct, data management and statistical analyses, were carried  
304 out by academic partners. ILiAD Biotechnologies and Inserm representatives were members  
305 of the Trial Steering Committee and as such were involved in the study design, the overview  
306 of the study conducts and analyses, the writing of the report and in the decision to submit the  
307 manuscript for publication. The corresponding author had full access to all the data in the  
308 study and had final responsibility for the decision to submit for publication.

309

310

311 **Results**

312 In total, 120 participants were assessed for eligibility, and 48 of them were enrolled and  
313 randomized to receive vaccine or placebo (figure 1). The first visit of the first participant was  
314 the 1<sup>st</sup> of September 2015 and the last visit of the last participant was the 1<sup>st</sup> of March, 2017.  
315 Baseline characteristics were similar in the participants receiving placebo or vaccine (Table  
316 1). Males and females were evenly distributed, except for the medium-dose group that had a  
317 higher number of males. During the trial, after review of the safety data, the DSMB  
318 recommended opening of the consecutive dose cohorts as planned by the protocol.

319

320 All participants came to scheduled appointments up to 6 months post-vaccination. Three  
321 participants (one placebo, one low-dose and one medium-dose recipient) could not participate  
322 during the extended follow up of 12 months. The 12-months immunogenicity data for a fourth  
323 participant (low-dose recipient) were excluded from the statistical analysis due to vaccination  
324 with acellular pertussis vaccine shortly after the 6 months follow up.

325

326 *Safety*

327 The primary safety endpoint between days 0 and 28 was reported by one participant in each of  
328 the placebo and low-dose groups, and two participants in each of the medium- and high-dose  
329 groups (Table 2).

330 AE following immunization (AEFI) are summarized in Table S2. No immediate AEFI was  
331 observed within the six-hour observation period at the investigational site. None of the  
332 participants experienced any spasmodic cough during the 28 days following immunization,  
333 nor did any of the participants report difficulties in breathing, and no AEFI was noted  
334 concerning vital signs during this period. Three participants (one medium-dose and two high-  
335 dose recipients) reported mild or moderate fever during the 28 days period.



336

337 One third of the participants (17/48) reported at least one day of cough after vaccination  
338 during the 28 days follow-up, without any marked difference between placebo and vaccine  
339 recipients. The highest number of cough events was reported during weeks 1 and 2 post-  
340 vaccination, with three cases in the placebo recipients, four in the low-dose, six in the  
341 medium-dose and four in the high-dose recipients. Most events resolved within a week. Three  
342 cases of cough (no case of spasmodic cough) were reported during the first three days post-  
343 vaccination, one in each group except for the high-dose group. In addition, four participants in  
344 the medium-dose group and one in the high-dose group reported a cough episode starting at  
345 week 3 or 4. The reported cough was mild or moderate, except for one severe case in the  
346 high-dose group. Overall, the occurrence of cough was similar among the groups.

347

348 Half of the participants reported solicited general symptoms during the 28 days follow-up.  
349 The most frequent solicited symptoms were sneezing, fatigue, headache, rhinorrhea and nasal  
350 congestion. Most of the symptoms were mild or moderate. Many participants reported  
351 common cold during 28 days follow-up. There were no apparent differences between the  
352 placebo and any of the dose arms.

353

354 Two SAEs were reported, both of which started after day 28 and were judged as not related to  
355 vaccination. The first SAE was pneumonia as a possible complication of suspected influenza  
356 in a participant 65 days after vaccination. The second SAE concerned another participant who  
357 was hospitalized for acute renal failure with pathological high serum levels of creatinine  
358 following intake of a high dose of the nonsteroidal anti-inflammatory drug Naproxen after  
359 dental surgery, 82 days after vaccination. Both participants recovered uneventfully.

360

361 *Colonization*

362 BPZE1 was isolated from 29/36 (81%; 95%CI 68-93) vaccinated participants at least at one  
363 time point between days 4 and 28. No placebo recipient was culture-positive at any time  
364 point. Ten out of 12 in the low- and the high-dose groups and 9/12 in the medium-dose group  
365 were culture-positive at least at one time point (Figure 2). Colonization profiles varied largely  
366 between participants.

367

368 The duration of the nasal colonization did not differ between the dose groups. However,  
369 BPZE1 was detected earlier with increasing dose levels with three, six and nine participants  
370 being culture-positive on Day 4 in the low-, medium- and high-dose groups, respectively.  
371 Four participants were culture-positive at Day 28, but new samples collected between days 43  
372 and 50 were negative. Cough occurrence was similar between colonized and non-colonized  
373 vaccine recipients.

374

375 *Immunogenicity at a dose group level*

376 The fold-increase in serum IgG and IgA levels against PT, PRN, FHA and Fim2/3 over pre-  
377 vaccination levels was measured at days 7, 14, 28 and months 6 and 12 after vaccination  
378 (Figure 3A and C). As expected, pre-vaccination antibody levels varied both within and  
379 between treatment groups (Tables S3 and S4). In general, increases in all four antigen-specific  
380 IgG levels were observed starting at Day 14 post-vaccination in all dose groups (Figure 3A).  
381 This increase usually peaked at Day 28 and remained above the pre-vaccination level during  
382 12 months follow-up. Similar results were also found for the IgA levels (Figure 3C).

383

384 Twenty-two of the 36 vaccinated participants (61%; 95% CI 45-77) responded with serum  
385 IgG against at least one *B. pertussis* antigen four weeks after vaccination and the vast majority

386 of those (20/21 [95%, 95% CI 86-100]) still responded after 12 months (one participant lost to  
387 follow-up at 12 month). For serum IgA, 24/36 (67%, 95% CI 51-82) responded to at least one  
388 *B. pertussis* antigen four weeks after vaccination. At 12 months, 17/23 (74%, 95% CI 56-92)  
389 of serum IgA responders had maintained their response (one participant lost to follow-up at  
390 12 month). At four weeks after vaccination 28/36 vaccinated participants (78%, 95% CI 64-  
391 91) had mounted IgG and/or IgA responses, including 11/12 in the high-dose group. Of the 28  
392 vaccinated participants that mounted IgG and/or IgA responses, 18 (64%, 95% CI 47-82)  
393 responded with both IgG and IgA.

394

#### 395 *Immunogenicity by colonization status*

396 When the antibody responses were examined in relation to the colonization status of the  
397 vaccine recipients, colonized participants (as defined by bacterial detection in at least one  
398 nasopharyngeal aspirate at any time point) had markedly higher increases in IgG and IgA  
399 titers than non-colonized vaccine recipients or placebo recipients (Figure 3B and D).

400

401 The individual profiles of IgG and IgA responses against the four *B. pertussis* antigens on  
402 Day 28 after vaccination in relation to colonization are shown in figure 2 and the frequency of  
403 responders in each dose group can be found in table S5. In the 29 colonized participants, the  
404 most frequently detected serum IgG responses were seen against PT and FHA. Anti-FHA IgA  
405 were also the most frequent response detected while, in contrast to IgG, anti-PT IgA  
406 responses were seen in the lowest frequency amongst the participants.

407

408 The majority of the culture-positive participants responded with either or both *B. pertussis*-  
409 specific IgG and IgA. Only four out of the 29 culture-positives did not mount any detectable  
410 IgG and/or IgA response at day 28 after vaccination. However, one of them did show a rise in

411 anti-PT IgG level from day 21 but did not reach the responding criteria until 6 months after  
412 vaccination. Interestingly, three of seven vaccinated culture-negative participants had positive  
413 IgG and/or IgA responses at day 28 post-vaccination.

414

#### 415 *Immunogenicity using pertussis case definition*

416 Investigating the positivity of the serological responses during the first 6 months post-  
417 vaccination showed that 28/36 BPZE1-vaccinated participants responded according to the  
418 case definition<sup>18</sup>, including all 12 participants in the high-dose group. Of the 28, 17 responded  
419 with both IgG and IgA, whereas five and six responded only with IgG or IgA, respectively  
420 (Figure 4). None of the placebo recipients responded.

421

## 422 **Discussion**

423 This single-center, dose-escalating, randomized placebo-controlled double-blind, clinical trial  
424 of the live genetically attenuated *B. pertussis* vaccine strain BPZE1 showed that the tested  
425 vaccine doses were well tolerated without any signal for differences in tolerance between the  
426 different active doses and placebo. Nasal colonization was frequent in vaccine recipients in all  
427 dose groups, while no colonization was observed in placebo recipients. Increases in  
428 serological responses against the four *B. pertussis* antigens tested were seen in all dose groups  
429 after intranasal vaccination, while no increase was apparent in placebo recipients.

430

431 This study was a follow-up trial after the first-in-man trial reported earlier.<sup>15</sup> In contrast to the  
432 previous trial this study escalated to higher doses, as the lowest dose here corresponds to the  
433 highest dose used in the previous study. Furthermore, the volume was increased to 0.4 ml per  
434 nostril to optimize bacterial adherence to the nasal epithelium. Unlike the previous study,  
435 females were also included. Finally, in this study participants with high serum IgG levels

436 against both PT and PRN were excluded, whereas previously, participants were only excluded  
437 based on high anti-PT IgG levels. The first study showed that high anti-PRN IgG levels were  
438 associated with lack of colonization. In the present study, which excluded subjects with either  
439 high anti-PT or anti-PRN IgG levels and used higher vaccine volumes, colonization rates with  
440 the  $10^7$  CFU dose were 10/12 (5/12 in the previous study).

441

442 In this trial the safety profile of BPZE1 showed no marked differences in the occurrence of  
443 solicited or unsolicited AEFI between the three active dose groups and placebo. Reported  
444 symptoms were usually mild or moderate. Spasmodic cough was not reported by any  
445 participant. There was no signal for differences in cough occurrence between groups.

446 However, as an inherent limitation of a phase I trial, given the small numbers of subjects  
447 included in this study, local tolerability and general safety need to be further investigated in  
448 future, larger-scale trials.

449

450 The overall proportion of colonization by the vaccine was 29/36 (81%, 95% CI 68-93) among  
451 vaccine recipients, without any apparent difference between dose groups. Culture positivity  
452 was examined between days 4 and 28 after vaccination, with a highly variable duration of  
453 positivity between participants. Some participants were culture-positive at only one time  
454 point, others were continuously positive over several time points and some showed  
455 discontinuously detectable colonization. These variations may be due to a suboptimal  
456 aspiration technique as the nasopharyngeal samples varied greatly in volume and mucus  
457 content. Bacteria were detected somewhat earlier in the high-dose group than in the other  
458 groups. Increases in antibody responses to the tested antigens tended to be more frequent in  
459 colonized than in non-colonized vaccine recipients, while cough occurrence was similar.

460

461 The increase of serum IgG against *B. pertussis* antigens is often used for the evaluation of  
462 immunogenicity of pertussis vaccines<sup>20</sup>, although no serological correlate of protection has  
463 been widely accepted for pertussis. Nevertheless, correlations between post-vaccination  
464 antibody levels and protective efficacy against pertussis disease have been reported for anti-  
465 PRN, anti-Fim2/3 and to lesser extent for anti-PT IgG.<sup>21,22</sup> All three antibody types were  
466 induced by BPZE1 in most of the vaccinees.

467

468 Generally, antibody responses were related to colonization. However, some culture-negative  
469 vaccinated participants showed increases in their IgG and/or IgA levels, although typically at  
470 a lower level than the culture-positive participants. It cannot be excluded that these  
471 participants may nevertheless have been colonized, but at levels below the detection limit  
472 and/or before the first sampling day (Day 4 post vaccination). Also, the suboptimal aspiration  
473 technique could possibly have rendered falsely negative colonisation results. In addition,  
474 although the doses were standardized and determined by CFU counting after thawing of the  
475 vaccine lot, it should be kept in mind that the vaccine suspension does not only contain live  
476 BPZE1 but also dead bacteria that died during the freeze/thaw cycle. It is therefore  
477 conceivable that the dead bacteria may have contributed to immunogenicity in a similar way  
478 as a killed whole-cell vaccine, especially in participants with a pre-existing immune response  
479 induced by prior exposure to *B. pertussis*.

480

481 The serum IgG levels to the four tested antigens observed here were low in relation to those  
482 induced by injectable acellular pertussis vaccines.<sup>23</sup> This is not unexpected, as acellular  
483 pertussis vaccines contain much larger amounts of these antigens than BPZE1. However, we  
484 have recently shown that BPZE1 induces a much broader and functional Th1-type antibody  
485 response than acellular vaccines<sup>24</sup>. In addition, the serological responses to the four tested

486 antigens were evaluated using the same case definition as used for patients with a natural  
487 infection to see if the BPZE1 vaccine could induce similar responses than those induced by  
488 natural infection.<sup>18</sup> This was seen in the vast majority of the vaccinated participants, including  
489 all participants in the high-dose group.

490

491 BPZE1 induced notable increases in IgA levels against the four antigens. As *B. pertussis* is a  
492 strictly mucosal pathogen, and disseminated bordetellosis is very rare<sup>25</sup>, IgA is expected to  
493 play a protective role against *B. pertussis* colonisation.<sup>26</sup> In contrast to whole-cell and  
494 acellular vaccines, which fail to prevent colonization by *B. pertussis*<sup>11</sup>, a single administration  
495 of BPZE1 has been shown to protect against both pertussis disease and *B. pertussis*  
496 colonization in baboons.<sup>14</sup> Recent studies in mice have confirmed the role of IgA in protection  
497 against nasal colonization by *B. pertussis*.<sup>27</sup> In future clinical studies it will therefore be  
498 important to evaluate the nasal IgA responses induced by BPZE1. However, this will require  
499 an improved and standardized method for the collection of nasopharyngeal specimens.

500

501 In summary, the three tested vaccine doses were safe, induced colonization, and were  
502 immunogenic in an adult population, which justifies further clinical development of BPZE1.  
503 Currently, a larger-scale phase 2 study (NCT03942406) with 10<sup>9</sup> CFU of BPZE1 is underway,  
504 which should gather additional safety data. This study will also examine the effect of prior  
505 acellular pertussis vaccination on BPZE1 take, as well as the booster effect of a second  
506 BPZE1 dose. If future studies confirm that BPZE1 can protect against infection by and  
507 transmission of *B. pertussis* in addition to protection against pertussis disease, this standalone  
508 pertussis vaccine may be useful for boosting adolescents and adults, and as an effective tool  
509 for cocoon vaccination. A limitation of the use of this vaccine may potentially be in

510 immunosuppressed subjects, including during pregnancy, although studies in mice have  
511 shown that BPZE1 is safe, even in severely immune-compromised mice.<sup>28</sup>  
512 Furthermore, if safety can be demonstrated in neonates, BPZE1 may potentially be used as a  
513 priming vaccine in early life. This may help to protect the most vulnerable population in  
514 conjunction with maternal vaccination. Maternal vaccination is not expected to interfere with  
515 BPZE1 take in neonates, since maternal antibodies are unlikely to prevent colonization by *B.*  
516 *pertussis*, as demonstrated in the baboon model.<sup>29</sup> Moreover, we have recently shown that  
517 BPZE1 vaccination results in significant protection within days in a murine model.<sup>28</sup> This is  
518 reminiscent of observations made with the live attenuated *Bordetella bronchiseptica* vaccine,  
519 shown to protect dogs against kennel cough as soon as two days after vaccination.<sup>30</sup> This  
520 provides strong hope that live attenuated *Bordetella* vaccines may effectively and quickly  
521 protect neonates against pertussis and, in conjunction with maternal immunization, may  
522 constitute a powerful tool to ultimately control the pertussis pandemic.

523

#### 524 **Acknowledgements**

525 The authors thank the participants for their participation in this clinical trial. The authors also  
526 thank the members of the Data Safety Monitoring Board (Patrick Olin, Marie-Blanche Rabier,  
527 Bernard Fritzell and Francois Spertini) and the Trial Scientific Committee (Brigitte Autran  
528 [Sorbonne-Université, cimi Paris, Inserm UMR-1135], Sonia Gueguen [Clinical Research  
529 Department, National Institute of Health and Medical Research, Paris, France] and Pierre van  
530 Damme) for invaluable advice during the study.

531

#### 532 **Conflicts of interest**



533 NM and CL hold patents on the BPZE1 vaccine, which is licensed to ILiAD Biotechnologies.  
534 MT, KS and KR are employees of ILiAD Biotechnologies. All other authors declare that they  
535 have no conflicts of interest.

536

537 **Author Contributions**

538 Study design: RT, JS, MJ, KS, KR, MT, CL, LR

539 Clinical data collection: JS, NaT

540 Laboratory data collection: MJ, RT

541 Statistical data analysis: LR, CC, CB

542 Data interpretation: MJ, RT, KS, KR, CL, LR, JS, NM

543 Manuscript writing and literature search: RT, JS, MJ, CL, LR

544 Figures: LR, CC, CB, MJ, RT

545 Manuscript review and critique: RT, KS, KR, LR, CC, CB, JS, NM, CL, NaT, MT

546

547 All authors contributed equally in specified tasks unless stated otherwise. All authors had full  
548 access to the data, and reviewed, revised, and gave final approval of the manuscript before  
549 submission.

550

551 **Funding:**

552 This study was funded by ILiAD Biotechnologies.

553

554

555

556

557

558 **Data sharing:**

Will individual participant data be available (including dictionaries)?	No. Only group data will be made available. Individual data requires currently the conformity to the GDPR and this was not provided for in the protocol and informed consent
What data in particular will be shared?	Group data that underlie the results reported in this article, after de-identification (text, table, figures and appendices including the study report)
What other documents will be available?	Study protocol
When will data be available? (start and end dates?)	Immediately following publication and ending 10 years following article publication.
With whom?	Researchers who provide a methodologically sound proposal.
For what types of analyses?	To achieve aims in the approved proposal.
By what mechanism will data be made available?	Proposals should be directed to <a href="mailto:promoteur.inserm@inserm.fr">promoteur.inserm@inserm.fr</a> ; to gain access, data requestors will need to sign a data access agreement.

559

560

561 **References**

- 562 1. Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical  
563 manifestations of respiratory infections due to *Bordetella pertussis* and other *Bordetella*  
564 subspecies. *Clin Microbiol Rev* 2005; **18**: 326-82.
- 565 2. Yeung KHT, Duclos P, Nelson EAS, Hutubessy RCW. An update of the global  
566 burden of pertussis in children younger than 5 years: a modelling study. *Lancet Infect*  
567 *Dis* 2017; **17**: 974-80.
- 568 3. WHO. Global routine vaccination coverage, 2015. *Wkly Epidemiol Rec* 2016; **65**:  
569 1270-73.
- 570 4. Allen A. Public health. The pertussis paradox. *Science* 2013; **341**: 454-5.
- 571 5. Skoff TH, Hadler S, Harari S. The epidemiology of nationally reported pertussis in  
572 the United States, 2000-2016. *Clin Infect Dis* 2018; **68**: 1633-40.
- 573 6. Tan T, Dalby T, Forsyth K, et al. Pertussis Across the Globe: Recent Epidemiologic  
574 Trends From 2000 to 2013. *Pediatr infect Dis J* 2015; **34**: e222-32.
- 575 7. Hozbor D, Ulloa-Gutierrez R, Marino C, Wirsing von König, CH, Tan T, Forsyth K.  
576 Pertussis in Latin America: recent epidemiological data presented at the 2017 Global  
577 Pertussis Initiative meeting. *Vaccine* 2019; **37**: 5414-21.
- 578 8. Burns DL, Meade BD, Messonnier NE. Pertussis resurgence: perspectives from  
579 the Working Group Meeting on pertussis on the causes, possible paths forward, and gaps  
580 in our knowledge. *J Infect Dis* 2014; **209 Suppl 1**: S32-5.
- 581 9. Althouse BM, Scarpino SV. Asymptomatic transmission and the resurgence of  
582 *Bordetella pertussis*. *BMC Med* 2015; **13**: 146.
- 583 10. Locht C. Will we have new pertussis vaccines? *Vaccine* 2018; **36**: 5460-9.
- 584 11. Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against  
585 disease but fail to prevent infection and transmission in a nonhuman primate model.  
586 *Proc Natl Acad Sci USA* 2014; **111**: 787-92.
- 587 12. Wearing HJ, Rohani P. Estimating the duration of pertussis immunity using  
588 epidemiological signatures. *PLoS Pathog* 2009; **5**: e1000647.
- 589 13. Mielcarek N, Debrie AS, Raze D, et al. Live attenuated *B. pertussis* as a single-dose  
590 nasal vaccine against whooping cough. *PLoS Pathog* 2006; **2**: e65.
- 591 14. Locht C, Papin JF, Lecher S, et al. Live Attenuated Pertussis Vaccine BPZE1  
592 Protects Baboons Against *Bordetella pertussis* Disease and Infection. *J Infect Dis* 2017;  
593 **216**: 117-24.
- 594 15. Thorstensson R, Trollfors B, Al-Tawil N, et al. A phase I clinical study of a live  
595 attenuated *Bordetella pertussis* vaccine--BPZE1; a single centre, double-blind, placebo-  
596 controlled, dose-escalating study of BPZE1 given intranasally to healthy adult male  
597 volunteers. *PloS One* 2014; **9**: e83449.
- 598 16. FDA. [Available from:  
599 <https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/UCM091977.pdf>.  
600
- 601 17. Gustafsson L, Hallander HO, Olin P, Reizenstein E, Storsaeter J. A controlled trial  
602 of a two-component acellular, a five-component acellular, and a whole-cell pertussis  
603 vaccine. *N Engl J Med* 1996; **334**: 349-55.
- 604 18. Ward JI, Cherry JD, Chang SJ, et al. Efficacy of an acellular pertussis vaccine among  
605 adolescents and adults. *N Engl J Med* 2005; **353**: 1555-63.
- 606 19. Buoen C, Holm S, Thomsen MS. Evaluation of the cohort size in phase I dose  
607 escalation trials based on laboratory data. *J Clin Pharmacol* 2003; **43**: 470-6.

- 608 20. Hallander HO. Microbiological and serological diagnosis of pertussis. *Clin Infect*  
609 *Dis* 1999; **28 Suppl 2**: S99-106.
- 610 21. Cherry JD, Gornbein J, Heininger U, Stehr K. A search for serologic correlates of  
611 immunity to *Bordetella pertussis* cough illnesses. *Vaccine* 1998; **16**: 1901-6.
- 612 22. Storsaeter J, Hallander HO, Gustafsson L, Olin P. Levels of anti-pertussis  
613 antibodies related to protection after household exposure to *Bordetella pertussis*.  
614 *Vaccine* 1998; **16**: 1907-16.
- 615 23. Edwards KM, Meade BD, Decker MD, et al. Comparison of 13 acellular pertussis  
616 vaccines: overview and serologic response. *Pediatrics* 1995; **96**: 548-57.
- 617 24. Lin A, Apostolovic D, Jahnmatz M, et al. Live attenuated pertussis vaccine BPZE1  
618 induces a broad antibody response in humans. *J Clin Invest* 2020; doi:  
619 10.1172/JCI135020.
- 620 25. Janda WM, Santos E, Stevens J, Celig D, Terrile L, Schreckenberger PC. Unexpected  
621 isolation of *Bordetella pertussis* from a blood culture. *J Clin Microbiol* 1994; **32**: 2851-3.
- 622 26. Solans L, Loch C. The Role of Mucosal Immunity in Pertussis. *Front Immunol*  
623 2018;**9**: 3068.
- 624 27. Solans L, Debie AS, Borkner L, et al. IL-17-dependent SIgA-mediated protection  
625 against nasal *Bordetella pertussis* infection by live attenuated BPZE1 vaccine. *Mucosal*  
626 *Immunol* 2018; **11**: 1753-62.
- 627 28. Debie AS, Mielcarek N, Lecher S, Roux X, Sirard JC, Loch C. Early protection  
628 against pertussis induced by live attenuated *Bordetella pertussis* BPZE1 depends on  
629 TLR4. *J Immunol* 2019; **203**: 3293-300.
- 630 29. Warfel JM, Papin JF, Wolf RF, Zimmerman LI, Merkel TJ. Maternal and neonatal  
631 vaccination protects newborn baboons from pertussis infection. *J Infect Dis* 2014; **210**:  
632 604-10.
- 633 30. Bey RF, Shade FJ, Goodnow RA, Johnson RC. Intranasal vaccination of dogs with  
634 live avirulent *Bordetella bronchiseptica*: Correlation of serum agglutination titer and the  
635 formation of secretory IgA with protection against experimentally induced infectious  
636 tracheobronchitis. *Am J Vet Res* 1981; **42**: 1130-32.
- 637
- 638
- 639

640 **Figure legends**

641 **Figure 1.** CONSORT Flow diagram. Number of participants assessed for eligibility, enrolled  
642 and randomized to study vaccine or placebo. Participants were included in a step-wise fashion  
643 with 16 participants in each dose group. 72 participants were not included (23 in group 1; 31  
644 in group 2; 18 in group 3). Three participants (one vaccinated in group 1, one vaccinated and  
645 one placebo in group 2) were unable to participate in the 12 months visit following the  
646 amendment of the study protocol. In addition, the 12-month immunogenicity data for a fourth  
647 participant (low-dose recipient) were excluded due to vaccination with an acellular pertussis  
648 vaccine shortly after the 6-month follow-up. Group 1 participants were vaccinated between 25  
649 September and 1 October, 2015; Group 2 participants were vaccinated between 12 and 18  
650 November, 2015; Group 3 participants were vaccinated between 28 January and 3 February,  
651 2016.

652  
653 **Figure 2.** Colonization of nasopharyngeal mucosa from day 4 to day 28 after vaccination and  
654 serum IgG and IgA responses on day 28. Culture-positive samples are shown in dark grey and  
655 are listed after time point of first positive sample. Samples with at least a two-fold increase of  
656 serum IgG and IgA from day 0 to day 28 post-vaccination and at least 4 times minimum level  
657 of detection are shown in light grey. M = missing sample; PT = pertussis toxin; Prn =  
658 pertactin; FHA = filamentous hemagglutinin; Fim = fimbriae 2/3.

659  
660 **Figure 3.** Fold change from day 0 of serum antibodies (geometric mean with 95% CI) against  
661 pertussis toxin (PT), pertactin, filamentous hemagglutinin (FHA) and fimbriae 2/3 per time  
662 point. The figure illustrates serum IgG (A) and IgA (C) per dose arm [placebo (blue circle),  
663 low dose (open circle), medium dose (grey circle), high dose (black circle)], respectively.  
664 Shown also is serum IgG (B) and IgA (D) in placebo recipient (●n=12), culture-positives (■

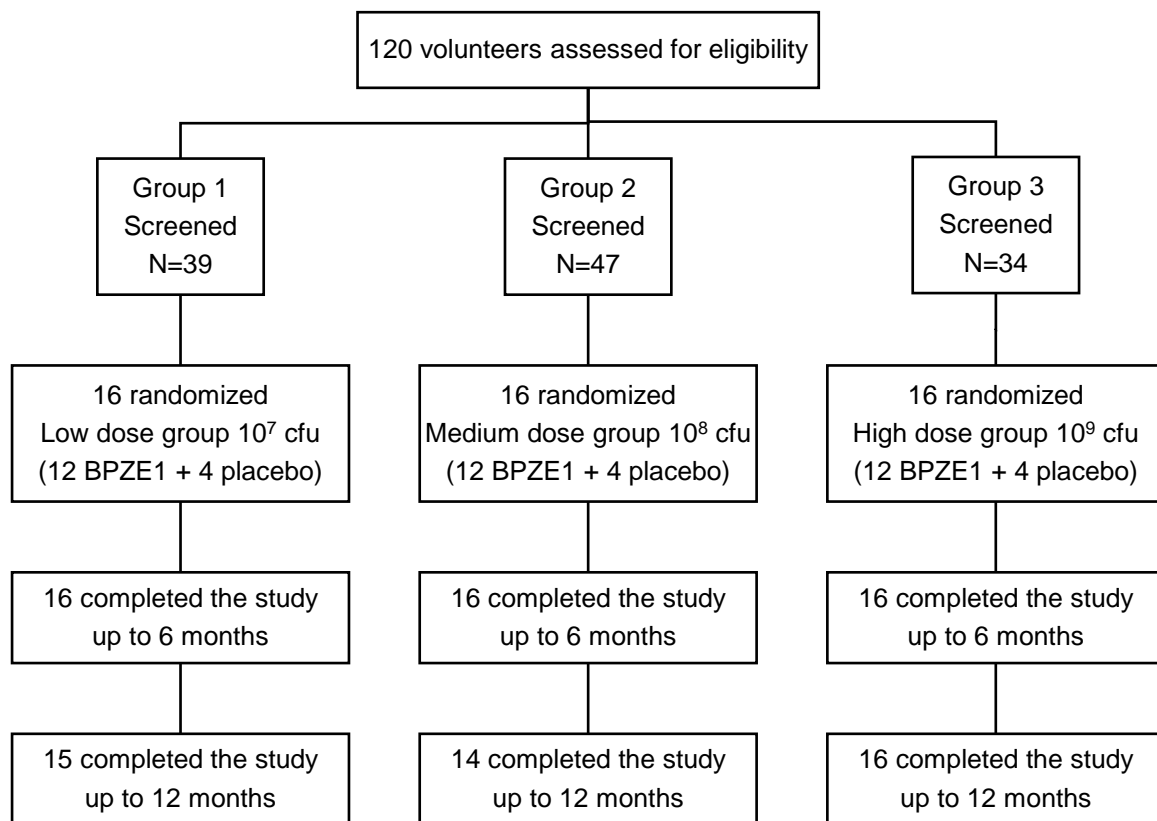
665 n=29) and culture-negatives (▲n=7) among active vaccine recipients from group 1-3. Culture  
666 positivity for a given participant is defined by at least one positive nasopharyngeal sample (at  
667 any time point).

668

669 **Figure 4.** Number of participants per dose group responding according to a pertussis case  
670 definition. A participant was considered to have a positive response when the antibody level  
671 (IgG or IgA) increased with a fold change of two or more between pre- and post-vaccination  
672 samples for either PT alone or for a combination of at least two other antigens (PRN+FHA;  
673 PRN+Fim2/3; FHA+Fim2/3). Responses were investigated up until 6 months post-  
674 vaccination. Twelve participants were included per dose group. Low dose group: in total eight  
675 participants responded, all with both IgG and IgA. Medium-dose group: in total eight  
676 participants responded, four with both IgG and IgA, three with IgG and one with IgA. High-  
677 dose group: all 12 participants responded, five with both IgG and IgA, two with IgG and five  
678 with IgA. No participant in the placebo group responded according to the case definition.

679

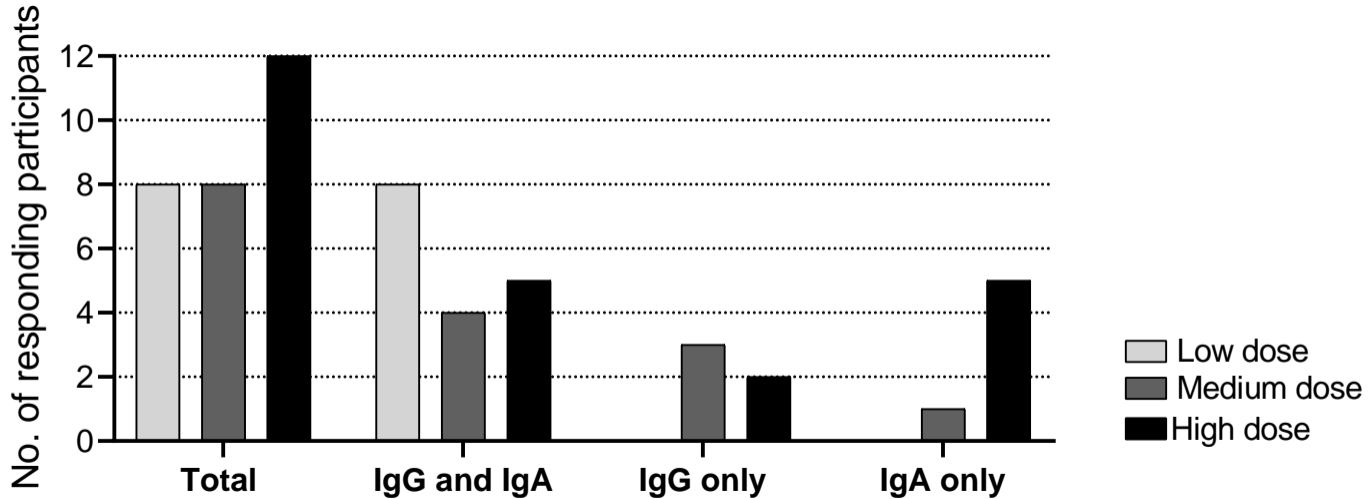
680











**Table 1.**

Baseline data per dose group and placebo.

	Placebo (n = 12)	Low dose (n = 12)	Medium dose (n = 12)	High dose (n = 12)
Females (n)	6	5	2	7
Age (years)	25 (22-27)	26 (25-30)	26 (25-28)	28 (24-30)
Height (cm)	174 (163-185)	177 (172-188)	180 (175-183)	172 (167-177)
Weight (kg)	69 (59-77)	71 (64-84)	74 (63-93)	69 (61-81)
BMI (kg/m <sup>2</sup> )	22 (20-24)	22 (22-25)	22 (20-28)	23 (22-26)
Systolic blood pressure (mmHg)	115 (109-124)	116 (110-130)	121 (117-126)	117 (115-122)
Diastolic blood pressure (mmHg)	72 (69-76)	73 (72-78)	71 (69-74)	73 (69-76)
Heart rate (bpm)	65 (54-74)	70 (59-75)	63 (57-72)	69 (59-79)
Respiratory rate (breath per minute)	15 (13-17)	14 (13-15)	15 (13-17)	15 (14-16)
Oral temperature (°C)	36.5 (36.4-36.9)	36.8 (36.6-37.0)	36.6 (36.3-36.9)	36.6 (36.2-37.0)

Results are presented as frequencies or median with interquartile ranges within parenthesis

**Table 2.**

Primary safety endpoint and its components between D0 and D28 by participant per active dose group and placebo

	Placebo (n = 12)	Low dose (n = 12)	Medium dose (n = 12)	High dose (n = 12)
Number of participants with:				
- Spasmodic cough	0	0	0	0
- cough of grade 2 or higher	1	1	1	2
- other respiratory tract AE related or possibly related to vaccination of grade 3 or higher	0	0	1*	1†
- any other AE related or possibly related to vaccination of grade 3 or higher	0	0	0	0
Composite primary endpoint (at least one of the events above), n	1	1	2	2
% [CI95%]	8.3% [0.2; 38.5]	8.3% [0.2; 38.5]	16.7% [2.1; 48.4]	16.7% [2.1; 48.4]

Results are presented as frequencies and percentages with exact binomial CI 95% within brackets.

\* Oropharyngeal pain

† Rhinorrhea and nasal congestion

AE: adverse events.