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Age-related immunogenicity and reactogenicity of live oral cholera vaccine CVD 103-HgR in a randomized, controlled clinical trial

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ABSTRACT

Aging is accompanied by a decline in immune function which can lead to decreased responses to vaccines. Attenuated recombinant *Vibrio cholerae* O1 vaccine strain CVD 103-HgR elicits a rapid serum vibriocidal antibody (SVA) response and protects against cholera diarrhea in volunteer challenge studies but has not been studied in older adults. We evaluated CVD 103-HgR (PXVX0200) in adults age 46–64, compared them to previously studied adults age 18–45, and studied age-related immunogenicity across adults 18–64 years of age. Volunteers were randomized to receive a single dose of 1×10^9 CFU of PXVX0200 or placebo. Immunogenicity endpoints included SVA and anti-cholera toxin (CT) antibody levels on days 1, 11, 29, 91 and 181 and lipopolysaccharide (LPS) and CT-specific IgA and IgG memory B cells on days 1, 91 and 181. Safety was assessed by comparing solicited signs and symptoms on days 1–8 and other adverse events through day 181. 2979 volunteers received vaccine, including 291 age 45–64. Day 11 seroconversion occurred in 90.4% of older adults vs 93.5%% of younger adults and met the endpoint of demonstrating non-inferiority between the two groups. Significant increases in LPS-specific IgG and IgA and CT-specific memory IgG memory B cells were seen at days 91 and 181. There appeared to be a continuous age-related decline in SVA seroconversion and geometric mean titers, but not memory B cell responses, across the 18–64 year age range. Most reactogenicity was mild and was more common in the placebo group. PXVX0200 appears safe and immunogenic in older adults.

Clinical Trials Registration: clinicaltrials.gov NCT02100631.

1. Background

Aging is accompanied by a decline in the function of the immune system, both humoral and cellular, in what is referred to as immunosenescence, a phenomenon noted as early as the fifth decade of life [1–4]. Studies of vaccines for prevention of hepatitis B, influenza and *Streptococcus pneumoniae* infection have shown both decreased seroconversion rates and lower antibody responses as measured by geometric mean titers (GMT) in the elderly, resulting in decreased protection of older populations against these vaccine-preventable diseases [5–7]. Thus, it is important that new vaccines are shown to be safe and effective in older populations. Limited information is available on oral cholera vaccines in older adults.

Serum vibriocidal antibodies (SVA) produced by natural or experimental *V. cholerae* infection correlate with protection against cholera [8,9]. Experimental infection results in protective immunity against re-challenge with both homologous and heterologous strains that lasts for at least three years [10]. This led to the development of attenuated strains of *V. cholerae* that produce similar immunity. CVD 103-HgR, a live, attenuated, recombinant *V. cholerae* O1 vaccine strain that does not produce active cholera toxin, was acquired by PaxVax, Inc. and given the research name PXVX0200 [11].

Vaccine efficacy of PXVX0200 was previously evaluated in a phase 3, placebo-controlled cholera Challenge Study in volunteers [12]. In the Challenge Study adults age 18–45 were protected against moderate to severe diarrhea, with efficacy of 90% at 10 days and 80% at 3 months following challenge with $1x10^5$ CFU wild type *V. cholerae* O1 El Tor Inaba strain 16961. SVA response, as assessed by seroconversion (≥4-fold increase in SVA) was shown to be a reliable correlate of protection, and vibriocidal antibody response was documented against all main biotypes and serotypes [12,13]. Vaccination with CVD 103-HgR induced antigen-specific memory B cell responses as well as antibodies targeting the *V. cholerae* O-specific polysaccharide and these also highly correlated with protection against cholera [14,15].

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A second placebo-controlled, phase 3 trial, a multi-center Lot Consistency Study in adults age 18–45 years, demonstrated the reproducibility of the production process and further documented the safety and immunogenicity of PXVX0200 in 2688 vaccine recipients [16]. Volunteers were randomized 8:1 to receive PXVX0200 from three production lots or placebo after which SVA and anti-cholera toxin (CT) antibodies, as well as safety parameters, were followed for 181 days. The SVA seroconversion rate 10 days after vaccination with PXVX0200 was 94% versus 4% in placebo recipients.

The Lot Consistency Study was conducted in a restricted (younger) adult population in order to have sufficient uniformity to meet the narrow immune response criteria required to demonstrate consistency of three consecutive lots. Therefore, a separate phase 3 study was performed to evaluate the safety and immunogenicity of PXVX0200 in adults age 46–64. Referred to as the Older Adult Study, this bridging trial evaluated the safety and immunogenicity of PXVX0200 in adults age 46–64, using adults 18–45 years of age from the Lot Consistency Study as comparators. Bridging studies are performed to extrapolate efficacy for vaccines in which a correlate of protection exists [17]. Here we report, for the first time, the safety and immunogenicity of PXVX0200 in adults age 46–64 from the Older Adult Study. We also combine data from three PXVX0200 trials, the Older Adult study, the Lot Consistency Study and the Challenge Study, including previously unpublished memory B cell data from the Lot Consistency Study, in order to evaluate age related immunogenicity across the entire adult population studied, ages 18–64.

2. Methods

2.1. Study design

The primary aim of the Older Adult Study, a randomized, double-blind, placebo-controlled phase 3 trial, was to evaluate SVA seroconversion rates and GMTs against the classical Inaba biotype in adults 46–64 years of age ten days after vaccination with PXVX0200 and to demonstrate that immunogenicity was non-inferior to that in adults age 18–45 from the Lot Consistency Study [16]. The Older Adult Study included healthy men and women age 46–64 without significant medical history, physical examination or abnormal laboratory test results at screening, as previously described in the Lot Consistency Study, from US sites between May 2014 and February 2015 [16].

Eligible participants in the Older Adult Study were randomized 3:1 (in permuted blocks of 8) by an Interactive Web Response System to receive either a single oral dose of PXVX0200 or 100 mL of physiological saline placebo. Subjects, clinical site personnel, investigators, and the sponsor were blinded to participant treatments. The placebo was not matched to the vaccine visually or by taste, therefore subjects were dosed by an unblinded staff member in order to maintain the blinding of staff performing post-vaccination assessments. Trial subjects were instructed not to discuss taste with the study staff.

A subset of participants was selected via a convenience sample from some sites and voluntarily enrolled in an immune sub-study with additional study visits and sample collections through 6 months after vaccination. Study visits, assessments, and safety data collection were the same as in the Lot Consistency Study [16].

2.2. Vaccine

Lyophilized PXVX0200 sachets from one production lot, P700.550-6BA03, were stored and reconstituted as previously described [16]. At reconstitution, the single-dose PXVX0200 solution contained 1×10^9 CFU of vaccine.

2.3. Immunology

Classical Inaba SVA titers were measured at baseline prior to vaccine administration (Day 1) and on Day 11. Day 1 and Day 11 serum samples were also tested for SVA titer against El Tor Inaba, classical Ogawa, and El Tor Ogawa strains to establish cross-strain protection.

To characterize immune response kinetics over time, subjects in the immune sub-study (36 PXVX0200 and 9 placebo recipients) were followed through additional time points at Days 29, 91 and 181. Additional sera collected from these immune sub-study participants were tested for anti-CT antibody levels at Days 1, 11, 29, 91, and 181, and for SVA on Days 29, 91 and 181. All assays were performed as previously described [16]. To characterize the memory B cell response following vaccination, immune sub-study participants were assessed at Days 1, 91 and 181 for the percentage of total IgA and IgG memory B cells specific for O1 lipopolysaccharide (LPS) or CT, performed as previously described [18]. Identical methods were used to assess the memory B cell response in a subset of 26 PXVX0200 and 6 placebo recipients in the Lot Consistency Study.

2.4. Statistical analysis

2.4.1. Immunogenicity

SVA and anti-CT antibody titers were summarized for each treatment group by GMT and 95% CI, and by the SVA and anti-CT seroconversion rates defined as the percentage of subjects who had a $\geq$4-fold increase in titer over Day 1. The 95% CI for the seroconversion rate was calculated by the Clopper-Pearson method [19]. Immune response kinetics over time were characterized by measurement of SVA and anti-CT antibody through 6 months post-vaccination at Days 1, 11, 29, 91 and 181 in the immune sub-study population.

2.4.2. Non-inferiority

The difference between the 46–64 year old and 18–45 year old groups' seroconversion rate was calculated along with its 95% Wald confidence interval (CI) [20]. To meet the first primary endpoint of the Older Adult Study, the lower limit of the CI on the difference had to be greater than −10 percentage points. Assuming the true seroconversion rate among adults aged 46–64 was no more than 3.5% lower than adults aged 18–45 plus a 5% dropout rate, a sample size of 295 vaccinees yielded approximately 90% power to test these non-inferiority criteria. This was rounded up to 300 vaccinees for convenience. Given the 3:1 ratio of vaccine to placebo recipients, the total required sample size was estimated to be 400 adults age 46–64.

To meet the second primary endpoint of the Older Adult Study the lower bound of the CI on the 46–64 year old adults' seroconversion rate could be no less than 70%. With an overall sample size of 300 vaccinees, and assuming a true seroconversion rate of 80% among the older adults, power was estimated to be 97% power to show that the lower 95% confidence bound on seroconversion is greater than 70%. No multiplicity adjustment was required to manage the first and second primary endpoints since both needed to be met to satisfy the overall primary objective.

2.4.3. Additional immunogenicity objectives

Outcomes included SVA seroconversion rates at Day 11 and SVA GMTs at Days 1 and 11 against each of the four biotypes and serotypes. The seroconversion rates in PXVX0200 recipients were compared to the rates in placebo recipients using Fisher's exact

tests while the GMTs from each group were compared using t-tests following log-transformation of the titer data. For the additional exploratory immunogenicity endpoint of characterizing the memory B cell response following vaccination, the percentage of anti-O1 LPS IgA memory B cells out of the total number of IgA memory B cells was summarized at each time point by descriptive statistics and the fold-rise over Day 1. Wilcoxon signed-rank tests were used to assess the within-subject differences between each pair of time points. Analogous methods were applied to anti-O1 LPS IgG, anti-CT IgA and anti-CT IgG memory B cells.

2.4.4. Age and immunogenicity

To assess the relationship between age and vibriocidal immune response, data from subjects age 46–64 in the Older Adult Study were pooled with data from the subjects age 18–45 in the Lot Consistency Study to create a large database of 2979 vaccinees spanning ages 18–64. The full age range was partitioned into 7 non-overlapping age categories (18–23, 24–30, 31–37, 38–45, 46–52, 53–59 and 60–64 years old). The first 6 categories span 7 years apiece while the oldest age category spans 5 years. The span of the categories was chosen to be narrow enough to detect age-related changes, to have the same length across almost the entire age range, and to ensure that no subjects from the Lot Consistency Study and Older Adult Study overlapped in the same category. Day 11 SVA seroconversion rates and GMTs were tabulated and analyzed for the PXVX0200 recipients in each age category to facilitate detection of age-related trends.

To explore the relationship between age and the induction of memory B cells across a wide age range, data for immune substudy PXVX0200 recipients from the Older Adult Study (N = 34), Lot Consistency Study (N = 23) and Challenge Study (N = 22) were combined into a single dataset [12,16]. Memory B cell studies in all three trials were performed at the same time points and by the same methods as described above. The fold-rise between Days 1 and 181 in each type of memory B cell was plotted against age. Spearman rank correlation and LOESS regression were used to characterize the relationship between age and fold-rise [21].

2.4.5. Reactogenicity and safety

Solicited reactogenicity was summarized in the adults age 46–64 in the Older Adult Study by the frequency in each treatment group of each of 7 solicited signs or symptoms of reactogenicity as previously described [16]. CIs for each frequency were calculated using the Clopper-Pearson method, and treatment groups were compared using Fisher's exact tests. Unsolicited treatment-emergent adverse events (AEs) and serious adverse events (SAEs) were summarized by System Organ Class (SOC) and Preferred Term (PT), by count and percent for PXVX0200 and placebo groups [16].

3. Results

3.1. Demographics

The Older Adult Study enrolled 398 adults ages 46–64, of whom 299 received PXVX0200 and 99 received a physiological saline placebo (Fig. 1). Participant demographics of the PXVX0200 and placebo recipients were similar and are described in Table 1. Participant characteristics of the PXVX0200 recipients in the Lot Consistency Study (age18-45) and the Older Adult Study (age 46–64) are presented in Supplemental Table 1.

3.2. Non-inferiority of older adults to younger adults

Day 11 classical Inaba serum vibriocidal antibody seroconversion rates (95% CI) for the 46–64 and 18–45 year old vaccine groups were 90.4% (86.4–93.5%) and 93.5% (92.5–94.4%), respectively (Table 2). Both co-primary endpoints were met. The lower bound of the two-sided 95% CI on the difference between the 46–64 and 18–45 year old seroconversion rates was −6.7 percentage points, and the lower bound of the two-sided 95% CI on 46–64 year old vibriocidal antibody seroconversion was 86.5%. Similar results were derived from a sensitivity analysis that used logistic regression to estimate the difference between older and younger adults while adjusting for the effects of sex, blood type, and baseline titer.

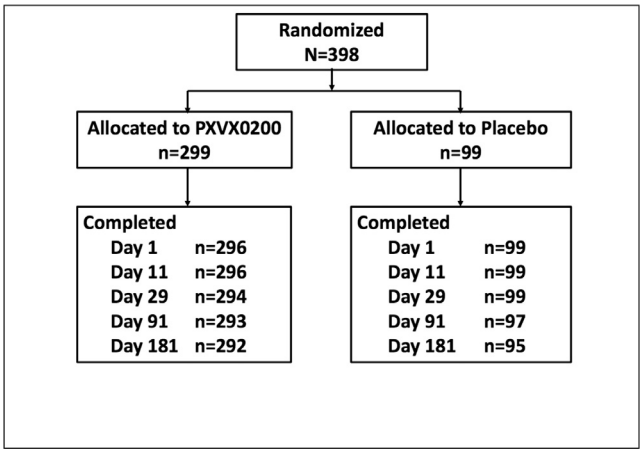

Fig. 1. Subject Disposition. CONSORT diagram for immunologic bridging study of adults ages 46–64. Seven subjects (2.3%) randomized to PXVX0200 discontinued the trial early; 3 were lost to follow-up, 1 withdrew consent and 3 discontinued for other reasons. Four subjects (4.0%) of subjects randomized to placebo were lost to follow up before trial completion.

Table 1
Older Adult Study Demographics.

Characteristic	PXVX0200 N = 299	Placebo N = 99	Total N = 398
Age in years			
Mean (SD)	53.8 (4.99)	54.1 (5.20)	53.8 (5.04)
Median (min, max)	54.0 (46, 64)	53.0 (46, 64)	53.5 (46, 64)
Sex, n (%)			
Male	135 (45.2%)	47 (47.5%)	182 (45.7%)
Female	164 (54.8%)	52 (52.5%)	216 (54.3%)
Race, n (%)			
White	221 (73.9%)	77 (77.8%)	298 (74.9%)
Black or African American	68 (22.7%)	19 (19.2%)	87 (21.9%)
American Indian or Alaskan Native	6 (2.0%)	1 (1.0%)	7 (1.8%)
Asian	0	2 (2.0%)	2 (0.5%)
Native Hawaiian or Other Pacific Islander	1 (0.3%)	0	1 (0.3%)
Multiracial	2 (0.7%)	0	2 (0.5%)
Other	1 (0.3%)	0	1 (0.3%)
Ethnicity, n (%)			
Hispanic or Latino	24 (8.0%)	6 (6.1%)	30 (7.5%)
Not Hispanic or Latino	274 (91.6%)	93 (93.9%)	367 (92.2%)
ABO Blood Type, n (%)			
Type O	111 (37.1%)	47 (47.5%)	158 (39.7%)
Not Type O	188 (62.9%)	52 (52.5%)	240 (60.3%)

Table 2
Primary Bridging Objective: Day 11 Classical Inaba Vibriocidal Antibody Seroconversion Non-Inferiority Analysis.

Statistic	PXVX0200 Recipients		Placebo Recipients	
	Older Adults (N = 291)	Younger Adults (N = 2688)	Older Adults (N = 99)	Younger Adults (N = 334)
N analyzable[a]	291	2687	99	334
N (%) seroconverted	263 (90.4%)	2513 (93.5%)	0	14 (4.2%)
95% CI on % seroconverted[b]	(86.4%, 93.5%)	(92.5%, 94.4%)	(0.0%, 3.7%)	(2.3%, 6.9%)
% Difference (younger-older)	−3.1%			
95% CI on % difference[c]	(−6.7%, 0.4%)			

[a] N analyzable was the number of subjects with any analyzable samples available at both Day 1 and Day 11. One subject from the younger adult study was missing a Day 1 vibriocidal antibody titer result.
[b] 95% CIs of seroconversion rate were based on the Clopper-Pearson method.
[c] The CI on the difference in seroconversion rates was calculated using the Wald method.

Table 3
Day 11 SVA Seroconversion and GMT by Age in PXVX0200 Recipients.

Ages	Lot Consistency Study				Older Adult Study		
	18–24	25–31	32–38	38–45	46–52	53–59	60–64
N analyzed	828	775	574	510	135	109	47
Seroconversion rate, % (95% CI)	95 (93–96)	93 (91–95)	94 (91–95)	92 (90–95)	92 (86–96)	90 (83–95)	87 (74–95)
Antibody Titer, GMT (95% CI)	12,519 (11,128–14083)	10,315 (9129–11,651)	8543 (7448–9800)	6692 (5706–7849)	4597 (3316–6738)	4231 (3005–5956)	3594 (1690–7640)

Note: Data are from PXVX0200 recipients only in order to examine the relationship between age and reponse to vaccination.

Table 4
Day 11 Cross-Strain Vibriocidal Antibody Seroconversion and Titers – Older Adults.

Cholera Strain	Seroconversion % (95% CI)*		GMT (95% CI)**	
	PXVX0200 N = 291	Placebo N = 99	PXVX0200 N = 291	Placebo N = 99
Classical Inaba	90.4 (86.4–93.5)	0 (0.0–3.7%)	4282 (3344–5484)	44 (36–56)
El Tor Inaba	91.0 (87.1–94.1)	5.1 (1.7–11.4)	4929 (3912–6209)	54 (39–76)
Classical Ogawa	73.2 (67.7–78.2)	2.0 (0.2–7.1)	1235 (944–1617)	53 (39–72)
El Tor Ogawa	71.4 (65.8–76.5)	6.1 (2.3–12.7)	1120 (863–1453)	50 (37–69)

* All p-values < 0.0001, calculated using Fisher's exact test comparing number of vaccine recipients with a 4-fold rise with placebo recipients.
** All p-values < 0.0001, calculated using a *t*-test comparing log-transformed titers from vaccine recipients to placebo recipients.

3.3. Immunogenicity

Day 11 SVA seroconversion rates and GMTs for PVXV0200 recipients in the different age groups are shown in Table 3. When evaluated across the Older Adult Study and Lot Consistency Study, immunogenicity decreased continuously in older versus younger adults without a strong breakpoint. Seroconversion was documented in 95% of 18–24 year-olds versus 90% in 53–59 year-olds and 87% in the relatively small (N = 47) 60–64 year-old age group. Peak GMTs of 12,519 were seen in the youngest age group and fell continuously with advancing age.

3.4. Cross-strain protection

Seroconversion by vibriocidal antibody for the four serotypes/biotypes of *V. cholerae* as well as GMTs in adults age 46–64 in the Older Adult Study are shown in Table 4. By Day 11, 90.4% of vaccine recipients had seroconverted against classical Inaba, 91.0% against El Tor Inaba, 73.2% against classical Ogawa and 71.4% against El Tor Ogawa.

3.5. Immune response kinetics

In the immune sub-study of the Older Adult Study, serum vibriocidal antibody seroconversion occurred among 35 of 36 (97.2%) PXVX0200 recipients by Day 11, with 100% seroconversion by Day 29. None of the 9 placebo recipients in the immune sub-study seroconverted at any visit through Day 181. SVA GMTs of PXVX0200 recipients were significantly higher than the respective GMT of the placebo recipients at each follow up visit (Table 5). Fig. 2 compares the SVA GMTs over time of vaccine recipients age 46–64 in the immune sub-study of the Older Adult Study with vaccine recipients age 18–45 from the immune sub-study in the Lot Consistency Study. Anti-CT antibody seroconversion rates and GMTs for vaccine recipients are presented in Table 5. These were significantly higher than those of placebo recipients at each follow-up visit.

3.6. Memory B cells

In adults age 46–64 in the Older Adult Study, significant increases in the percentage of anti-O1 LPS IgA and IgG memory B cells and anti-CT IgG, but not anti-CT IgA, memory B cells were seen at days 91 and 181 (Table 6). Fig. 3 depicts the fold-rise from Days 1 to 181 in memory B cells as a function of age after combining data for vaccinees from the sub-study populations in the Challenge Study, Lot Consistency Study and Older Adult Study [12,16]. There was no relationship between age and memory B cell response with the strongest correlation among the four memory B cell types reaching only r = 0.173 (Fig. 3).

3.7. Reactogenicity and safety

In adults age 46–64 in the Older Adult Study, reactogenicity signs and symptoms after vaccine administration were reported by 36.3% (95% CI: 30.8–42.0%) of vaccine recipients and 50.5%

Table 5
Immune Sub-Study SVA and Anti-CT through Day 181 – Older Adults.

	SVA GMT (95% CI)				
	Day 1	Day 11	Day 29	Day 91	Day 181
PXVX0200	39	4740	2412	177	124
	(29, 52)	(2541–8845)	(1410–4126)	(114–273)	(85–179)
Placebo	54	54	54	37	37
	(16, 190)	(17–174)	(16–190)	(16–84)	(16–84)
p value*	0.5741	<0.0001	<0.0001	0.0015	0.0045
Anti-CT Seroconversion % (95% CI)					
	Day 1	Day 11	Day 29	Day 91	Day 181
PXVX0200	–	55.6%	68.6%	68.6%	57.1%
		(38.1%-72.1%)	(50.7%-83.1%)	(50.7%-83.1%)	(39.4%-73.7%)
Placebo	–	0	0	11.1%	0
		(0.0%–33.6%)	(0.0%, 33.6%)	(0.3%-48.2%)	(0.0%, 33.6%)
p value*	–	0.0025	0.0002	0.0028	0.0021
Anti-CT GMT (95% CI)					
	Day 1	Day 11	Day 29	Day 91	Day 181
PXVX0200	514	2540	4140	2870	2049
	(422, 626)	(1485–4345)	(2512–6833)	(1985–4148)	(1380–3044)
Placebo	432	467	432	485	432
	(362, 516)	(327–666)	(362–516)	(340–692)	(362–516)
p value*	0.1715	<0.0001	<0.0001	<0.0001	<0.0001

Note: The Immune Sub-study consisted of 36 PXVX0200 recipients and 9 placebo recipients in the Older Adult Study.
* PXVX0200 versus placebo.

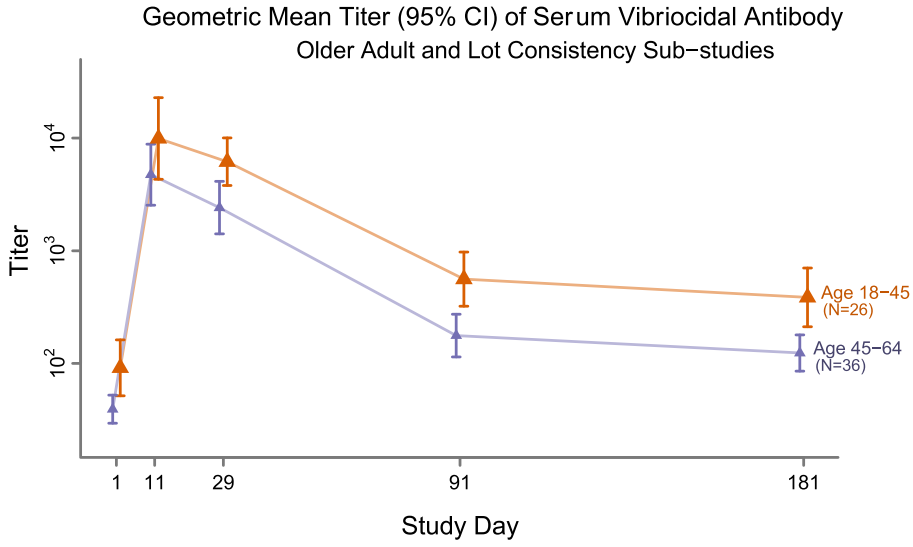

Fig. 2. SVA GMT (95% CI) by Age Through Day 181. The symbols mark the SVA GMT of the subjects in the sub-study of the Older Adult Study (age 46–64) or the sub-study of the Lot Consistency Study (age 18–45) at the indicated time point. The vertical bars delineate the extent of the 95% CI for each GMT.

(40.3–60.7%) of placebo recipients (p = 0.0174) (Table 7). The most commonly reported symptoms were headache, tiredness, and abdominal pain and most reactogenicity was mild and resolved within 1–2 days. Unsolicited AEs were reported by 20.6% of vaccine recipients and 27.3% of placebo patients, with no clinically meaningful differences in the frequency and severity of AEs between the two groups, and there were no study related SAEs.

4. Discussion

Cholera is a potentially lethal infection which occurs worldwide and represents a risk to travelers from industrialized countries, particularly those with underlying medical conditions. To protect against cholera, an ideal vaccine for travelers must have a rapid onset of action and be convenient, safe and effective in all age groups, including older adults, a group known to respond poorly to some immunizations [1–3,11,22,23].

Immunosenescence, the phenomenon of decreased immune response with age, has been noted in humans beginning in middle age [1–4]. It is associated with the increased severity of common infections such as influenza, the increased prevalence of cancer and the decreased response to influenza, hepatitis B and *S. pneumoniae* vaccinations seen with aging [1,5,6,23–31]. Both innate and adaptive immune responses are affected. Higher cholera case fatality rates have been observed in older adults beginning as early as the forties [32,33]. For example, in a cholera outbreak in Nigeria, Dalhat observed age-specific case fatality rates of 14.5% in individuals 65 years of age and older and 9.5% in those 45–64 years of age, versus 4–5% in those 5–44 years of age [32]. However, the relative impacts of age related decreased immune response versus age related increased vulnerability to the physiological effects of cholera infection would be difficult to sort out.

The Older Adult Study reported here documents the safety and immunogenicity of single dose, live oral cholera vaccine PXVX0200

Table 6
Memory B Cells: Summary Statistics and Fold-rise over Day 1 – Older Adults.

Part A: IgA and IgG Memory B Cells Specific for O1 Lipopolysaccharide (LPS)				
Study Day	Anti-O1 LPS IgA		Anti-O1 LPS IgG	
Statistics*	PXVX0200	Placebo	PXVX0200	Placebo
Day 1				
N	34	7	34	8
Mean	0.16%	0.15%	0.00%	0.01%
Median	0.12%	0.14%	0.00%	0.00%
95% CI for Median	(0.06%, 0.16%)	(0.05%, 0.32%)	(0.00%, 0.00%)	(0.00%, 0.02%)
Day 91				
N	34	6	34	7
Mean	0.29%	0.17%	0.01%	0.00%
Median	0.18%	0.16%	0.00%	0.00%
95% CI for Median	(0.11%, 0.34%)	(0.01%, 0.41%)	(0.00%, 0.01%)	(0.00%, 0.01%)
Fold-rise (95% CI)†	2.0 (1.0, 4.2)	1.2 (0.1, 4.5)	1.4 (1.0, 2.3)	0.9 (0.2, 16.0)
Fold-rise p-value	0.0163	0.5625	0.0487	1
Day 181				
N	34	5	34	6
Mean	0.28%	0.05%	0.01%	0.01%
Median	0.25%	0.04%	0.01%	0.00%
95% CI for Median	(0.18%, 0.32%)	(0.01%, 0.10%)	(0.00%, 0.01%)	(0.00%, 0.02%)
Fold-rise (95% CI)†	2.0 (1.5, 4.1)	0.4 (0.1, 2.0)	2.1 (1.6, 2.8)	1.0 (0.9, 38.8)
Fold-rise p-value	0.0009	0.1875	0.0003	0.4375

Part B: IgA and IgG Memory B Cells Specific for Cholera Toxin (CT)				
Study Day	Anti-CT IgA		Anti-CT IgG	
Statistics*	PXVX0200	Placebo	PXVX0200	Placebo
Day 1				
N	34	7	34	8
Mean	0.46%	0.33%	0.04%	0.02%
Median	0.30%	0.25%	0.04%	0.02%
95% CI for Median	(0.28%, 0.49%)	(0.00%, 1.06%)	(0.02%, 0.06%)	(0.01%, 0.05%)
Day 91				
N	34	6	34	7
Mean	0.51%	0.32%	0.10%	0.04%
Median	0.43%	0.31%	0.07%	0.03%
95% CI for Median	(0.35%, 0.56%)	(0.11%, 0.61%)	(0.05%, 0.10%)	(0.01%, 0.11%)
Fold-rise (95% CI)†	1.2 (1.0, 1.6)	1.6 (0.8, 155)	2.3 (1.9, 3.0)	1.3 (0.8, 3.1)
Fold-rise p-value	0.1668	0.3125	<0.0001	0.1563
Day 181				
N	34	5	34	6
Mean	0.55%	0.13%	0.10%	0.06%
Median	0.42%	0.10%	0.07%	0.02%
95% CI for Median	(0.33%, 0.59%)	(0.06%, 0.27%)	(0.05%, 0.08%)	(0.00%, 0.17%)
Fold-rise (95% CI)†	1.2 (0.9, 1.7)	0.9 (0.2, 67.1)	1.9 (1.3, 3.6)	1.3 (0.5, 3.3)
Fold-rise p-value	0.1615	0.6250	<0.0001	0.4375

* Of the 36 PXVX0200 recipients enrolled in the Immune Sub-study, statistics were calculated from the 34 who had memory B cell data available at Day 1, 91 and 181. Of the 9 placebo recipients in the Immune Sub-study, data were available from between 5 and 8 subjects depending on the time point and type of memory B cell.
† Fold-rise is the ratio of the memory B cell percentage from the indicated day to the percentage at Day 1.

in adults age 46–64. Since *V. cholerae* challenge studies are not feasible in an older adult population, we undertook an immunologic bridging study with SVA seroconversion as a surrogate marker of protection. The first primary objective of demonstrating that classical Inaba vibriocidal antibody seroconversion on Day 11 in adults age 46–64 was non-inferior to seroconversion in adults age 18–45 was met. Classical Inaba vibriocidal seroconversion at Day 11 was selected as a primary immunogenicity endpoint in the Older Adult Study since it was shown to be the best serological correlate of protection in the Challenge Study [12,34]. Seroconversion, defined as a ≥4-fold rise in vibriocidal antibody against homologous classical Inaba, occurred in 90.4% of vaccine recipients age 46–64 ten days after vaccination (Day 11) versus 93.5% of vaccine recipients age 18–45 , suggesting that protection against *V. cholerae* infection should be similar in the two age groups.

Continuous variables afford a relatively sensitive assessment of the magnitude of age-related decreases in antibody response. While a comparison of SVA seroconversion rates in older adults age 46–64 with younger adults age 18–45 demonstrated non-inferiority, our evaluation of age related immunogenicity on a continuous basis across the entire adult population of almost 3000 subjects from the Older Adult Study and Lot Consistency Study revealed a trend towards decreased seroconversion with increasing age. More notable was the evaluation of GMT by age, which demonstrated an almost linear association between advancing age and decreasing GMTs. Day 11 titers in the 60–64 year old age group were 4 times lower than those in the 18–24 year old age group, demonstrating that younger adults generate a more robust immune response to vaccination. Similar continuous declines in seroconversion and GMT with age have been noted with hepatitis B, pneumococcal polysaccharide and influenza immunization [7,24,28,35]. The immune sub-studies demonstrated that GMT response in the 18–45 and 46–64 year-old age groups followed a similar trajectory over 181 days but was lower in the older subjects. Although GMTs were noted to be lower in older adults, a correlate-of-protection analysis performed using data from the Challenge Study demonstrated that vibriocidal seroconversion had a nearly one-to-one correlation with the categorical outcome of protection against moderate-to-severe diarrhea and was a better correlate than GMT [12,34]. Based on this correlate-of-protection analysis and the non-inferiority of seroconversion, it is expected that, despite lower GMTs, the efficacy of PXVX0200 in an older

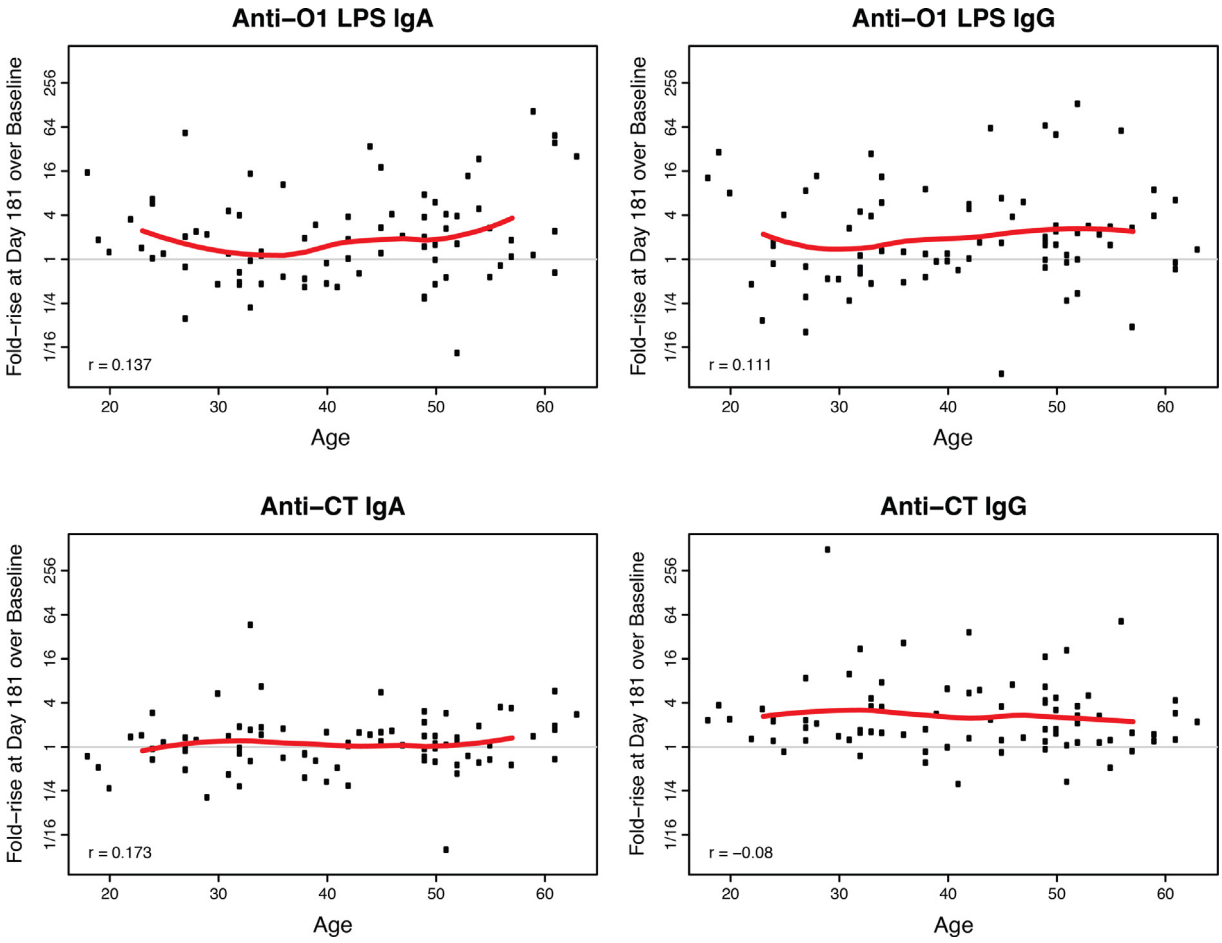

Fig. 3. Memory B cell Responses by Age Through Day 181. Data from PXVX0200 recipients in the Challenge Study (N = 22), Lot Consistency Study (N = 23) and Older Adult Study (N = 34) Combined. Each dot represents the fold-rise between Day 181 and baseline in the percentage of antigen-specific memory B cells of total memory B cells. Data are plotted on a $\log_2$ scale where dots above the horizontal line at $y = 1$ represent subjects with an increase in memory B cells while dots below the line represent a decrease. The red, flexible curve is a nonparametric regression line fit by the LOESS method; r is the Spearman rank correlation coefficient. Data are from PXVX0200 recipients in the sub-studies of the Challenge Study (N = 22), Lot Consistency Study (N = 23) and Older Adult Study (N = 34) combined. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

adult population would be similar to the efficacy in younger adults. Additionally, as the morbidity and mortality of cholera in older adults is greater than in younger adults, the benefit-risk of PXVX0200 in older adults is favorable.

Age-related changes in the GI tract include delayed gastric emptying, increased gastric pH, reduced GI blood flow and, possibly, slowed transit time and bacterial overgrowth [36,37]. While the lower SVA GMTs with age seen in these studies may reflect immunosenescence, since vaccine shedding studies were not performed we cannot rule out the possibility that they may be the result of decreased vaccine replication in the small intestine.

In adults age 46–64 years, a robust vibriocidal antibody response was seen against all four types of *V. cholerae* tested, which comprise the majority of the *V. cholerae* O1 serogroup. Previously conducted challenge studies of subjects vaccinated with 10^8 CFU/-dose CVD 103 (a precursor strain to the CVD 103-HgR strain used in PXVX0200) demonstrated 87% vaccine efficacy against challenge with 10^6 CFU of classical Inaba, 82% efficacy against challenge with 10^6 CFU of classical Ogawa, and 67% efficacy against challenge with 10^6 CFU of El Tor Inaba while 10^8 CFU CVD 103-HgR demonstrated 62% vaccine efficacy against challenge with a high challenge dose of 10^6 CFU of El Tor Inaba [38]. The PXVX0200 clinical development program only used the El Tor Inaba strain in the Challenge Study, therefore effectiveness against the other three strains can only be inferred. Nonetheless, vibriocidal antibody seroconversion of 90.4%, 73.2% and 71.4% for the classical Inaba, classical Ogawa, and El Tor Ogawa strains, respectively, in subjects age 46–64 years in the Older Adult Study, versus 90.3%, 87.1% and 88.2%, respectively, in adults age 18–45 years in the Challenge Study, suggests that substantial protection against these strains in older adults may be expected [12].

In the immune sub-study of the Older Adult Study, subjects age 46–64 years' anti-CT GMT peaked on day 29 and remained significantly above placebo throughout the study, with results similar to those seen in the PXVX0200 phase I study and in the Lot Consistency Study in subjects age 18–45 years [16,39]. While anti-CT antibodies may add to protection, SVA is most strongly associated with protection against cholera [12,38]. Vaccination with PXVX0200 also resulted in significant LPS-specific IgA, LPS-specific IgG and CT-specific IgG memory-B cell responses which lasted 6 months and were not affected by age. LPS-specific memory B cells have been shown to protect against cholera in both endemic and experimental challenge settings [14,40]. While serum vibriocidal antibodies decline substantially by 6 months after infection or vaccination, memory B cells remain significantly elevated for at least one year after cholera infection and may mediate long-term protective immunity against cholera [40–42]. While not tested in this study, vaccination with PXVX0200 also results in *V. cholerae* anti-O-specific polysaccharide antibodies which protect against cholera after experimental challenge [15]. Thus, there are multiple measures of immunity against cholera following vaccination, similar to those seen with wild-type infection.

Table 7
Reactogenicity Signs and Symptoms by Highest Reported Severity, Adults Age 46–64.

Sign or Symptom	Severity	PXVX0200 N = 296		Placebo N = 99		P-value*
		n	%	n	%	
Any Reactogenicity	Any	107	36.3%	50	50.5%	0.0174
Tiredness	Any	59	20.0%	36	36.4%	0.0017
	Mild	37	12.5%	20	20.2%	
	Moderate	20	6.8%	16	16.2%	
	Severe	2	0.7%	0	0	
Headache	Any	60	20.3%	30	30.3%	0.0522
	Mild	41	13.9%	19	19.2%	
	Moderate	17	5.8%	11	11.1%	
	Severe	2	0.7%	0	0	
Abdominal Pain	Any	42	14.2%	13	13.1%	0.8679
	Mild	34	11.5%	10	10.1%	
	Moderate	7	2.4%	3	3.0%	
	Severe	1	0.3%	0	0	
Nausea/Vomiting	Any	35	11.9%	12	12.1%	1.0000
	Mild	27	9.3%	9	9.1%	
	Moderate	7	2.4%	3	3.0%	
	Severe	1	0.3%	0	0	
Lack of Appetite	Any	24	8.1%	12	12.1%	0.2320
	Mild	14	4.7%	11	11.1%	
	Moderate	8	2.7%	1	1.0%	
	Severe	2	0.7%	0	0	
Diarrhea	Any	7	2.4%	2	2.0%	1.0000
	Mild	4	1.4%	1	1.0%	
	Moderate	2	0.7%	0	0	
	Severe	1	0.3%	1	0.3%	
Fever	Any	2	0.7%	0	0	1.0000
	Mild	2	0.7%	0	0	
	Moderate	0	0	0	0	
	Severe	0	0	0	0	

* P-value is from a Fisher's exact test comparing the PXVX0200 recipients to the placebo recipients on the frequency of the corresponding sign or symptom of reactogenicity.

The Older Adult Study demonstrates the safety of PXVX0200 in adults age 46–64. Reactogenicity signs and symptoms after vaccine administration were reported by over a third (36.3%) of vaccine recipients and half (50.5%) of placebo recipients (p = 0.0174). Most reactogenicity signs and symptoms were mild and lasted 1 or 2 days. Reactogenicity signs and symptoms after PXVX0200 administration were reported by 51.9% of vaccine recipients age 18–45 years in the Lot Consistency Study [16]. It is not clear why reactogenicity, in particular tiredness and, possibly, headache were more common in placebo recipients in adults age 46–64 but this may reflect the poor palatability of physiological saline. Diarrhea is the most important adverse effect with live cholera vaccine and was reported with equal frequency by vaccine and placebo recipients in the 46–64 year old age group. This contrasts with the results of the Lot Consistency Study in adults age 18–45 years of age where diarrhea was seen more frequently with vaccine [16].

Based on the Challenge Study, the Lot Consistency Study and the Older Adult Study, PXVX0200 was approved for use in adults 18–64 in the US in 2016 under the trade name Vaxchora®.

5. Conclusion

The live, oral cholera vaccine PXVX0200 is safe in adults 46–64 years of age and the SVA seroconversion rate following immunization of older adults was non-inferior to that seen in adults age 18–45 (90.4% vs 93.5%). A more detailed analysis by age demonstrated a relatively continuous age related decline in SVA seroconversion rates and GMTs, but not specific memory B cells, over the 18–64 year age range. Since SVA seroconversion, the correlate of protection against cholera disease, remains high enough to be non-inferior in adults age 46–64, it is expected that PXVX0200 will provide protection against cholera in older adults from developed countries who are at increased risk of infection when traveling or residing in at-risk countries.

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JM and KH participated in data analysis and report writing.

ML, MG, JS and SB participated in study development, study conduct, data analysis and report writing.

JM, KH and ML are consultants for PaxVax, Inc.

SB is employed by PaxVax, Inc.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.01.077.

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