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Reviewer Name(s)	Tina Khoie Mongeau, MD, MPH
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Supervisory Concurrence	Jeffrey Roberts, MD, Branch Chief, CRB1
	Roshan Ramanathan, MD, MPH, Team Leader, CRB1
Applicant	Pax Vax Bermuda Ltd.
Established Name	Cholera Vaccine, Live, Oral
(Proposed) Trade Name	Vaxchora
Pharmacologic Class	Vaccine
Formulation(s), including Adjuvants, etc	Suspension for oral use supplied as a packet of the buffer component and a packet of the active component (lyophilized 4×10^8 to 2×10^9 CFU live attenuated <i>Vibrio cholerae</i> CVD 103-HgR). After preparation, a single dose of Vaxchora is 100 mL. Other ingredients in the active component packet include sucrose (≤ 165.37 mg), sodium chloride (≤ 17.11 mg), Hy-Case SF (hydrolyzed casein) (≤ 17.11 mg), ascorbic acid (≤ 8.55 mg), and dried lactose (≤ 2.09 g). The buffer packet includes sodium bicarbonate (2.16-2.41 g), sodium carbonate (0.24-0.49 g), ascorbic acid (1.50-1.80 g), and dried lactose (0.18-0.22 g).
Dosage Form(s) and Route(s) of Administration	Vaxchora is prepared by reconstituting the buffer component in 100 mL of purified bottled water and adding the active component (lyophilized <i>V. cholerae</i> CVD 103-HgR) for oral administration.
Dosing Regimen	Single dose
Indication(s) and Intended Population(s)	For active immunization against disease caused by <i>V. cholerae</i> serogroup O1. VAXCHORA is approved for use in adults 18 through 64 years of age traveling to cholera-affected areas.
Orphan Designated (Yes/No)	No

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GLOSSARY

AE	Adverse Event
AESI	Adverse Event of Special Interest
BLA	Biologics License Application
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CRF	Case Report Form
CVD	Center for Vaccine Development
CFU	Colony Forming Unit
CI	Confidence Interval
CT	Cholera Toxin
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ETEC	Enterotoxigenic <i>Escherichia coli</i>
ER	Emergency Room
FDA	Food and Drug Administration
FD&C	Food, Drug & Cosmetic
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
IgA	Immunoglobulin alpha
IgG	Immunoglobulin gamma
IND	Investigational New Drug
ITT	Intent-to-treat
LLOQ	Lower Limit of Quantification
LPS	Lipopolysaccharide
LTFU	Lost to Follow-Up
NaHCO ₃	Sodium Bicarbonate
NIH	National Institutes of Health
NSAID	Non-Steroidal Anti-Inflammatory Drugs
OR	Odds Ratio
PBMC	Peripheral Blood Mononuclear Cells
PeRC	Pediatric Review Committee
PLA	Product License Application
PMC	Postmarketing Commitment
PMR	Postmarketing Requirement
PREA	Pediatric Research Equity Act
PT	Preferred Term
PXVX0200	Investigational Live, Oral Cholera Vaccine, Strain CVD 103-HgR
REMS	Risk Evaluation and Mitigation Strategy
SAE	Serious Adverse Event
SOC	System Organ Class
SSVI	Swiss Serum and Vaccine Institute
U.S.	United States
UMB	University of Maryland, Baltimore
VRBPAC	Vaccines and Related Biologics Products Advisory Committee
WHO	World Health Organization

1. Executive Summary

Pax Vax Bermuda Ltd. has submitted a Biologics License Application (BLA) for their Cholera Vaccine, Live, Oral under the proposed proprietary name Vaxchora. The proposed indication is active immunization against disease caused by *Vibrio cholerae* serogroup O1, including El Tor Inaba, classical Inaba, El Tor Ogawa, and classical Ogawa subtypes. The proposed use is in adults 18 through 64 years of age traveling to cholera-affected areas. Vaxchora is a suspension for oral administration. It is supplied as a foil packet of the buffer component and an accompanying foil packet of the active component (lyophilized *V. cholerae* CVD 103-HgR). Vaxchora is prepared by reconstituting the buffer component in 100 mL of purified bottled water and then reconstituting the active component in the buffer solution. The proposed dosage regimen is a single dose for oral administration at least 10 days before potential exposure to cholera. Priority review was granted for this BLA, because Vaxchora targets a serious condition (cholera) and, if approved, Vaxchora would be a significant improvement in the safety and effectiveness of cholera prevention strategies recommended for United States (U.S.) adults who are traveling to cholera-affected areas.

Cholera is caused by toxin producing strains of *V. cholerae* serogroups O1 and O139. It is characterized as an acute, painless watery diarrhea which can be voluminous and lead to severe dehydration if adequate rehydration and electrolyte replacement are not initiated promptly. Serogroup O1 has 2 biotypes (classical and El Tor), and each biotype has 2 major serotypes (Inaba and Ogawa). *V. cholerae* O1 El Tor is the predominant cause of cholera globally. Although most cases are unreported, the World Health Organization (WHO) estimates that there are 1.4 to 4.3 million cases of cholera and 28,000 to 142,000 deaths per year worldwide due to cholera. Cholera primarily occurs in resource limited settings where minimal requirements of clean water and sanitation are not met. It is not endemic in the United States, however persons traveling to cholera endemic or epidemic areas may be at risk. Travelers at highest risk are those who are visiting remote areas where access to safe food and water and access to medical care is likely to be limited. Risk is also higher among persons with underlying medical conditions that predispose them to increased morbidity due to mild or worse diarrhea.

Vaxchora is a live, recombinant attenuated bacterial vaccine. The CVD 103-HgR vaccine strain is characterized by two prominent genetic modifications to the wild-type *V. cholerae* O1 classical Inaba strain 569B. Ninety-four percent of the gene encoding the enzymatically active A subunit of cholera toxin was deleted, thus removing the toxigenicity of the vaccine strain. The vaccine strain retains the ability to synthesize the non-toxic B subunit of cholera toxin. The second prominent genetic modification was insertion of a *S. flexneri* mercury resistance operon into a hemolysin gene locus, enabling differentiation of the vaccine strain from wild type.

PaxVax submitted data from 4 clinical studies to this BLA. One phase 3 clinical study used a human cholera challenge model to demonstrate the efficacy of a single dose of Vaxchora in preventing moderate to severe diarrhea in 18 through 45 year old adults (PXVX-VC-200-003). Another two phase 3 clinical immunogenicity studies supported manufacturing consistency (PXVX-VC-200-004) and effectiveness of a single dose of Vaxchora in adults 46 through 64 years of age (PXVX-VC-200-005). One phase 1 study provided shedding and transmission data (PXVX-VC-200-002). Safety data from the four clinical studies contributed to the Vaxchora safety database.

This BLA is noteworthy as it is the first time that a human challenge study has demonstrated vaccine efficacy in lieu of a field efficacy trial as a basis for licensure. In 1993 and 1998, the Agency convened Vaccines and Related Biologics Products Advisory Committee (VRBPAC) meetings to consider whether data from human cholera challenge studies in U.S. subjects could be sufficient to demonstrate the efficacy of a cholera vaccine for use in U.S. travelers to cholera affected areas. In 1998, in light of a failed field efficacy study in Indonesia, the Committee agreed that human challenge studies could suffice to demonstrate efficacy of a cholera vaccine for use in U.S. travelers provided that studies were adequate and well-controlled and conducted under the provisions of Good Clinical Practice (GCP).

Vaccine efficacy in adults 18 through 45 years of age was established in study PXVX-VC-200-003, a randomized, double-blind, placebo-controlled challenge study. A total of 197 subjects 18 through 45 years of age, with no prior history of cholera infection or travel to a cholera-endemic area in the previous 5 years, were randomized according to a 1:1 ratio to receive a single dose of Vaxchora (5×10^8 colony forming units (CFU)/dose) or a saline placebo. A total of 68 Vaxchora recipients and 66 placebo recipients were challenged with 1×10^5 CFU of heterologous live wild type *V. cholerae* O1 El Tor Inaba strain N16961 at either 10 days (35 vaccine, 33 placebo) or 3 months (33 vaccine and 33 placebo) post-vaccination. Immunogenicity was assessed using a vibriocidal antibody assay to measure serum levels of neutralizing antibodies against the vaccine strain. The vibriocidal assay measured activity from any immunoglobulin isotype. Anti-cholera toxin antibody responses were also measured using an anti-cholera toxin immunoglobulin G (IgG) (b) (4)

The primary study objectives were to demonstrate vaccine efficacy of a single dose of Vaxchora in preventing moderate to severe diarrhea following challenge at 10 days and 3 months post-vaccination. Moderate to severe diarrhea was defined as cumulative diarrheal purge ≥ 3 Liters through 10 days post-challenge. The success criterion was a lower, two-sided 95.1% confidence bound on vaccine efficacy $\geq 30\%$. Vaxchora recipients challenged at 10 days post-vaccination and 3 months post-vaccination were each compared against a pooled group of placebo recipients challenged at either post-vaccination time point. Both co-primary objectives were met, as the lower limit of the 95% CI of vaccine efficacy was $\geq 30\%$ at both 10 days and 3 months post-vaccination. Efficacy was 90.3% (95.1% CI 62.7%, 100.0) at 10 days post-vaccination and 79.5% (95.1% CI 49.9%, 100.0%) at 3 months post-vaccination.

An exploratory objective of the challenge study was to evaluate whether there is an association between post-vaccination/pre-challenge vibriocidal antibody titer against the vaccine strain and the incidence of moderate to severe diarrhea following challenge with *V. cholerae*. Serum vibriocidal antibody seroconversion, defined as a ≥ 4 -fold rise in serum vibriocidal antibodies from baseline, at 10 days post-vaccination was associated with protection against moderate to severe diarrhea (Day 10 challenge adjusted OR=50, $p < 0.001$; 3 month challenge adjusted OR=39, $p < 0.001$) and was selected as the immunologic parameter for bridging effectiveness to adults > 45 years of age. Among subjects who were challenged, 91% of vaccinees seroconverted at 10 days post-vaccination and 9% developed moderate to severe diarrhea following challenge, while 2% of placebo recipients seroconverted at 10 days post-vaccination and 59% developed moderate to severe diarrhea.

Study PXVX-VC-200-004 was a phase 3 study demonstrating lot consistency and safety in 18 through 45 year olds not previously exposed to cholera. A total of 3146 subjects were randomized according to an 8:1 ratio to receive Vaxchora (1×10^9 CFU/dose) or saline placebo. The primary objective was to demonstrate immunologic equivalence of three different production lots of Vaxchora. The primary objective was met, as the 95% confidence interval for each geometric mean titer (GMT) ratio was within 0.67 and 1.5.

Vaccine effectiveness and safety in adults 46 through 64 years of age were established in study PXVX-VC-200-005, a randomized, double-blind, placebo-controlled study conducted in the U.S. A total of 398 subjects 46 through 64 year of age not previously exposed to cholera were randomized according to a 3:1 ratio to receive a single dose of Vaxchora (1×10^9 CFU/dose) or saline placebo. Vaxchora recipients 18 through 45 years of age from study PXVX-VC-200-004 served as a historical comparator group for the purpose of bridging effectiveness based on a non-inferiority immunogenicity comparison. Both primary objectives were met, as the lower limit of the 2-sided 95% CI on the difference in the seroconversion rates between Vaxchora recipients 46-64 years of age in study -005 and Vaxchora recipients 18-45 years of age in study -004 was greater than -10% (lower limit was -6.7%), and the lower bound of the 2-sided 95% confidence interval on seroconversion in 46 through 64 year olds was 86.4%. Seroconversion was defined as a ≥ 4 -fold rise in serum vibriocidal antibodies from baseline to 10 days post-vaccination.

The safety of Vaxchora was evaluated in adults 18 through 64 years of age across the 4 clinical studies submitted in this BLA. A total of 3235 Vaxchora recipients and 562 placebo recipients contributed to the safety database. The safety data reviewed raised no safety concerns that would preclude licensure. Within 6-months post-vaccination, 0.6% (20/3235) of Vaxchora recipients and 0.5% (3/562) of placebo recipients experienced a serious adverse event. No vaccine-related serious adverse events occurred, and no safety signals were identified. One death due to suicide in a 38 year old occurred 84 days after receipt of Vaxchora; this death was not considered to be caused by vaccination.

In accordance with the Pediatric Research Equity Act (PREA), the Agency is waiving the pediatric study requirement for children less than 2 years of age, because Vaxchora is not likely to be used by a substantial number of children in this age group and does not represent a meaningful therapeutic benefit over existing measures recommended by the Centers for Disease Control (CDC) for the prevention of cholera in this age group. Children younger than 2 years of age traveling to cholera affected areas whose parents follow CDC's recommendations for the prevention of cholera, would have little chance of exposure to contaminated food or water. Breastfeeding and/or feeding formula prepared with sterile water would further reduce the possibility of exposure to contaminated food or water. Furthermore, the low incidence of cholera among US children suggests that Vaxchora is not likely to be used by a substantial number of children less than 2 years of age.

The Agency is deferring submission of the pediatric study for children 2 through 17 years of age, because the product is ready for approval for use in adults and the pediatric studies have not been completed. FDA's Pediatric Review Committee (PeRC) concurred with this decision on May 11, 2016. The deferred pediatric study in 2 through 17 year old children and adolescents is a postmarketing requirement under PREA.

The routine pharmacovigilance plan proposed by the Applicant is adequate. No safety signals were identified in this BLA which would require a postmarketing requirement (PMR) or a Risk Evaluation and Mitigation Strategy (REMS). The Applicant has committed to conduct a post-licensure U.S. based pregnancy registry for 5 years to prospectively collect safety data on spontaneously reported exposures to Vaxchora occurring within 28 days prior to the last menstrual period or at any time during pregnancy.

The data submitted by the Applicant in this BLA support the approval of Vaxchora for the active immunization against disease caused by *V. cholerae* serogroup O1 in adults 18 through 64 years of age traveling to cholera-affected areas. The safety and effectiveness of Vaxchora have not been established in persons living in cholera-affected areas. The effectiveness of Vaxchora has also not been established in persons who have pre-existing immunity to *V. cholerae* due to prior exposure to *V. cholerae* or receipt of a cholera vaccine.

1.1 Demographic Information: Subgroup Demographics and Analysis Summary

Among the 3235 Vaxchora recipients and 562 placebo recipients 18 through 64 years of age in all four clinical trials submitted to this BLA, the mean age was 32.5 years; 53.8% of trial participants were female; by race, 67.1% were white, 27.3% black or African American, 1.8% Asian, 1.7% multiracial, 1.3% other, 0.6% American Indian or Alaskan Native and 0.3% Native Hawaiian or Pacific Islander; by ethnicity, 9.3% were Hispanic or Latino; by blood type, 47.3% had type O blood and 52.6% had non-O type blood.

As expected, seroconversion rates appeared to diminish slightly with age in study PXVX-VC-200-005, reflecting diminished antibody responses with increasing age in the 46-64 year old population. Otherwise, subgroup analyses based on sex, race and blood type did not reveal any clear and consistent differences in effectiveness or safety between subgroups. However subsets were generally small and analyses were not powered adequately for hypothesis testing.

2. CLINICAL AND REGULATORY BACKGROUND

2.1 Disease or Health-Related Condition Studied

Infectious Agent

Cholera is an acute, secretory diarrheal disease caused by intestinal infection with toxin producing strains of *Vibrio cholerae* serogroups O1 and O139.^{1,2} *V. cholerae* is classified based on the structure of the O-antigen of the lipopolysaccharide complex associated with the outer membrane of the bacterial cell wall. Serogroup O1 consists of 2 biotypes, classical and El Tor.³ Classical strains produce an inactive truncated haemolysin protein, whereas El Tor strains can be hemolytic or non-hemolytic. Each biotype has 2 major serotypes: Inaba and Ogawa; Hikojima is a rare third serotype.⁴ There are data in published scientific literature demonstrating cross-protection across the four major *V. cholerae* O1 subtypes following natural infection or vaccination with another non-US licensed live oral cholera vaccine (also see section 2.3).^{5,6,7,8}

V. cholerae are typically non-invasive gram-negative, rod-shaped facultative anaerobic, non-spore forming bacteria with a single polar sheathed flagellum. Although there are more than 200 *V. cholerae* serogroups that can cause a cholera-like illness, only toxigenic strains of serogroups O1 and O139 have caused widespread epidemics and

are reportable to the WHO as “cholera.”^{3,9} Toxin-producing strains of *V. cholerae* non-O1/non-O139 can cause sporadic cases of severe dehydrating diarrheal illness, but these strains have not caused large cholera outbreaks. Non-toxin-producing strains of *V. cholerae* non-O1/non-O139 are associated with sporadic cases of gastroenteritis, sepsis, and rare cases of wound infection.¹⁰

V. cholerae O1 is the predominant cause of cholera globally, and most O1 cases are caused by El Tor organisms.³ *V. cholerae* O1 is endemic¹ in much of Africa and South and Southeast Asia.³ The O139 serogroup, which was derived from the El Tor biotype¹, emerged in 1992 in the Bay of Bengal as the cause of an outbreak that spread through Asia. It has since remained localized to a few areas in SE Asia³ and has not emerged as a significant threat. In 2013, only 37 O139 cases were reported from China.¹¹

Symptoms of classical and El Tor biotype infections are indistinguishable, although El Tor infections are more often asymptomatic or result in mild illness.³ In recent years, El Tor variant strains have emerged that express the toxin produced by classical strains. These El Tor variant strains were first identified in Bangladesh and have since been reported from several African countries, Asia and Hispaniola.^{1,3,11} They appear to have displaced typical El Tor cholera since 2002¹, and they cause more severe cholera with higher case fatality rates.^{1,11} An El Tor variant strain was responsible for the 2010 epidemic on the island of Hispaniola (Haiti and the Dominican Republic).³

Clinical Presentation

Most persons infected with *V. cholerae* are asymptomatic or have only mild diarrhea. When illness does occur, about 80-90% of cases are of mild or moderate severity and are difficult to distinguish clinically from other types of acute diarrhea. Less than 20% of ill persons develop typical cholera with signs of moderate to severe dehydration.^{12,13}

Severe cholera, or cholera gravis, is characterized by acute, painless diarrhea which leads rapidly (within 4 to 18 hours) to moderate or profound dehydration.¹ Ten or more voluminous stools may be passed within a few hours, at first liquid in consistency and then becoming like rice water (i.e., resembling water in which rice has been washed).^{1,2} Vomiting is a common feature, particularly early in illness.² Fever and abdominal cramps are typically absent. Complications from cholera arise from the loss of fluid volume and electrolytes, especially sodium, potassium and bicarbonate, resulting in hypovolemia, metabolic acidosis, and potassium deficiency.¹ Secondary complications may include renal failure and, in children, severe hypoglycemia due to depletion of glycogen stores.^{1,2} If left untreated, the disease may lead to severe dehydration, hypovolemic shock and death within hours.³ However, with timely and adequate rehydration, case-fatality rates are < 1%.^{3,10}

Transmission

Cholera transmission is closely linked to resource limited settings where minimal requirements of clean water and sanitation are not met.¹³ Dense populations (e.g., refugee camps) and populations affected by natural disasters are at a particular high risk.^{1,2} The main reservoirs of *V. cholerae* are humans and environmental waters globally.^{1,13} Transmission occurs through consumption of contaminated water or food¹

1 WHO defines endemic as having reported cholera cases in at least 3 of the 5 past years(textbook).

(i.e., food washed with contaminated water or exposed to contaminated irrigation water, or raw or undercooked shellfish from contaminated waters).¹

V. cholerae thrive in brackish aquatic environments, where fresh and saltwater merge, especially in warmer climates. The bacterium is able to form biofilms on water surfaces that can resist a variety of environmental stresses. Chitin serves as a source of carbon and nitrogen and potentially a surface for the formation of biofilms. Thus, the bacteria associate with chitinous exoskeletons of zooplankton, other surface-associated plankton, and chitinous shelled animals (e.g., crustaceans) and vegetation.¹ Shellfish that ingest zooplankton may also result in human exposure to *V. cholerae*.

The bacterium may be present in the feces of infected persons as planktonic cells and as biofilm aggregates.² Asymptomatic infected individuals generally shed the bacterium for a few days; however, symptomatic individuals shed the bacterium for between 2 days and 2 weeks, and rarely longer.² *V. cholerae* O1 that are shed from individuals are hyperinfective (i.e., the infectious dose is 10-100 times lower than for non-human shed organisms).² This hyperinfectivity phenotype persists in water for 5-24 hours. This phenomenon is thought to explain the explosive nature of cholera outbreaks.

Disease Pathogenesis

The infectious dose of *V. cholerae* O1 has been estimated to be 10^5 to 10^8 vibrios based on experimental human infection studies; but it could be as low as 10^3 vibrios in the presence of achlorhydria (i.e., young children, the elderly and those taking antacids).² Cholera has a short incubation period, from 2 hours to 5 days.^{10,13} After ingestion, most of bacteria are killed by gastric acid.² Surviving organisms colonize the proximal small intestine using pili, such as toxin-coregulated pili.¹ Bacterial motility allows penetration of the mucous lining¹ where it expresses cholera toxin, a protein exotoxin. Cholera toxin activity is limited to the intestinal mucosa, because cholera toxin is not systemically absorbed and *V. cholerae* are typically non-invasive bacteria.¹

Cholera toxin consists of one central A subunit associated with a pentameric B subunit.¹ The A subunit is composed of an enzymatically active A1 fragment and a helical A2 fragment which forms a link to the pentameric B subunit. When the pentameric B subunit binds to intestinal epithelial cell surface GM1 ganglioside, the A subunit is translocated intracellularly, where A1 activates adenylate cyclase.^{1,2} This initiates a cascade of biochemical events that lead to hypersecretion of chloride and bicarbonate into the intestinal lumen. The resulting osmotic gradient results in concomitant water loss and diarrhea.¹

Risk Factors

Individuals with type O blood are less likely to become infected with *V. cholerae* O1 but, once infected, are more likely to develop severe illness;¹⁴ this risk appears to apply to the El Tor biotype only.¹ The risk of illness is increased in persons with gastric hypochlorhydria, non breast-fed infants, childhood, and travelers who drink untreated water or eat poorly cooked or raw seafood in disease-endemic areas).³ In areas of high endemicity in Asia, the incidence of *V. cholerae* infection follows a seasonal distribution with peaks before and after rainy seasons. The incidence of cholera in cholera-affected areas is highest in children < 5 years of age^{15,16}, likely reflecting the lack of protective immunity. In populations with more limited immunity, massive epidemics may occur with similar attack rates in children and adults.²

Travelers who follow the usual tourist itineraries and who observe safe food and water recommendations and hygiene precautions while in countries reporting cholera have virtually no risk of acquiring cholera.³ Although rare, several cases of cholera have been reported among U.S. health care workers caring for cholera patients in Haiti and in the United States.³ Travelers at highest risk are those who are visiting remote areas where access to safe food and water and medical care is likely to be limited. Risk is also higher among persons with underlying medical conditions that predispose them to increased morbidity due to moderate or mild diarrhea.

Epidemiology

Descriptions of a disease thought to be cholera are found in Sanskrit writings, and the disease is believed to have been endemic in the Indian subcontinent, particularly in the Ganges region, since 500 BC.^{2,15} Since 1817, seven worldwide cholera pandemics have occurred. The seventh pandemic, which is still in effect, began in 1961 in Indonesia.² This pandemic is caused by the El Tor biotype, which was first isolated in 1905 in El Tor, Egypt.² Molecular epidemiology shows that this pandemic has occurred in 3 successive waves, with each one spreading from South Asia to other regions in Asia, Africa and the Western Pacific Islands (Oceania).^{2,10} In 1991, epidemic cholera caused by the El Tor biotype appeared in Peru and spread to most countries in South, Central and North America.¹⁰

In October 2010, the El Tor biotype was introduced into Haiti. A large cholera epidemic, caused by *V. cholerae* O1 El Tor Ogawa, occurred 10 months after an earthquake in the capital city of Port-au-Prince and surrounding areas.³ Within 3 years, 696,794 cases of infection, 389,903 hospitalizations, and 8531 deaths from cholera were reported in Haiti.¹¹ Cholera has persisted in Haiti at endemic levels. After it emerged in Haiti, cholera spread to a number of other countries, including the Dominican Republic and Cuba.

Globally, most cases of cholera are unreported.³ The WHO estimates that officially reported cases represent only 5-10% of the actual number of cases occurring worldwide.¹⁶ In 2014, the WHO estimated that there are 1.4 to 4.3 million cases of cholera and 28,000 to 142,000 deaths per year worldwide due to cholera.^{11,16} In 2013, 47 countries reported a total of 129,064 cases of cholera including 2012 deaths in 26 countries, giving a case fatality rate of 1.63%. Of all cholera cases reported globally in 2013, 47.3% were reported from the island of Hispaniola, where an outbreak began in October 2010; 43.6% of cases were reported from Africa, and 9.0% were reported from Asia.¹¹ Of all deaths reported globally in 2013 due to cholera, 65% were from Africa (CFR 2.43%) and 30% were from Haiti and the Dominican Republic (CFR 1.04%). Case fatality rates ranged from < 1% to 13.61%.¹¹

From 2001 through 2013, 123 confirmed cases of cholera in the United States were acquired abroad; of these, 63 were associated with the epidemic in Hispaniola.³

2.2 Currently Available, Pharmacologically Unrelated Treatments/Interventions for the Proposed Indication

Prevention strategies primarily include ensuring access to safe water (i.e., water for consumption, washing and preparing food, or brushing teeth), making food safe for consumption (i.e., peeling fruits and vegetables, thoroughly cooking high risk foods, particularly seafood), improving sanitation and ensuring appropriate hand hygiene after

defecating and before preparing or eating food. Water can be disinfected through chlorination or boiling. Foods such as fish, rice, or grain gruels should be refrigerated promptly after meals and thoroughly reheated before eating.¹⁷ Cholera vaccines, in addition to the preventive strategies discussed above, can assist in cholera control. See Section 2.3.

Cholera can be treated with prompt and adequate rehydration and electrolyte replacement (i.e., correct the acidosis and potassium deficiency); antibiotic therapy can be initiated in moderate to severe cases.¹ Oral rehydration salt solution should be considered as the first treatment for cholera, because of ease of administration and because more potassium, bicarbonate and glucose are available in oral rehydration solution than in standard intravenous fluids.² Intravenous fluids with polyelectrolyte solution (e.g., Ringer's lactate) are needed in cases of severe dehydration or shock.¹ The most common errors in caring for patients with cholera include underestimating the amount of fluid needed or the use of non-isotonic, fluids.

Antibiotics can be used to reduce the volume of diarrhea and the length of time the organism is excreted in the stool.¹ The choice of antibiotic should be based on availability and local resistance patterns. Although *V. cholerae* strains remain susceptible to commonly used antibiotics, strains resistant to one or more antibiotics have been reported. In addition, unlike other enteric organisms, predominant strains often lose their resistance; so antibiotic resistance can vary from time to time and from place to place.¹ Doxycycline or tetracycline is the antibiotic of choice for sensitive strains.¹ Either erythromycin or azithromycin is an appropriate first line regimen for children and pregnant women.¹⁸ Other clinically effective antibiotics include ciprofloxacin, cotrimoxazole, chloramphenicol, and furazolidone.^{1,18}

Children in resource-limited areas may additionally benefit from supplementation of zinc.^{3,19} Zinc supplementation reduces the duration and volume of stool in children with cholera.^{3,19,20}

2.3 Safety and Efficacy of Pharmacologically Related Products

Vaccines against cholera have been in development and use since the late 19th century.¹⁴ Killed whole-cell parenteral cholera vaccines have not been recommended by the WHO for over 40 years, and the manufacture and sale of these vaccines in the U.S. have been discontinued.¹ Parenteral vaccines fell out of favor for several reasons, including limited efficacy (45% for 3 months) and short duration of protection thereby necessitating frequent administration (every 6 to 12 months) to maintain clinically significant protection.^{1,11}

Consequently, research shifted to the development of oral cholera vaccines.¹ Two oral killed vaccines, prequalified by WHO, are internationally-licensed and commercially available in several countries.² Both vaccines require > 1 dose for protection, and booster doses are recommended for each.¹⁵ From 1994 until 2004, an oral, live attenuated single-dose vaccine containing a *V. cholerae* O1 strain (CVD 103-HgR) was licensed in several countries outside of the U.S. as Orochol/Mutachol Berna. No cholera vaccine is currently licensed in the United States (U.S.).

Killed Oral Vaccines

1. Dukoral (WC-rBS, Crucell, Sweden): Dukoral, the first oral killed whole cell vaccine (licensed in 1991 in Sweden)¹⁵, is a monovalent oral vaccine based on formalin and heat-killed² whole cells (WC) of *V. cholerae* O1 (classical and El Tor biotypes, Inaba and Ogawa serotypes) plus recombinant cholera toxin B subunit.^{1,2} Each dose consists of 1.25×10^{11} killed bacteria with 1 mg of recombinant cholera toxin subunit B. The vaccine requires administration with a sodium bicarbonate buffer to protect the acid labile cholera toxin B subunit component from gastric acid. The vaccine is indicated for children ≥ 2 years of age. Children 2-5 years of age are given 3 doses 7- 14 days apart, and persons ≥ 6 years of age are given 2 doses 7-14 days apart. Booster doses are needed every 6 months for persons 2-5 years of age and every 2 years for persons ≥ 6 years of age.^{15,21} WHO pre-qualification was received in October 2001.¹⁵ However, Dukoral has not been routinely adopted for public health use due to its high cost, limited duration of protection and logistical issues with administration (e.g., need for buffer).
2. Shanchol (Shantha Biotechnics, Sanofi Pasteur, India): This bivalent oral, killed whole cell cholera vaccine contains several biotypes and serotypes of *V. cholerae* O1 and *V. cholerae* O139 without the cholera toxin B subunit. It was licensed in 2009 in India. The vaccine is indicated for use in persons ≥ 1 year of age. Two doses are to be administered 14 days apart.¹⁵ Recent data from a randomized, double-blind, placebo-controlled study suggest that a single dose of Shanchol among persons ≥ 1 year of age in a highly endemic area provides 40% (95% CI 11, 60) protection against all cholera for at least 6 months; however, there was a trend toward decreasing vaccine efficacy with decreasing age.²² Booster doses are needed every 2 years for all age groups.¹⁵ Initially, a monovalent (O1) formulation of the vaccine was licensed in Vietnam as ORCVAX in 1997. Following the emergence of O139 in India and Bangladesh in 1992, the vaccine was modified to include killed *V. cholerae* O139 cells. The bivalent vaccine was licensed as mORCAX in Vietnam by VaBiotech for domestic use).^{1,15} WHO pre-qualification was received in November 2011.¹⁵

Live Oral Vaccines

Live oral cholera vaccines are being developed because they are likely to produce a mucosal immune response and require only one dose. Live oral cholera vaccines have focused on incorporating the classical biotype as the vaccine strain. In field studies, an initial cholera infection due to the classical biotype is almost 100% protective against natural clinical infection due to either classical or El Tor biotypes. Infection with the El Tor biotype, however, results in a lower immune response. It has also been hypothesized that antibacterial immunity is critical in protection, and that antitoxin immunity while helpful is not critical to protection.²³

Oral Live Monovalent CVD 103-HgR Vaccine (Orochol/Mutachol Berna)

Orochol/Mutachol Berna was a previously licensed oral, live attenuated vaccine that contained the same CVD 103-HgR vaccine strain used to manufacture Vaxchora. The vaccine consisted of a single dose of 2×10^8 to 1×10^9 CFU CVD 103-HgR for use in

2 Formalin killing better preserves protein antigens and heat-killing better expresses LPS antigens.

travelers to cholera endemic areas who are at high risk for infection and who are aged two years or older. This vaccine, which was first licensed in 1994 in Switzerland, was marketed mainly in Switzerland, Canada, New Zealand and Australia; it was also registered in Austria, Finland, Sri Lanka, the Philippines, and several Central and South American countries. It was marketed as Mutachol Berna in North America and as Orochol elsewhere. In 2004, the manufacturer ceased production of Orochol/Mutachol Berna for business reasons. Over 500,000 doses were distributed worldwide.

- *U.S. Regulatory History*

In February 1997, a Product License Application (PLA) for Mutachol Berna (CVD 103-HgR) was submitted to the FDA by SSVI [SSVI later became Berna Biotech AG (Bern, Switzerland) and was subsequently acquired by Crucell NV (Leiden, The Netherlands)]. The vaccine was studied in multiple clinical trials, including challenge studies. The proposed indication was prevention of cholera in travelers to cholera-affected areas. A VRBPAC meeting was held in May 1998 (see below). While the committee agreed that challenge studies are acceptable to support licensure of a cholera vaccine for use in travelers, their assessment was that the clinical studies submitted to the PLA were insufficient due to lack of blinding and randomization.²⁴ In response, an additional randomized, double-blind, placebo controlled trial was conducted with 85 U.S. subjects, demonstrating an efficacy of 80% against all diarrhea and 91% against severe disease caused by the heterologous *V. cholerae* O1 El Tor Inaba strain N16961 over at least 3 months.²⁵ (b) (4)

[REDACTED], and the application process was never completed by the Applicant. Crucell ultimately made a business decision to focus on the production and marketing of its oral killed cholera vaccine (Dukoral) and returned the license for CVD 103-HgR to the Center for Vaccine Development at University of Maryland, Baltimore (CVD UMB).

VRBPAC Meetings

In January 1993, the Vaccines and Related Biologics Products Advisory Committee (VRBPAC) met to discuss whether data from human challenge studies are sufficient to demonstrate efficacy of cholera vaccines for use in travelers in cholera-affected areas. Presentations to the Committee noted that oral, live cholera vaccines induce higher immune responses in immunologically naïve volunteers from developed countries compared to residents in cholera-endemic areas. Two possible reasons for this difference in immune response include: (1) higher levels of pre-existing immunity to cholera in endemic areas limit the replication of the vaccine organisms resulting in lower immune response(s) and (2) ileal microflora in persons from endemic areas compete with the vaccine strain limiting its replication. Given the differences in immune response between immunologically naïve and putative seropositive populations and the impracticability of conducting an efficacy study in U.S. travelers, the Committee agreed that while a challenge study in U.S. volunteers not previously exposed to *V. cholerae* is a potentially valid approach for demonstrating the efficacy of cholera vaccines for use in a U.S. traveler population. However, for a cholera vaccine indicated for use in an endemic population, the Committee expressed the need to confirm vaccine efficacy in a field trial.²³

On May 27, 1998, the agency convened the VRBPAC to review data from studies submitted in the Mutachol Berna PLA and consider whether data from human challenge studies in U.S. subjects were sufficient to support the efficacy of a cholera

vaccine for use in travelers to endemic areas (VRBPAC transcript). The degree of protection observed in the studies varied depending upon the nature of the challenge strain. The highest protection was seen against the classical biotype challenge strain (569B) and lower efficacy was seen against an El Tor biotype challenge strains. In addition, results from a large-scale field trial of CVD103-HgR in Indonesia (dose of 3×10^9 CFU) did not demonstrate efficacy.²⁴

The VRBPAC agreed that in theory, a challenge study can be sufficient for demonstrating efficacy of a cholera vaccine for the prevention of cholera in U.S. travelers to cholera-affected areas, provided that the studies are adequate and well-controlled and conducted under the provisions of GCP. The committee recommended that a randomized, blinded human challenge study be conducted in addition to the submitted studies. In addition, the committee emphasized the importance of evaluating the duration of the protective response and revaccination in the U.S. traveler population. The total number of subjects evaluated in the challenge studies submitted at the time was noted to be small (< 30 subjects); thus, larger numbers of challenged subjects were recommended as well.²⁴

- *CVD 103-HgR*

The CVD 103-HgR strain was developed at the CVD UMB in the early 1980s. It is characterized by two prominent genetic modifications to the parent wild-type *V. cholerae* O1 classical Inaba strain 569B, which was first isolated from a patient in 1948 in India. Ninety-four percent of the gene encoding the enzymatically active A subunit of cholera toxin was deleted, thus removing the toxigenicity of the strain. The strain retained the ability to synthesize the non-toxic B subunit of cholera toxin, which is immunogenic. The second prominent genetic modification was insertion of a *S. flexneri* mercury resistance operon into a hemolysin gene locus, which inactivates hemolysin A toxin and enables differentiation of the vaccine strain from wild type *V. cholerae* O1.

The 569B parent strain produces large amounts of cholera toxin in vitro, but it is less virulent compared to the N16962 strain based on challenge studies (Levine 1988a, Tacket 1992, Tacket 1999). The reason for this difference is not known but may be due to reduced motility and adherence of the 569B strain. Antibiotic sensitivity of the CVD 103-HgR strain is identical to the parent 596B strain. The strain is sensitive to chloramphenicol, tetracycline, ampicillin, ciprofloxacin, ceftriaxone, gentamicin, trimethoprim/sulfamethoxazole, norfloxacin, nitrofurantoin, erythromycin and doxycycline. It is of intermediate sensitivity to ampicillin.

- *CVD 103-HgR Efficacy and Immunogenicity in Developed Countries*

The efficacy of Orochol/Mutachol Berna was evaluated in a series of non-randomized, placebo-controlled challenge studies in adults in the U.S. and Switzerland. A total of 103 subjects who received a single dose of $3\text{-}5 \times 10^8$ CFU CVD 103-HgR and 86 controls were exposed to El Tor Inaba N16961, El Tor Ogawa E7946 or 3008, and classical Inaba 569B challenge strains at various time intervals following vaccination (8 days to 6 months classical Inaba, 10-30 days for El Tor Ogawa and 30-90 days for El Tor Inaba N16961). Controls received an initial dose of 5×10^8 CFU *E. coli* K12 or were unvaccinated prior to challenge. These studies demonstrated 100% efficacy against the classical strain (there were no cases among vaccinees); 54% (95% CI -2, 80) to 64% (95% CI 21, 83) % efficacy against El Tor Ogawa and 62% (95% CI -22, 88) efficacy against El Tor Inaba N16961 in

preventing any diarrhea (defined as ≥ 2 unformed stools of ≥ 200 mL or single stool ≥ 300 mL over 48 hour period).²⁶ These cross-protection data are consistent with other human challenge and field study data in the scientific literature.[reference Bangladesh study.^{5,6,7,8}

In early open-label studies in U.S. adults, rates of seroconversion (defined as a ≥ 4 -fold increase in anti-classical Inaba vibriocidal antibodies from baseline) after administration of a single 3×10^8 to 5×10^8 CFU dose of CVD 103-HgR ranged from 94%-100%.⁶ Further studies performed in the U.S. and Switzerland had similar results (81%-97%) using 4×10^8 to 5.8×10^8 CFU/dose.^{25,27-30} A lower seroconversion rate (67%) was observed in a study performed in U.S. military personnel in Panama wherein 40% of participants had elevated levels of baseline immunity to *V. cholerae* O1.³¹ Lower seroconversion rates (29% homologous Inaba serotype, 23% heterologous Ogawa serotype) were observed in a separate study that evaluated a booster dose of 4×10^8 CVD 103-HgR when administered 15-24 months after an initial dose of 5×10^8 CVD 103-HgR in Swiss adults 23-48 years of age; Slightly lower seroconversion rates were observed for heterologous Ogawa vibriocidal antibodies (53%-68%).^{28,29,31}

The duration of infection-derived immunity to cholera was studied in four subjects who were rechallenged with 10^6 classical Ogawa 395 organisms 33-36 months after an initial induced cholera infection (3 with classical Ogawa and one with classical Inaba). None of the four cholera veterans and four of the five controls developed diarrhea ($p=0.04$).³² Of the four subjects rechallenged 3 years after their initial infection, two had significant rises in levels of serum vibriocidal and IgG antitoxic antibody as well as a rapid anamnestic secretory IgA antitoxin response in jejunal fluid. With a longer duration between prior infection and ingestion of vibrios (3 years), antibacterial mechanisms limit proliferation, but not always before sufficient toxin has been produced to stimulate a local anamnestic antitoxin response. Although the protective antigens are not known, the antibacterial and antitoxic mechanisms presumably work synergistically.³²

- *CVD 103-HgR Efficacy and Immunogenicity in Developing Countries*
Studies evaluating a 5×10^8 CFU dose of CVD 103-HgR in subjects from countries with endemic/epidemic cholera generally resulted in lower seroconversion rates. Two Thai trials were conducted; one trial (N=12) resulted in a high seroconversion rate (92%)³³ and the other trial (consisting of a set of three trials, N=168) conducted in Thai soldiers resulted in lower rates (20%-39%).³⁴ Further investigation showed a correlation between socioeconomic status of vaccine recipients and the proportion with vibriocidal seroconversion. It is hypothesized that exposure to *V. cholerae* causes elevated baseline vibriocidal antibody titers which do not increase robustly in response to a 5×10^8 CFU dose. In addition, increased levels of small intestinal microflora in lower socioeconomic groups may diminish vaccine immunogenicity. This was also seen in studies conducted in Peru³⁵ and these findings are consistent with studies with other orally administered vaccines in developing countries, leading to a need for higher doses in such countries.³⁶ This led to a 10-fold higher dose marketed as Orochol E (specification: 2×10^9 to 1×10^{10} CFU of CVD 103-HgR) in endemic countries, which resulted in significantly greater rates of Inaba vibriocidal antibody conversion.

Field efficacy studies conducted in cholera endemic or epidemic areas have had differing results. The largest field efficacy study was a Phase 3 trial in Jakarta, Indonesia. This was a randomized, double-blind, placebo-controlled efficacy trial of one dose (5×10^9 CFU) of CVD 103-HgR live oral cholera vaccine was conducted between July 1993 and December 1997 in Jakarta in 67,508 persons 2 to 41 years of age.³⁷ The placebo was 5×10^8 E. coli K12 C600 bacteria. The primary objective was to evaluate efficacy against endemic cholera disease caused by *V. cholerae* O1 El Tor that caused a participant to seek medical care. A nested-immunogenicity study (N=657) showed vibriocidal seroresponses (≥ 4 -fold rise in vibriocidal titer 10 days after vaccination) in 64-70% of vaccinees vs 1-2% of controls. A nested reactogenicity study (538 vaccinees, 535 controls) revealed no vaccine-attributable side effects. Long term safety follow up showed no impact on overall mortality or morality due to diarrheal disease. Cholera incidence was lower than expected; 93 evaluable cases of *V. cholerae* O1 El Tor diarrhea were detected (43 vaccinee, 50 placebo). Only 18 cases of severe cholera were evaluable, 11 among vaccine recipients and 7 among placebo recipients.³⁷ The vaccine did not confer significant protection over a 4-year follow up period (13.5% vaccine efficacy for all ages (lower limit of 95% confidence interval -24%). Protection in the first year of follow up was 17.7% (lower limit of 95% CI -89%).³⁷ Protection within the first 4-6 months could not be assessed, as there was an insufficient number of cases. All *V. cholerae* isolates were El Tor Ogawa.³⁷

- *CVD 103-HgR Safety*

The safety of Orochol was assessed in many clinical trials. Vaccinees were monitored for adverse reactions 7–9 days after the ingestion of a single dose of 10^8 to 10^{10} CFU of CVD 103-HgR. Overall, CVD 103-HgR did not exhibit significantly higher rates of adverse reactions compared to placebo (bacteriological media). Adverse reactions were generally mild, self-resolving, and transient.

Solicited adverse reactions following Orochol were monitored in 339 U.S. adult subjects in one phase 1 study (5×10^8 and 5×10^9 CFU/dose).²⁶ The placebo consisted of 5×10^8 heat inactivated *E. coli* K12 cells suspended in the Orochol buffer. The most commonly reported adverse reactions included headache (43%), abdominal gurgling (37-41%), malaise (35-37%), abdominal cramps (25-31%) and anorexia (15-23%). Rates of solicited adverse reactions generally appeared to be higher following the 10^9 dose compared to the 10^8 dose and generally appeared higher following the 10^8 dose compared to placebo. Diarrhea (defined as ≥ 4 stools/24 hours) was reported in 3% in the 10^8 dose group, 4% in the 10^9 dose group and 1% in the placebo group.²⁶

The safety of a booster dose was evaluated in 31 adults immunized 15-24 months previously. Only one subject presented with a mild transient adverse reaction consisting of slightly soft stools (not meeting definition of diarrhea) at increased frequency.²⁶

One study evaluated Orochol in HIV positive (N=38) and HIV negative (N=387) adults in Mali; in this study, there were similar rates of adverse reactions among vaccine and placebo recipients.³⁸ Although significant rises in vibriocidal antibody GMTs were observed in all study groups, responses (peak GMTs) were significantly lower among HIV seropositives compared to HIV seronegatives, and lower among HIV seropositives with CD4+ counts < 500 per μ L compared to HIV seropositives with CD4+ counts ≥ 500 per μ L). Seroconversion rates were slightly, but not

significantly, higher among HIV seronegative participants than seropositives (71% vs 58%, $p=0.41$ Chi squared test). The CD4+ count and post-immunization titer among HIV seropositives were not significantly correlated (Spearman's test, $r=0.21$, $p=0.2$).³⁸

In the post-marketing experience of Orochol, two adverse reactions were reported from Europe. One 2 year old female reported vomiting and gastroenteritis, and one 50 year old female reported facial angioedema. Both individuals recovered.

- *CVD 103-HgR Shedding & Transmission*

The strain contained in the vaccine was minimally excreted with a geometric mean number of 200 *V. cholerae* per gram of stool.⁶ Data from clinical trials have demonstrated that up to 30% of *V. cholerae* CVD 103-HgR recipients excrete detectable numbers of the modified bacteria. Excretion peaked on day 3 post-vaccination (11.7%) and was undetectable after day 7 post-vaccination.³⁰

In one Indonesian study conducted by Simanjuntak et al, pairs of children 24-59 months of age in the same household were given either vaccine or placebo. Transmission to unvaccinated close contacts was monitored by directly culturing stool to detect vaccine strain and indirectly monitoring for seroconversion. The vaccine strain was not recovered from the 81 placebo recipients among the household pairs. CVD 103-HgR was isolated from 1 out of 174 (0.6%) other unvaccinated household contacts.³⁹ A similar study with pairs of children in the same household conducted in Chile demonstrated a low rate of transmissibility, with 5% of unvaccinated contacts shown to shed vaccine strain bacteria.⁴⁰

In the Indonesian study conducted by Simanjuntak et al, Moore swabs (4 cm thick gauze rolls attached to a nylon string) were used in an attempt to isolate the CVD 103-HgR cholera vaccine strain from toilets and sewers near 97 households of people who had received the vaccine.³⁹ Samples were taken from where the household effluent entered the sewage drains and from toilets. The vaccine strain was not isolated from any of the samples. Non-O1 *V. cholerae* (i.e. strains other than the vaccine strain) were isolated from 46 of the samples.

2.4 Previous Human Experience with the Product (Including Foreign Experience)

There is no previous human experience with Vaxchora. Vaxchora is not licensed in any country at present. Please refer to section 2.3 for information regarding previous human experience with Orochol/Mutachol Berna, a vaccine similar to Vaxchora previously manufactured and licensed outside of the U.S.

2.5 Summary of Pre- and Post-submission Regulatory Activity Related to the Submission

In 2009, PaxVax acquired a worldwide exclusive license to CVD 103-HgR from the University of Maryland at Baltimore with the aim to redevelop the cholera vaccine and introduce it into the U.S. market where there is no cholera vaccine currently available for use in persons traveling to cholera affected areas. PaxVax was able to access information in Investigational New Drug (IND) Application number 2112 (IND for CVD 103-HgR held by the National Institutes of Health (NIH)). However, PaxVax was not able to acquire Orochol/Mutachol Berna production assets or cross reference the Mutachol

Berna PLA. The Vaxchora clinical development program was designed to stand alone, without using Orochol/Mutachol Berna data to support licensure.

In June 2010, PaxVax submitted a pre-IND briefing document describing the anticipated overall development plan for PXVX0200 with supporting material describing the new manufacturing process for CVD 103-HgR. In a September 8, 2010 written response, CBER informed PaxVax that clinical study data from publications alone are not sufficient for submission as supportive information to a BLA. For each study intended to support licensure, we would expect to receive a final study report along with the raw data, provided as electronic datasets.

In February 2012, PaxVax submitted IND 15010 under the name PXVX0200. The Vaxchora development program received Fast Track designation from the FDA in December 2012, because it was intended for the prevention of a serious or life-threatening condition (cholera) and it demonstrated the potential to address an unmet medical need for the condition. There was no vaccine for the prevention of cholera in the United States, particularly for U.S. travelers at increased risk of exposure or at increased risk of morbidity if infected.

In April 2013, an End of Phase 1 meeting was held to obtain CBER input regarding the phase 3 clinical development plan. CBER agreed with the general design of the challenge study, including the criteria for successfully demonstrating vaccine efficacy. CBER agreed that immunologic bridging from challenge efficacy trials in healthy adults could be an acceptable pathway for licensure in other populations. However, we advised that PaxVax explore several potential immunologic measurements and comparison strategies to develop clinically relevant and appropriate endpoints for bridging to older adults.

In a letter dated December 18 2014, CBER concurred with PaxVax's general proposal to use vibriocidal antibody seroconversion (defined as ≥ 4 -fold increase over baseline titer) rate by 10 days post-vaccination with Vaxchora to bridge vaccine efficacy to established in the challenge study of younger adults to older adults in study PXVX-VC-200-005. CBER also concurred with the pre-specified non-inferiority margin for the primary non-inferiority comparisons. CBER recommended that PaxVax also pre-specify a minimum lower bound of the 95% CI for the seroconversion rate in the older adult population.

Upon approval of this BLA, PaxVax would qualify to receive a Tropical Disease Priority Review Voucher. The application was eligible to receive a tropical disease priority review voucher because it met the following provisions of Section 524 of the Federal Food, Drug and Cosmetic Act: 1) cholera is a listed tropical disease; 2) the application was submitted under section 505(b)1 of the Federal Food, Drug and Cosmetic Act; 3) Vaxchora is a new molecular entity which has never been approved by FDA in any other application; 4) the application was submitted after enactment of the Food and Drug Administration Amendments Act of 2007; and 5) the application was granted priority review.

3. SUBMISSION QUALITY AND GOOD CLINICAL PRACTICES

3.1 Submission Quality and Completeness

The submission was adequately organized and integrated to accommodate the conduct of a complete clinical review without unreasonable difficulty.

3.2 Compliance With Good Clinical Practices And Submission Integrity

The 4 studies included for review in this application were conducted in accordance with GCP and according to the requirements of 21 CFR Part 56 (Institutional Review Boards) and 21 CFR Part 50 (Informed Consent).

A total of 44 sites, including 38 sites in the U.S. and 6 sites in Australia, participated in the three phase 3 trials. Four principal investigators were selected for inspection of a total of 7 clinical sites (three sites participated in two clinical protocols), representing 15.9% of the total clinical sites in the phase 3 studies. The clinical sites selected for inspection were based on the following factors: participation in more than one study, number of subjects enrolled, date last inspected, and geographic location. No significant inspection findings were identified, and no sponsor findings were identified.

3.3 Financial Disclosures

Covered clinical study (name and/or number): 4 clinical studies (see section 6)		
Was a list of clinical investigators provided:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request list from applicant)
Total number of investigators identified: <u>280</u>		
Number of investigators who are sponsor employees (including both full-time and part-time employees): <u>0</u>		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): <u>0</u>		

4. SIGNIFICANT EFFICACY/SAFETY ISSUES RELATED TO OTHER REVIEW DISCIPLINES

4.1 Chemistry, Manufacturing, and Controls (CMC)

The CMC reviewer concludes that the BLA content regarding the active component and buffer component of the drug product are acceptable. Please refer to the CMC review for additional details CMC Review issues which are addressed in the package insert (see section 11.5) are summarized below:

1. The applicant originally proposed use of 100 mL of any type of bottled water to reconstitute the buffer component of Vaxchora. However, data in the BLA do not provide confidence that the potency of Vaxchora would be maintained regardless of the type of bottled water used for reconstitution. Five different types of bottled water were tested for reconstitution of the vaccine (purified, spring, spring/mineral, artesian, or sparkling). Use of purified water maintained a higher potency (CFU/dose) through 30 minutes after reconstitution compared to the other types of water tested. Artesian water

resulted in potency dropping below 4×10^8 CFU (the lower limit of specification). Due to concerns about active component packet lots at the lower end of the potency specification falling below the lower potency specification following reconstitution, CBER requested that the package insert specify use of purified bottled for reconstitution. Specification of purified water also avoids the possibility that other beverages that could be mistaken for bottled water (e.g., flavored, tonic or nutrient enhanced water) will be used for reconstitution. Finally, purified bottled water must meet USP standards that would be expected to be of more consistent quality than the other types of bottled water evaluated. In particular, mineral water is not subject to the same restrictions as other bottled waters regarding allowable levels of certain substances, and therefore may contain high levels of zinc, which is known to possess antimicrobial activity.

2. The applicant originally proposed that the active component be reconstituted in the buffer solution within 30 minutes after removal of the two component packets from the freezer, and that Vaxchora be ingested within 30 minutes of reconstitution. However, in practice, the active component packet was reconstituted within an average of 14 minutes in the clinical studies submitted to BLA 125597, and the vaccine was ingested an average of 9 minutes after reconstitution. Therefore, the package insert was revised to reflect data which support a timeframe up to 15 minutes for reconstitution and ingestion. packet
3. Instructions for preparation, reconstitution and administration of Vaxchora are complex, and the following items are needed: a freezer that goes down to -25°C to -15°C , a graduated measuring cup that measures 100 mL, a stirrer, scissors, 70% isopropyl alcohol or 10% bleach solution, and standard procedures for handling medical waste. In addition, the order of reconstitution of the two component packets and stirring time are important for maintaining the potency of the product within its specifications. The package insert will therefore specify that the preparation, reconstitution and administration of Vaxchora occur in a healthcare setting equipped to dispose of medical waste.

Vaxchora Drug Product Description and Comparison to Orochol/Mutachol Berna
[Please see section 2.3 for a description of the CVD 103-HgR vaccine strain.]

Table 2 shows a comparison of the clinical lots of Vaxchora used in pre-licensure clinical trials, the proposed commercial Vaxchora formulation and the commercial Orochol/Mutachol Berna formulation. The PXVX-VC-200-002 Phase 1 trial utilized a lot produced as part of the PXVX0200 Working Seed Lot. The vaccine dose differed between some trials, as shown in Table 1. There were no other major formulation differences between clinical study lots of Vaxchora which would be expected to affect the safety or immunogenicity of the vaccine.

There are two main differences in the ingredients that are contained in Orochol/Mutachol Berna and Vaxchora. First, aspartame, an artificial sweetener included in Orochol, was omitted in the Vaxchora formulation. Second, sodium chloride was added as an excipient for optimal survival and proliferation of *V. cholerae* during manufacturing. The concentration of other excipients, common to Vaxchora and Orochol/Mutachol Berna, differ based on manufacturing process. The active ingredient of each vaccine is viable *V. cholerae* CVD 103-HgR.

Table 1. PXVX0200 Drug Product Formulation Used in Clinical Trials, Vaxchora Commercial Formulation and Orochol Drug Product Formulation

Ingredient	Orochol Drug Product	PXVX-VC-200-002	PXVX-VC-200-003	PXVX-VC-200-004	PXVX-VC-200-005	Commercial Vaxchora Formulation
Viable CVD 103-HgR	2x10 ⁸ – 1x10 ⁹ CFU	4.43x10 ⁸ CFU ^a	5x10 ⁸ CFU	1 x10 ⁹ CFU	1 x10 ⁹ CFU	4x10 ⁸ – 2x10 ⁹ CFU
Hy-Case SF	0.15-3 mg	4.66 mg	0.33 mg	0.32-0.51 mg	0.32 mg	(b) (4) -17.11 mg
Ascorbic Acid	0.16-1 mg	None	0.16 mg	0.16-0.25 mg	0.16 mg	(b) (4) -8.55 mg
Sucrose	1.4-30 mg	46.6 mg	3.25 mg	3.12-4.90 mg	3.12 mg	(b) (4) -165.37 mg
Sodium Chloride	None	None	0.33 mg	0.32-0.51 mg	0.32 mg	(b) (4) -17.11 mg
Dried Lactose	1.8-2.1 g	None	1.99 g	1.99 g	1.99 g	(b) (4) -2.09 g
Aspartame	20-30 mg	None	None	None	None	None
Manufacture Site	Berna Biotech Switzerland	(b) (4)	PaxVax, Inc (b) (4)			
Container Closure	Packet	Vial with stopper	Packet			
Storage Conditions	Refrigerated 2-8°C	Frozen (b) (4)	Frozen -15°C to -25°C			

^a Potency data taken from the 18 month stability time point which was prior to the start of the Phase 1 study. The original release titer is 6.9x10⁸ CFU.

Source: STN 125597_0, m3.2.P.2.2-pharm-dev, drug product.pdf, Table 1.

Vaxchora is supplied as two multilayer foil packets co-packaged in a single-dose carton: a buffer component packet for reconstitution in 100 mL purified bottled water and a active component packet containing lyophilized *Vibrio cholerae* CVD 103-HgR for reconstitution in the buffer solution. The carton is to be stored frozen at -20°C. The reconstitution procedure first requires dissolution of the buffer component in 100 mL of purified bottled water (cold or room temperature), followed by the addition of the active component and mixing for at least 30 seconds to ensure that the potency exceeds the lower specification limit.^{3,4} The reconstituted vaccine appears as a slightly cloudy solution that may contain some white particulates (the active component may not dissolve completely); it is to be administered orally within 15 minutes after reconstitution.

The buffer formulation was adopted from buffer used for Orochol/Mutachol Berna. It provides an (b) (4) during reconstitution and administration. The buffer also serves to neutralize stomach acid to facilitate passage of the acid labile vaccine strain from the stomach to the intestinal tract, where the bacteria can induce an immune response. The buffer composition is shown in Table 2. The final buffer solution is clear and colorless once completely dissolved.

³ The potency of the vaccine reconstituted in reverse order was immediately below the lower level of potency criterion of 4x10⁸ CFU/dose.

⁴ STN 125597_1, m3.2.P.2.6.7 Vaccine Reconstitution Preparation.

Table 2. Quantitative Composition of Buffer

Ingredient	Function	Quantity (per dose)
Sodium Bicarbonate and Sodium Carbonate	Buffer	2.4-2.9 g
Ascorbic Acid	Buffer, Water Chlorine Neutralizer	1.5-1.8 g
Dried Lactose	Manufacturing Flowability	0.18-0.22 g

Source: STN 125597_0, m3.2p-drug-prod, Buffer-Granule Description and composition, Table 1

4.2 Clinical Serology Assays

The clinical serology assay reviewer considered the cholera serum vibriocidal antibody assay (LLOQ= (b) (4) for each of the four O1 subtypes evaluated) validated for the detection and measurement of antibacterial antibodies against *V. cholerae* O1 classical and El Tor biotypes in test sera derived from clinical studies. The cholera toxin IgG (b) (4) quantitative assay was also considered validated for the detection and measurement of serum IgG antibodies against cholera toxin. The clinical serology assay reviewer concluded that performance of the assays was adequate for their intended use. Memory B cell response assays (b) (4) against cholera toxin B subunit and cholera lipopolysaccharide were not formally reviewed as they were used for exploratory endpoints and they were not qualified. The clinical serology assay reviewer concluded that vibriocidal seroconversion (defined as a 4-fold increase over baseline) at day 11 is an acceptable non-mechanistic correlate of vaccine efficacy for the purpose of bridging responses in the Vaxchora lot consistency study (PXVX-VC-200-004) to responses following vaccination of 46-64 year old adults in study PXVX-VC-200-005.

4.3 Nonclinical Pharmacology/Toxicology

No nonclinical animal safety or toxicology studies are included in this license application, because *V. cholerae* is a strictly human pathogen with no valid animal model available to assess safety or predict mucosal immune response. Thus, Module 4 was omitted. This plan was discussed and agreed upon with CBER during the pre-IND and pre-BLA correspondence with CBER.

4.4 Clinical Pharmacology

4.4.1 Mechanism of Action

The mechanism of protection against cholera is not completely understood, and there is no known correlate of protection. Intestinal secretory IgA, directed primarily against lipopolysaccharide (LPS) and cholera toxin, is thought to be a primary mediator of protection. Since intestinal secretory IgA is not easily measured in clinical trials, serum vibriocidal antibodies are used instead as a proxy of intestinal secretory antibodies for oral cholera vaccines. It is not known, however, if the presence of elevated serum vibriocidal antibodies may be indicative of local gut immunity. Of note, the usefulness of serum vibriocidal antibody response to determine the response to revaccination is unknown.

CVD 103-HgR is a live attenuated vaccine strain administered orally; it is expected to induce a local mucosal immune response in the small intestine. Because mucosal antibodies are difficult to measure, serum vibriocidal antibody titers were measured as a

marker of immune response. Serum vibriocidal antibody seroconversion, defined as a \geq 4-fold rise in serum vibriocidal antibodies from baseline to 10 days post vaccination, was used for bridging effectiveness based on its association with protection against cholera in the challenge study (PXVX-VC-200-003). CBER agreed with the selection of seroconversion rate as the primary endpoint in the bridging analysis conducted in study PXVX-VC-200-005. A serum vibriocidal antibody assay was used to measure serum levels of neutralizing antibodies against the 4 strains of the O1 serogroup (El Tor Inaba, classical Inaba, El Tor Ogawa and classical Ogawa). The assay measures activity from any immunoglobulin isotype.

4.5 Statistical

The statistical reviewer verified that the safety and effectiveness analyses cited by the applicant were supported by the submitted data. No major statistical issues were identified in this submission. For additional details, please see Dr. Sang Ahnn's review.

4.6 Pharmacovigilance

The Pharmacovigilance reviewer agrees with the Applicant's postmarketing plans. Please refer to the pharmacovigilance review for more details.

5. SOURCES OF CLINICAL DATA AND OTHER INFORMATION CONSIDERED IN THE REVIEW

5.1 Review Strategy

This review focused on three phase 3 trials submitted to BLA 125597: 1) Study PXVX-VC-200-003, which demonstrated safety and efficacy of Vaxchora in 18 through 45 year old adults; 2) study PXVX-VC-200-004, which demonstrated safety and manufacturing consistency of Vaxchora in 18 through 45 year old adults; and 3) study PXVX-VC-200-005, which demonstrated safety and effectiveness of Vaxchora in 46 through 64 year old adults. An integrated summary of safety, consisting of safety data from each of the four studies submitted to this BLA, was also evaluated.

5.2 BLA/IND Documents That Serve as the Basis for the Clinical Review

IND 15010 – PXVX0200 (Vaxchora) Investigational New Drug File
IND 6476 – *V. cholerae* Strain 16961 (Challenge Strain). Sponsor of IND 6476 provided a letter authorizing FDA to cross-reference IND 6476 in support of IND 15010.

The following general modules of the BLA were reviewed:

- m1.3 Administrative Information
- m1.6 Meetings
- m1.9 Pediatric Administrative Information
- m1.12 Environmental Analysis
- m1.14 Labeling
- m1.16 Risk Management Plan
- m2 Common Technical Document Summaries
- m5 Clinical Study Reports

Information reviewed in the BLA is provided below by amendment number:

Amendment 0: Date Submitted 10/16/2015

Amendment 17: Date Submitted 3/4/2016

m1.9.2 Request for deferral of pediatric studies (estimated dates for protocol submission, study completion and study submission). First partial response to clinical request dated 2/12/16

m1.11.3 Efficacy information amendment

Amendment 20: Date Submitted 3/10/16

m1.9.4 Proposed pediatric study synopsis

Amendment 21: Date Submitted 3/11/16

m1.11.3 Clinical information amendment: Orochol data in pregnancy; demographic information. Second partial response to clinical request dated 2/12/16.

Amendment 24: Date Submitted 3/17/16

m1.9.2 Updated timeline for deferred pediatric study submission.

Amendment 26: Date Submitted 3/23/16

m1.11.3 & m1.14.1 Third and final partial response to clinical request dated 2/12/16.

Amendment 30: Date submitted 4/6/16

m1.11.3 & m1.14.1.3 Response and revision to package insert regarding the need to reconstitute Vaxchora in a pharmacy or health care provider's office

Amendment 32: Date Submitted 4/20/16

m1.11.3 & m5.4 Pregnancy Registry Protocol Synopsis and Timeline

Amendment 34: Date Submitted 4/22/16

m1.11.4, m1.14.1, m3.2.P.2, & m5.4 Water type and procedure used for reconstitution of Vaxchora.

Amendment 35: Date Submitted 4/28/16

m1.11.3 Clinical information amendment: Randomization procedure used in PXVX-VC-200-003 challenge trial.

Amendment 39: Date Submitted 5/18/16

m1.11.3, m1.14.1.3, and m5.3.5.1 Partial response to first round of labeling comments sent by CBER on 5/5/16.

Amendment 42: Date Submitted 5/26/16

m1.11.3, m1.14.1.1. Completion of response to CBER's 5/5/16 labeling comments.

Amendment 43: Date Submitted 5/31/16

m1.11.3 and m1.14.1.3 Response to CBER's 5/23 and 5/27/16 labeling comments.

Amendment 44: Date Submitted 6/1/16

m1.11.3 Response to CBER's 5/31/16 labeling comments.

Amendment 45: Date Submitted 6/6/16

m1.11.3 and m1.14.1.3 Response to CBER's 6/3/16 labeling comments.

Amendment 46: Date Submitted 6/7/16

m1.11.3 and m1.14.1.3 Response to CBER's 6/6 and 6/7/16 labeling comments.

Amendment 47: Date Submitted 6/8/16

m1.11.3 and m1.14.1.3 Revised carton and packet labels.

5.3 Table of Studies/Clinical Trials

The clinical section of the BLA contains study reports from 4 randomized, double-blind, placebo-controlled clinical studies (Table 3). Each study will be referred to in this review using the last 3 digits of the full study number (e.g., PXVX-VC-200-003 may be referred to as study -003).

Table 3. Clinical Studies Included in the Vaxchora Biologics License Application^a

Study Number	Study Description and Objectives	Subject Age	Test Product Dosage	Number of Subjects Vaccinated		
				Vaccine	Placebo	Total ^b
PXVX-VC-200-002^c (NCT01585181)	Phase 1, Randomized, Double-Blind, Placebo-Controlled Study - Safety & Immunogenicity	≥ 18 to ≤ 50 year olds	4.43 x 10 ⁸ CFU/dose; oral single dose	55	11	66
PXVX-VC-200-003 (NCT01895855)	Phase 3, Randomized, Double-Blind, Placebo-Controlled Study - Efficacy study demonstrating protection after live cholera challenge ^d	≥ 18 to ≤ 45 year olds	5 x 10 ⁸ CFU/dose ^e ; oral single dose	95	102	197
PXVX-VC-200-004 (NCT02094586)	Phase 3, Randomized, Double-Blind, Placebo-Controlled Study - Demonstrate clinical lot consistency	≥ 18 to ≤ 45 year olds	1 x 10 ⁹ CFU/Dose; oral single dose	2789	350	3139
PXVX-VC-200-005 (NCT02100631)	Phase 3, Randomized, Double-Blind, Placebo-Controlled Study - Demonstrate safety in older adults and equivalence in immune response of older and younger adults	≥ 46 to ≤ 64 year olds	1 x 10 ⁹ CFU/dose; oral single dose	296	99	395
Total # Subjects Vaccinated				3235	562	3797

^a Complete full reports provided for each study. Vaxchora contains live attenuated CVD 103-HgR.

^b A total of 3235 subjects received the investigational vaccine.

^c Study PXVX-VC-200-002 used part of the working seed lot. Thus, the vaccine formulation differed slightly from the formulation used in the phase 3 studies, because it did not include dried lactose, ascorbic acid or sodium chloride.

^d Heterologous Live *Vibrio cholerae* El Tor Inaba challenge strain N16961.

^e A low dose was used in the challenge study in order to confirm a low, effective dose and set a minimum level for the end of shelf life specification.

Source: 125597_0 module 5.2, Table 1, Tabular listing of all clinical studies.

5.4 Consultations

5.4.1 Advisory Committee Meeting

As our review of information submitted in this BLA did not raise concerns or controversial issues that would have benefited from an advisory committee discussion, we did not refer this application to the VRBPAC. Please refer to section 2.3 for more details regarding the 1993 and 1998 VRBPACs pertaining to use of human cholera challenge studies in U.S. volunteers to support licensure of cholera vaccines for use in U.S. adults who are traveling to cholera-affected areas and who are at increased risk of exposure or at increased risk of morbidity if infected.

5.4.2 External Consults/Collaborations

There were no external consultations or collaborations during the review of this BLA.

5.5 Literature Reviewed

1. Clemens JD, Shin S, Sah BK, Sack DA. Cholera Vaccines. In: Plotkin SA, Orenstein WA, Offit PA eds. *Vaccines*. 6th ed. Elsevier;2013:[141-152].
2. Harris JB, LaRocque RC, Qadri F et al. Cholera. *Lancet*. 2012;379:2466-2476.
3. Routh JA, Newton AE, Mintz E. Cholera. In: Burnette GW. *Yellow Book*. Oxford University Press;2016. Available at: <http://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/cholera> [accessed June 7, 2016].
4. Chatterjee SN, Chaudhuri K. Lipopolysaccharides of *Vibrio cholerae*, physical and chemical characterization. *Biochimica et Biophysica Acta*. 2003;1639:65-79.
5. Clemens JD, van Loon F, Sach DA et al. Biotype as determinant of natural immunising effect of cholera. *Lancet*. 1991;337:883-884.
6. Levine MM, Kaper JB, Herrington D et al. Safety, immunogenicity, and efficacy of recombinant live oral cholera vaccines, CVD 103 and CVD 103-HgR. *Lancet*. 1988;8609:467-740.
7. Levine MM, Nalin DR, Craig JP et al. Immunity of cholera in man: relative role of antibacterial versus antitoxic immunity. *Trans R. Soc Trop Med Hyg*. 1979;73:3-9.
8. Tacket CO, Losonsky G, Nataro JP et al. Onset and duration of protective immunity in challenged volunteers after vaccination with live oral cholera vaccine CVD 103-HgR. *JID*. 1992;166:837-841.
9. Dutta E, Chowdhury G, Pazhani GP et al. *Vibrio cholerae* non-O1, non-O139 serogroups and cholera-like diarrhea, Kolkata, India. *EID*. 2013;19:464-467.
10. American Academy of Pediatrics [Cholera.] In: Kimberlin DW, Brady MT, Jackson MA, and Long SS eds. *Red Book: 2015 Report of the Committee on Infectious Diseases*. 30th ed; Elk Grove Village, IL: American Academy of Pediatrics;2015:[860-863].
11. World Health Organization. Cholera, 2013. *Weekly epidemiological record*. 2014;89(31):345-356.
12. World Health Organization [Internet]. Cholera. Available at: <http://www.who.int/topics/cholera/about/en/> [accessed June 7, 2016].
13. World Health Organization [Internet]. Cholera Fact Sheet. Geneva: WHO;2015. Available at: <http://www.who.int/mediacentre/factsheets/fs107/en/> [accessed June 7, 2016]

14. Tacket CO and Sack DA. Cholera Vaccines. In: Plotkin SA, Orenstein WA, Offit PA eds. *Vaccines*. 5th ed. Elsevier; 2008.
15. Lopez AL, Gonzales MLA, Aldaba JG, et al. Killed oral cholera vaccines: history, development and implementation challenges. *Ther Adv Vaccines*. 2014;2(5):123-136.
16. Ali M, Lopez AL, You YA et al. The global burden of cholera. Bulletin of the World Health Organization. 2012;90:209-218A. Available at: <http://www.who.int/bulletin/volumes/90/3/11-093427/en/> [accessed June 7, 2016].
17. Centers for Disease Control [Internet]. Cholera Prevention and Control. Available at: <http://www.cdc.gov/cholera/five-messages.html> [accessed June 7, 2016].
18. Centers for Disease Control [Internet]. Recommendations for the Use of Antibiotics for the Treatment of Cholera. Available at: <http://www.cdc.gov/cholera/treatment/antibiotic-treatment.html> [accessed June 7, 2016].
19. Centers for Disease Control [Internet]. Zinc Treatment. Available at: <http://www.cdc.gov/cholera/treatment/zinc-treatment.html> [accessed June 7, 2016].
20. Roy SK, Hossain MJ, Khatun W et al. Zinc supplementation in children with cholera in Bangladesh: randomized controlled trial. *BMJ*. 2008;336(7638):266-271.
21. Dukoral product monograph, Valneva Canada Inc.
22. Qadri F, Wierzba TF, Ali M et al. Efficacy of a single-dose, inactivated oral cholera vaccine in Bangladesh. *NEJM*. 2016;374:1723-1732.
23. VRBPAC meeting transcript, January 26. , 1993.
24. VRBPAC meeting transcript, May 26-27, 1998. Available at: <http://www.fda.gov/ohrms/dockets/ac/98/transcpt/3422t1.pdf> and <http://www.fda.gov/ohrms/dockets/ac/98/transcpt/3422t2a.pdf>
25. Tacket CO, Cohen MB, Wasserman SS et al. Randomized, double-blind, placebo-controlled, multicentered trial of the efficacy of a single dose of live oral cholera vaccine CVD 103-HgR in preventing cholera following challenge with *Vibrio cholerae* O1 El Tor Inaba three months after vaccination. *Infect. Immun*. 1999;67(12):6341-6345.
26. Orochol Product Monograph, CSL Vaccines Ltd, 2000.
27. Kotloff KL, Wasserman SS, O'Donnell SA et al . Safety and immunogenicity in North American of a single dose of live oral cholera vaccine CVD 103-HgR: Results of a randomized, placebo-controlled, double-blind cross-over trial. *Infect Immun* 1992, 60:4430-4432.
28. Cryz Jr. SJ, Levine MM, Kaper JB et al. Randomized double-blind placebo controlled trial to evaluate the safety and immunogenicity of the live oral cholera vaccine strain CVD 103-HgR in Swiss adults. *Vaccine*. 1990;9:577-580.
29. Cryz Jr SJ, Levine MM, Losonsky G et al. Safety and Immunogenicity of a booster dose of *Vibrio cholerae* CVD 103-HgR live oral cholera vaccine in Swiss adults. *Infect. Immun*. 1992;60(9):3916-3917.
30. Cryz Jr. SJ, Kaper J, Tacket C et al. *Vibrio Cholerae* CVD 103-HgR Live Oral Attenuated Vaccine: Construction, Safety, Immunogenicity, Excretion and Non-Target Effects. *Dev Biol Stand*. 1995;84:237-244.
31. Taylor, DN, Sanchez JL, Castro JM et al. Expanded safety and immunogenicity of a bivalent, oral, attenuated cholera vaccine, CVD 103-HgR plus CVD 111, in United States Military Personnel Stationed in Panama. *Infect. Immun*. 1999;67(4):2030-2034.
32. Levine MM, Black RE, Clements ML et al. Duration of infection-derived immunity to cholera. *JID*. 1981;143(6):1-3.

33. Migasena S, Pitisuttitham P, Prayurahong B et al. Preliminary assessment of the safety and immunogenicity of live oral cholera vaccine strain CVD 103-HgR in healthy Thai adults. *Infect. Immun.* 1989;57(11):3261-3264.
34. Su-Arehawaratana P, Singharaj P, Taylor DN et al. Safety and immunogenicity of different immunization regimens of CVD 103-HgR live oral cholera vaccine in soldiers and civilians in Thailand. *JID.* 1992;165:1042-1048.
35. Gotuzzo E, Butron B, Seas C et al. Safety, immunogenicity, and excretion pattern of single-dose live oral cholera vaccine CVD 103-HgR in Peruvian adults of high and low socioeconomic levels. *Infect. Immun.* 1993;61(9):3994-3997.
36. Levine MM. Immunogenicity and efficacy of oral vaccine in developing countries: lessons from a live cholera vaccine. *BMC Biology.* 2010;8:129.
37. Richie E, Punjabi NH, Sidharta Y et al. Efficacy trial of single-dose live oral cholera vaccine CVD 103-HgR in North Jakarta, Indonesia, a cholera-endemic area. *Vaccine*;18:2399-2410.
38. Perry RT, Plowe CV, Koumaré B et al. A single dose of live oral cholera CVD 103-HgR is safe and immunogenic in HIV-infected and HIV-noninfected adults in Mali. *Bulletin of the World Health Organization.* 1998;76:63-71.
39. Simanjuntak CH, O'Hanley P, Punjabi NH et al. Safety, immunogenicity, and transmissibility of single-dose live oral cholera vaccine strain CVD 103-HgR in 24- to 59-month old Indonesian children. *JID.* 1993;168:1169-1176.
40. Lagos R, Losonsky G, Abrego P et al. Tolerancia, inmunogenicidad, excreción y transmisión de la vacuna anti-colera oral viva-atenuada, CVD 103-HgR estudio pareado doble ciego en niños chilenos de 24 a 59 meses. *Bol Med Hosp Infant Mex.* 1996;53(5):214-220.
41. Russo TA, Johnson JR. Diseases Caused by Gram-Negative Enteric Bacilli. In Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J (Eds), *Harrison's Principles of Internal Medicine*, 19e. New York, NY: McGraw-Hill; 2015. Available at: <http://accessmedicine.mhmedical.com/content.aspx?bookid=1130&Sectionid=79735990> [accessed June 7, 2016]
42. Keystone JS, Kozarsky PE. Health Recommendations for International Travel. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J. eds. *Harrison's Principles of Internal Medicine*, 19e. New York, NY: McGraw-Hill; 2015. <http://accessmedicine.mhmedical.com/content.aspx?bookid=1130&Sectionid=63652517>. Accessed June 07, 2016.
43. Camilleri M and Murray JA. Diarrhea and Constipation. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J. eds. *Harrison's Principles of Internal Medicine*, 19e. New York, NY: McGraw-Hill; 2015. <http://accessmedicine.mhmedical.com/content.aspx?bookid=1130&Sectionid=79726207>. Accessed June 09, 2016.
44. DuPont HL and the Practice Parameters Committee of the American College of Gastroenterology. Guidelines on Acute Infectious Diarrhea in Adults. *The American Journal of Gastroenterology.* 1997;92(11): 1962-1975.
45. Garratty G, Glynn SA, and McEntire R. ABO and Rh(D) phenotype frequencies of different racial/ethnic groups in the United States. *Transfusion.* 2004;44:703-706.
46. Sack DA, Tacket CO, Cohen JB et al. Validation of a volunteer model of cholera with frozen bacteria as a challenge. *Infect. Immun.* 1998;66(5):1968-1972.
47. Kollaritsch H, Cryz Jr SJ, Lang AB, et al. Local and systemic immune responses to combined *Vibrio cholerae* CVD 103-HgR and *Salmonella typhi* Ty21a live oral vaccines after primary immunization and reimmunization. *Vaccine.* 2000;18(2000): 3031-3039.
48. Watson JC, Hlavsa MC, Griffin PM. Food and water precautions. In: Burnette GW.

- Yellow Book*. Oxford University Press;2016. Available at: <http://wwwnc.cdc.gov/travel/yellowbook/2016/the-pre-travel-consultation/food-water-precautions> [accessed June 7, 2016].
49. Weinberg N, Weinberg MS, Maloney SA. Traveling safety with infants & children. In: Burnette GW. *Yellow Book*. Oxford University Press;2016. Available at: <http://wwwnc.cdc.gov/travel/yellowbook/2016/international-travel-with-infants-children/traveling-safely-with-infants-children> [accessed June 7, 2016].
50. Hirschhorn N, Chowdhury AK, Lindenbaum J. Cholera in pregnant women. *Lancet*. 1969;1:1230–2.
51. Khan PK. Asiatic cholera in pregnancy. *Int Surg*. 1969;51:138–41.
52. Ayangade O. The significance of cholera outbreak in the prognosis of pregnancy. *Int J Gynaecol Obstet*. 1981;19:403–7.
53. Proegler C. Cholera, and its relation to pregnancy and child-birth. *Boston Med Surg J* 1871 85: 200–2.2.
54. Nguyen-Toan Tran, Taylor R, Antierens A, Staderini N. Cholera in Pregnancy: A Systematic Review and Meta-Analysis of Fetal Neonatal and Maternal Mortality. *PLoS One*. 2015 Jul 15;10(7):e0132920. doi: 10.1371/journal.pone.0132920.
55. Schillberg E, Ariti C, Bryson L, Delva-Senat R, Price D, GrandPierre R, Lenglet A. Factors Related to Fetal Death in Pregnant Women with Cholera, Haiti, 2011-2014. *Emerg Infect Dis*. 2016 Jan;22(1):124-7
56. Ciglenecki I, Bichet M, Tena J, Mondesir E, Bastard M, Tran NT. Cholera in pregnancy: outcomes from a specialized cholera treatment unit for pregnant women in Léogâne, Haiti. *PLoS Negl Trop Dis*. 2013;7:e2368.

6. DISCUSSION OF INDIVIDUAL STUDIES/CLINICAL TRIALS

6.1 Study PXVX-VC-200-003 (NCT01895855): Primary Efficacy Study

Study PXVX-VC-200-003 was entitled “A Phase 3 Randomized, Double-Blind, Placebo-Controlled, Efficacy Trial of a Single Dose of Live Oral Cholera Vaccine Candidate, PXVX0200 CVD 103-HgR Strain, in Preventing Cholera following Challenge with *Vibrio cholera* O1 El Tor Inaba 10 Days or 3 Months after Vaccination.”

Study enrollment began on September 13, 2013, and the last subject completed the last study visit on July 28, 2014.

6.1.1 Objectives

10-Day Challenge Co-Primary Objective

Demonstrate that the lower limit of the 95.1% confidence interval on vaccine efficacy of a single dose of PXVX0200 is $\geq 30\%$ following a challenge with virulent *V. cholerae* O1 El Tor Inaba 10 days post-vaccination. PXVX0200 recipients challenged at 10 days post-vaccination were compared with a pooled group of placebo (saline) recipients challenged at either 10 days or 3 months.

- Primary Endpoint (Attack rate): Proportion of subjects in each treatment arm who experienced moderate or severe diarrhea (cumulative diarrheal purge ≥ 3.0 L) after challenge through 10 days post-challenge.
- Vaccine efficacy = [(attack rate in combined placebo group – attack rate in vaccine group)/attack rate in combined placebo group x 100].

3-Month Challenge Co-Primary Objective

Demonstrate that the lower 95.1% confidence limit on the efficacy of a single dose of PXVX0200 is $\geq 30\%$ following a challenge with virulent *V. cholerae* O1 El Tor Inaba 3 months post-vaccination. PXVX0200 recipients challenged at 3 months post-vaccination were compared with a pooled group of placebo recipients challenged at either 10 days or 3 months. The primary endpoint and vaccine efficacy are defined as above.

Secondary Objectives and Endpoints

1. Evaluate the impact of vaccination on post-challenge disease severity.
 - a. Total weight (converted to volume) of diarrheal stools
 - b. Incidence of diarrhea of any severity⁵
 - c. Incidence of fever
 - d. Incidence of fecal shedding of wild type *V. cholera*
 - e. Peak concentration of *V. cholerae* challenge strain detected in stool
2. Evaluate the tolerability of vaccine prior to challenge.
 - a. Incidence and severity of fever, abdominal pain, diarrhea, headache, lack of appetite, nausea/vomiting and tiredness
 - b. Incidence and severity of unsolicited AEs

Tertiary Objectives and Endpoints

3. Evaluate the pre-challenge immunologic response to PXVX0200.
 - a. Serum vibriocidal antibody and serum IgG anti-CT geometric mean titer (GMT) at all available pre-challenge time points
 - b. Percentage of subjects with a ≥ 4 -fold rise in vibriocidal antibody prior to challenge
 - c. Percentage of subjects with a ≥ 4 -fold rise in anti-CT prior to challenge
 - d. Percentage of subjects with a vibriocidal antibody titer ≥ 2560 at any time point prior to challenge
4. Evaluate the post-challenge immunologic response to PXVX0200.
 - e. Percentage of subjects with a ≥ 4 -fold rise in vibriocidal antibody or anti-CT antibody, when comparing last pre-challenge levels to levels following challenge

Clinical Reviewer Note: The Applicant specified at an end of phase 1 meeting that a vibriocidal titer cutoff of 2560 is not considered seroprotective. This value was chosen as a measure of vaccine “response” based on results from a previous cholera vaccine trial in which all subjects, except for one, who achieved a vibriocidal titer of ≥ 2560 did not go on to experience a diarrheal purge of ≥ 3 liters.

Exploratory Objectives and Endpoints

1. Explore the relationship between post-vaccination, pre-challenge vibriocidal and/or anti-cholera toxin (CT) antibody titer and the incidence of moderate/severe diarrhea, mild diarrhea, any diarrhea, or measures of diarrhea severity such as total number or total volume of diarrheal stools.
2. Explore the association between age and immunologic response and whether the relationship between immunologic response and outcome varies with age.
3. Explore the impact of blood type on incidence and severity of diarrhea.

⁵ Severity will be based on 2 continuous endpoints: the total weight of diarrheal stools and the total number of diarrheal stools.

4. Explore the relationship between pre-challenge memory B cell concentration and the incidence or severity of diarrhea [vaccine recipients challenged at 3 months].
5. Explore whether the memory B cell response to PXVX0200 vaccination is similar to the response induced by cholera infection [non-challenged vaccine recipients and in placebo recipients challenged at 10 days].

6.1.2 Design Overview

This was a Phase 3 randomized, double-blind, placebo-controlled 3-center study to evaluate vaccine efficacy, immunogenicity, and safety of PXVX0200 compared with placebo following challenge with virulent *V. cholerae* O1 El Tor Inaba at 10 days or 3 months post-vaccination in healthy subjects aged 18 to 45 years. A total of 197 subjects (95 vaccine recipients; 102 placebo recipients) were randomized according to a 1:1 ratio to receive PXVX0200 vaccine (5×10^8 CFU/dose) or placebo, administered orally as 100 mL of liquid.

The co-primary objectives were to demonstrate the efficacy of a single dose of VAXCHORA in the prevention of moderate to severe diarrhea following a challenge with 1×10^5 CFU of virulent wild type *V. cholerae* O1 El Tor Inaba (strain N16961) at 10 days and 3 months post-vaccination. Moderate to severe diarrhea was defined as cumulative diarrheal purge ≥ 3 liters (L) within 10 days after challenge. Diarrheal stool post-challenge was defined as ≥ 2 unformed stools (takes shape of container) over a 48 hour period that was ≥ 200 g or a single unformed stool that totaled ≥ 300 g (1 gram is equivalent to 1 mL). The success criterion was a lower limit of the two-sided 95.1% confidence interval on vaccine efficacy $\geq 30\%$. PXVX0200 recipients challenged at 10 days post-vaccination and PXVX0200 recipients challenged at 3 months post-vaccination were compared with a pooled group of placebo (saline) recipients challenged at either 10 days or 3 months.

Subjects were instructed to collect every stool from the time of challenge until discharge from the inpatient unit. Nursing staff or study personnel inspected all stool, graded the consistency of the stool and calculated the total weight of diarrheal stool per day. VAXCHORA recipients challenged at 10 days post-vaccination and VAXCHORA recipients challenged at 3 months post-vaccination were compared with a pooled group of placebo (saline) recipients challenged at either 10 days or 3 months post-vaccination.

On the day of vaccination (day 1), subjects did not take food or water for 60 minutes prior to vaccination and 60 minutes after vaccination. All subjects were observed for 90 minutes post-vaccination for acute side effects. Solicited adverse reactions were recorded through Day 8, unsolicited adverse events (AEs) through Day 29, and medically attended events, new onset chronic medical conditions, and unsolicited serious adverse events (SAEs) through Day 181. Blood was collected for immunological assessments on days 1, 8, 11, 29, and 181 after vaccination and 1, 11, 28, and 170 days after each challenge. Immunogenicity was assessed by analyzing serum vibriocidal and anti-CT antibody responses. Subjects also provided whole blood specimens for the assessment of antigen-specific memory B cells at Day 1 pre-vaccination, Day 91 pre-challenge in subjects challenged in the 3-Month Challenge group, Day 181 in non-challenged vaccine recipients, and Day 181 placebo recipients in the 10-Day Challenge group.

More subjects were vaccinated than needed for the challenge phase in case some subjects dropped out or became ineligible prior to the challenge. A subset of vaccinated

subjects were selected for the challenge phase using four randomly ordered lists of subjects 1) to ensure roughly equal numbers of vaccine and placebo recipients and 2) to ensure that at least 60% of challenged subjects in each treatment group had blood type O (see more details below under *Randomization*). Subjects were administered a written comprehension exam to ensure the informed nature of their consent prior to challenge.

The challenges were conducted sequentially in 2 cohorts, one at 10 days post-vaccination and one at 3-months post-vaccination. Subjects were admitted to an inpatient unit on the day of or day prior to challenge. Subjects who underwent challenge at 10 days or 3 months post-vaccination were admitted to an inpatient unit. They had a 1- to 2-day acclimation in-patient period prior to challenge, during which blood was collected for pre-challenge immunogenicity. On the day of challenge, the challenge strain (virulent *V. cholerae* O1 El Tor Inaba strain N16961 (NIH under IND 6476) was thawed and 1×10^5 CFU was diluted in 30 milliliters (mL) sodium bicarbonate (NaHCO_3) solution. The subjects ingested 120 mL NaHCO_3 solution and approximately 1 minute before ingestion of the challenge strain inoculum. Subjects had nothing by mouth from midnight before ingestion of the challenge strain, except for water, and took nothing by mouth 90 minutes after ingestion of the challenge strain.

All challenged subjects were carefully monitored on the in-patient unit, had intravenous access placed when they had stool output that met the definition of diarrhea, and were administered antibiotics when they had stool output that met the definition of severe diarrhea. All subjects who did not receive antibiotics due to diarrhea received antibiotics starting 96 hours after challenge. Safety monitoring continued daily until the subject was discharged from the in-patient unit. Discharge criteria included: 1) three consecutive negative stool culture results for *V. cholerae* at least 12 hours apart, 2) no diarrhea, and 3) completion of a full course of antibiotics.

Blinding

An unblinded site pharmacist maintained the randomization list and used it to dispense the PXVX0200 vaccine or placebo as appropriate. The placebo was not matched to the vaccine visually or by taste, therefore subjects were dosed by an unblinded staff member (not involved in post-vaccination assessments) in order to maintain the blind of staff performing post-vaccination assessments. Subjects were asked not to discuss the taste of the product with the clinic staff.

Subjects and clinical site personnel were blinded. In addition to the site pharmacist and dose administrator, there was an unblinded statistician and an unblinded site monitor who knew subjects' treatment assignments and blood group status. Per-protocol unblinding occurred after data were collected through 28 days after the final subject in each group was challenged (Day 39 for the 10-day challenge group and Day 119 for the 3-month challenge group). PaxVax, study statisticians and the DSMB were unblinded at this time, while investigators and site staff remained blinded until all safety follow up visits were completed at Day 191.

Randomization

A total of 197 subjects were randomized according to a 1:1 ratio to receive PXVX0200 or placebo. In order to identify the subset of subjects to be challenged, an unblinded statistician prepared four randomly ordered lists of subjects per site, one list each for vaccine recipients with blood type O, vaccine recipients with non-O blood types, placebo recipients with blood type O, and placebo recipients with non-O blood types. This was

done to obtain balance across treatment groups maintaining a minimum of 60% blood group O subjects. Individuals with type O blood are less likely to be infected, but if infected, they are at greater risk for developing severe cholera. Each site was provided with a blinded version of the four lists specific to its site and advised on the number of subjects from each list to challenge. In the event that a subject was determined to be ineligible for challenge, the site was instructed to select the next subject from the same list as the ineligible subject.

Clinical Reviewer Comment:

- The design of the PXVX-VC-200-003 challenge study was based on a study conducted by Tacket et al.²⁵ The Tacket study in turn was designed following advice received from the VRBPAC in 1998. The blinding procedure was determined by this reviewer to be acceptable given the logistical challenge of ensuring that the vaccine and placebo appear and taste similarly. It is noted that it is possible that subjects may have been able to guess which treatment they received which would have introduced bias. However, the study results, which show similar rates of self-reported adverse reactions among subjects assigned to vaccine and placebo groups, do not seem to suggest that bias had been introduced in this manner. The randomization procedure was determined by this reviewer to be acceptable.
- Conducting a study with a challenge at 6 or 12 months post-vaccination was considered by the Applicant, but subject retention was a concern. A 3 month duration of protection was considered sufficient, since most travelers are likely to conclude their travel within that time. Similarly, a demonstration of onset of protection at 10 days post-vaccination is considered to be appropriate, since most travelers are likely to schedule travel vaccination more than a week in advance of their travel date. The Applicant's assumption regarding the length of U.S. travelers' stay in cholera-affected areas and the assumption that U.S. travelers are likely to schedule travel more than 1 week in advance of their travel date are reasonable assumptions to this reviewer.
- The study population was "enriched" for subjects with type O blood, since individuals with type O blood who become infected with *V. cholerae* O1 typically develop cholera of greater severity than those with type A or B blood. An estimated 44% of the U.S. population has type O blood. By enriching to a target of 60%, the size of the logistically complex challenge study was limited as a result of the expected increase in the placebo group attack rate. Ultimately, 56% of subjects challenged in study PXVX-VC-200-003 had type O blood.

6.1.3 Population

Inclusion Criteria:

1. Able to understand the study and give written consent.
2. Healthy males and females, age 18 to 45 years (inclusive), without clinically significant medical history, physical exam abnormalities, clinical laboratory abnormalities (as per protocol-defined acceptable ranges), or protocol-defined abnormal electrocardiogram results at screening and pre-challenge.
3. Women of childbearing potential (e.g., neither surgically sterile nor postmenopausal for < 1 year) had to meet the following criteria:
 - a. Negative pregnancy test at screening, prior to vaccination and challenge
 - b. Agree to practice abstinence or use an effective licensed method of birth control within 2 months of vaccination, and

- c. Agree to continue such precautions during the study and for 30 days post-challenge.
Males had to agree not to father a child for 30 days post-vaccination.
- 4. Pass a written examination, with a score of $\geq 70\%$ correct, demonstrating comprehension of study procedures and possible side effects. If the subject scored $\geq 50\%$ correct, he/she could retake the test after undergoing re-education, but could not participate if the second score was $< 70\%$.
- 5. Agreed to not participate in another investigational vaccine or drug trial during the duration of the study.
- 6. Willing and able to comply with the study requirements and procedures.

Exclusion Criteria:

- 1. Clinically significant history of immunodeficiency, cardiovascular disease, respiratory disease, endocrine disorder, liver disease, renal disease, gastrointestinal disease, anal or rectal disorders, neurologic illness, psychiatric disorder requiring hospitalization, or current drug or alcohol abuse.
- 2. History of hospitalization for psychiatric illness, suicide attempt, or confinement for danger to self or others within the past 10 years. Subjects with other psychiatric disorders that had been controlled for a minimum of 3 months and whose mental status the investigator determined would not compromise their ability to comply with protocol requirements could be enrolled.
- 3. Elevated blood pressure, ≥ 150 systolic or ≥ 90 diastolic mm Hg, before vaccination.
- 4. Use of any of the following psychiatric drugs: aripiprazole, carbamazepine,
 - a. chlorpromazine, chlorprothixene, clozapine, divalproex sodium, fluphenazine, haloperidol, lithium carbonate, lithium citrate, loxapine, mesoridazine, molindone, olanzapine, perphenazine, pimozide, quetiapine, risperidone, thioridazine, thiothixene, trifluoperazine, triflupromazine, or ziprasidone.
- 5. Use of > 1 antidepressant drug not included in the list above. Subjects taking only one antidepressant drug not listed above who had been stable for at least 3 months prior to enrollment without decompensating were allowed enrollment into the study provided the investigator determined the subject's mental status would not compromise the subject's ability to comply with protocol requirements.
- 6. Abnormal stool pattern defined as:
 - a. < 3 stools per week or > 2 stools per day in the past 6 months, *and*
 - b. loose stools during the 1–2 day acclimation period before challenge.
- 7. Regular use of laxatives in the past 6 months.
- 8. History of eating disorders (ie, bulimia) within the past 10 years.
- 9. Known allergy to, or known medical condition that precludes the use of both tetracycline and/or ciprofloxacin.
- 10. Previously received a licensed or investigational cholera vaccine.
- 11. History of cholera or enterotoxigenic *E. coli* infection (natural infection or experimental challenge).⁶
- 12. Travel to a cholera-endemic area in the previous 5 years.
- 13. Recipient of bone marrow or solid organ transplant.

⁶ The heat-labile enterotoxin of enterotoxigenic *Escherichia coli* (ETEC) is similar to cholera toxin and causes secretory diarrhea by the same mechanism. Dukoral has been shown to provide some cross-protection against ETEC.^{2,41,42}

14. Use of systemic chemotherapy in the previous 5 years prior to the study.
15. Malignancy (excluding non-melanotic skin cancers) or lymphoproliferative disorders diagnosed or treated during the past 5 years.
16. Received or planned to receive systemic immunosuppressive therapy, radiation therapy, parenteral or high-dosage inhaled steroids (> 800 ug/day of beclomethasone dipropionate or equivalent) within 6 months prior to the study vaccination through 28 days post-vaccination or challenge, whichever is longer.
17. History of Guillain-Barré Syndrome.
18. Pregnant or nursing.
19. Positive serology for HIV, hepatitis B antigen, or hepatitis C.
20. Clinically abnormal screening electrocardiogram, defined as pathologic Q waves or significant ST-T wave changes; criteria for left ventricular hypertrophy; and any non-sinus rhythm excluding isolated premature atrial contractions.
21. A clinically significant abnormality detected on physical examination, including, but not limited to a pathologic heart murmur, lymphadenopathy, hepatosplenomegaly, or a large abdominal scar of unclear origin.
22. Poor venous access, defined as the inability to withdraw the required amount of blood for screening tests after 4 attempts.
23. Received or planned to receive any other licensed vaccines, except for seasonal influenza vaccine, from 14 days prior to the study vaccination until 28 days post-vaccination or challenge, whichever is longer.
24. Received or planned to receive antibiotics (other than protocol-specified) or chloroquine within 14 days prior to the study vaccination through 28 days post-vaccination or challenge, whichever is longer.
25. Febrile illness (temperature > 100.4°F) within 48 hours prior to challenge and/or vaccine administration.
26. Taking any prescription or over the counter medications containing aspirin, acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), antacids, proton pump inhibitors, anti-diarrheals, etc. within 48 hours prior to challenge and/or vaccine administration.
27. Other condition(s) that in the opinion of the investigator would jeopardize the safety or rights of a subject participating in the trial or would render the subject unable to comply with the protocol.

In-patient Challenge Eligibility

1. Continue consent to participate in the study.
2. Pass a written examination, with a score of $\geq 70\%$ correct, before inoculation with the challenge strain to demonstrate comprehension of study procedures and possible side effects. If subjects scored $\geq 50\%$ correct, they could retake the exam after undergoing re-education, but were ineligible if the second score was $< 70\%$.
3. If study personnel deemed a subject unsuitable for in-patient stay (psychological evaluations could be conducted at the site's discretion), the pregnancy test was positive, or the subject was no longer available for other reasons, the subject was excluded from participating in the challenge.
4. The stratified randomization scheme used to select subjects for challenge was designed to ensure that at least 60% of subjects in each group in the challenge had blood type O.

6.1.4 Study Treatments or Agents Mandated by the Protocol

The placebo and vaccine were dispensed in an opaque cup. 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. Subjects took nothing by mouth (food and water) for 60 minutes before and 60 minutes after vaccination and were asked not to discuss the taste of the product with the clinic staff.

PXVX0200 is derived from the wild type *V. cholerae* O1 classical Inaba parent strain 569B. Clinical trial material was manufactured from PaxVax's working seed Lot No. (b) (4), which was derived from the CVD 103-HgR expanded progenitor seed obtained from the (b) (4). Lyophilized PXVX0200 was manufactured according to current GCP at (b) (4) and was

(b) (4) Packets were filled at PaxVax, Inc. (b) (4). The packets were stored at -20 °C (± 5°C) until used. PXVX0200 was reconstituted in 100 mL of bicarbonate buffer solution. All subjects received a single dose of the final vaccine suspension (buffer packet + bacteria packet + water). PXVX0200 lot number administered in study -003: B701.550-8WA02. Each single-dose packet contained 5×10^8 CFU lyophilized CVD 103-HgR at release in study -003. The sodium bicarbonate buffer solution was prepared by reconstituting the contents of a single-use buffer packet (b) (4) containing 2.5 g NaHCO₃, 1.5 g of ascorbic acid, and 0.2 g dried lactose in 100 mL of sterile Water for Irrigation USP.

Placebo consisted of 100 mL of physiological saline administered orally.

Challenge consisted of 1×10^5 CFU of wild type *V. cholerae* O1 El Tor Inaba strain N16961 (NIH under IND 6476) diluted in 30 mL NaHCO₃. The challenge strain was isolated originally from the stool of a cholera patient in Dacca, Bangladesh on October 15, 1975. NIH prepared a batch of frozen *V. cholerae* strain N16961 with a large number of aliquots so that identical vials from the lot could be used for volunteer challenge studies.⁴⁶ One minute prior to challenge, subjects ingested 120 mL NaHCO₃. Subjects ingested nothing by mouth from midnight before administration of the challenge strain, except for water, and took nothing by mouth 90 minutes after ingestion of the challenge strain.

Clinical Reviewer Note: A single challenge strain was used in study -003 for the following reasons: 1) O1 is the dominant serogroup worldwide, 2) El Tor is the dominant O1 biotype causing disease worldwide; 3) strain N16961 is the only clinical grade challenge strain available for human use; and 4) the product indication will specify that the vaccine does not protect against serogroup O139. The same challenge strain and dose produced a 91% attack rate for any diarrhea and a 39% attack rate for moderate to severe diarrhea among unvaccinated subjects in a prior study (Tacket et al 1999).

6.1.5 Directions for Use

Study sites were instructed to reconstitute the vaccine within 30 minutes after removing the product from the freezer/refrigerator. The buffer component packet was first mixed in 100 mL of sterile water for irrigation; subsequently the active component packet was

added and mixed. The product was to be administered orally within 30 minutes of reconstitution.

The investigational vaccine and placebo were administered by unblinded study personnel. Compliance with study-prescribed vaccine or placebo administration was monitored and recorded. If a subject failed to receive the complete volume of the assigned dose, or if there was difficulty with administration, this non-compliance was documented along with the reason for the incomplete dose.

6.1.6 Sites and Centers

The trial was conducted at 3 sites in the U.S. (Baltimore, MD; Cincinnati, OH; and Burlington, VT). Due to the space constraints and safety considerations at each site, the challenges were split into 3 cohorts in Vermont, 4 cohorts in Maryland and 4 cohorts in Cincinnati. Cohorts 1 and 2 were composed of subjects who participated in the 10-Day Challenge and Cohorts 3 and 4 were composed of subjects who participated in the 3-Month Challenge.

6.1.7 Surveillance/Monitoring (Table 5)

- 90 minute observation period post-vaccination for acute side effects.
- Solicited adverse reactions: recorded on days 1-8 following vaccination by subjects on a daily memory aid and graded by severity. Solicited adverse reactions post-challenge were recorded daily via physician assessment during the inpatient period (about 10 days). Study personnel reviewed the memory aid to confirm relationship and grade the severity of each reaction. Adverse reactions were those signs/symptoms considered by the Investigator as at least possibly related to the study vaccine. Signs/symptoms determined to be definitely unrelated to study vaccine by the Investigator were reported as an AE.
 - Fever (temperature), fatigue, headache, lack of appetite, nausea/vomiting, diarrhea, and abdominal pain. Diarrhea was defined as passage of ≥ 2 unformed stools (Grade 3-5) over a 48 hour period that was ≥ 200 grams or a single unformed stool that totaled ≥ 300 g. Subjects with diarrhea had all diarrheal stools weighed and recorded using a weight to volume conversion of 1g = 1 mL.
 - *Pre-challenge/post-vaccination* diarrhea grading: 1 (mild): 4 loose stools/24 hours; 2 (moderate): 5 loose stools/24 hours; 3 (severe): ≥ 6 loose stools/24 hours; 4 (potentially life threatening): emergency room (ER) visit or hospitalization.
 - Fever grading scale: 1(mild): 38.0-38.4°C; 2 (moderate): 38.5-38.9°C; 3 (severe): 39-40°C; 4 (potentially life threatening) $> 40^\circ\text{C}$.
 - Vomiting grading scale: 1 (mild): 1-2 episodes/24 hours; 2 (moderate): > 2 episodes/24 hours; 3 (severe): requires IV hydration; 4 (potentially life threatening): ER visit or hospitalization for hypotensive shock.
 - Headache, fatigue, myalgia, abdominal pain, and lack of appetite grading: 1 (mild): mild, no interference with activity; 2 (moderate): some interference with activity; 3 (severe): significant, prevents daily activity; 4 (potentially life threatening): ER visit or hospitalization.
 - *Post-challenge diarrhea grades* were assigned based on cumulative diarrheal volume as follows:
 - Mild: < 3 L; Moderate: ≥ 3 L to 5 L; Severe: > 5 L.
 - Post-challenge diarrhea grades were assigned based on cumulative diarrheal volume and consistency as follows:

- Mild: < 3 L; Moderate: ≥ 3 L to 5 L; Severe: > 5 L.
- Post-challenge stools were graded as follows:
 - 1: Normal – formed (normal, does not take shape of container);
 - 2: Soft (normal, does not take shape of container);
 - 3: Thick (liquid diarrheal, takes shape of container);
 - 4: Opaque watery;
 - 5: Rice water (clear water).
- Unsolicited AEs were recorded from the day of vaccination through 28 days post-vaccination or 28 days after challenge, whichever was later.
- SAEs and medically attended AEs were recorded through 6 months post-vaccination.
- AEs were classified by MedDRA version 15.0.
- Fresh stool samples were obtained from the first two specimens per subject each day for quantitative and qualitative culture. If a stool sample was not available, a rectal swab was collected for qualitative culture.
- At each visit, details of prior and concomitant medication usage were collected and recorded in the electronic case report form (CRF).
- The unblinded site monitor performed investigational vaccine accountability (i.e., compliance with study prescribed vaccine or placebo administration).
- See Table 6 for more details regarding inpatient procedures/monitoring.
- Clinical safety laboratory evaluations were performed at screening, prior to receipt of study product for the 10-day challenge and within 1 week prior to challenge for the 3-month challenge group. Safety laboratory tests included:
 - Chemistry: direct and total bilirubin, alkaline phosphatase, albumin, AST, ALT, creatinine, potassium, sodium
 - Hematology: Hemoglobin, WBC and differential
 - Urinalysis

Clinical Reviewer Note:

- The grading scale used for grading solicited adverse reactions was based on FDA's Guidance entitled *Toxicity Grading Scale for Healthy Adult and Adolescent Subjects Enrolled in Preventative Vaccine Clinical Trials*. However, the scale was modified to accommodate the historical definition of diarrhea in cholera vaccine studies. Diarrhea post-vaccination/pre-challenge was defined as ≥ 4 loose stools/24 hours rather than ≥ 2 loose stools/24 hours.
- The definition of diarrhea above is consistent with the definition of diarrhea used in previous cholera challenge studies. Although in clinical practice, the definition of diarrhea is loosely defined as passage of abnormally liquid or unformed stools at an increased frequency^{43,44}, for adults on a typical Western diet, stool weight > 200 grams per day is a generally accepted definition of diarrhea (Harrisons, 18th edition). This definition is considered acceptable to the FDA clinical reviewer for the purpose of the pre-licensure clinical trials with Vaxchora.

Table 4. PXVX-VC-200-003 Study Flowchart for Vaccination Phase

Study Days	Screen (-60 to -1 d)	Baseline 1	8	10 Day Challenge ^a	11	29	3 Month Challenge ^a	181
Visit Number	1	2	3		4	5		6
Informed Consent	X							
Medical History	X	X						
Physical Examination ^b	X							
Vital Signs	X	X						
Safety Laboratory Tests	X							
ABO Blood Typing	X							
HBsAg, anti-HCV (b) (4), HIV (b) (4)	X							
Pregnancy Test	X	X ^c						
Randomization		X						
Vaccination		X						
Observe 90 min for acute reactions		X						
Adverse Event Evaluation		X	X		X	X		X
Prior/Concomitant Medication	X	X	X		X	X		X
Memory Aid: Solicited Adverse Reactions		Distriabuted	Collected					
Serology ^d		X ^c	X		X	X		X
PBMC: Memory B Cell (Immune Subset)		X						

^a Refer to Table 6 for Challenge Phase Study Flowchart.

^b A physical e xam could be performed at additional time points if indicated by AE monitoring.

^c Conducted or collected before vaccination.

^d Vibriocidal Assay and Anti-CT (b) (4)

Source: STN 125597_0. Module 5.3.5.1. Study PXVX-VC-200-003 Clinical Study Report, Table 5.

Table 5. PXVX-VC-200-003 Study Flowchart for Challenge Phase

10 Day Challenge Study Days (Post-Vaccination)	9-10	11	12	13	14	15	16	17	18	19	20	21	Discharge	39	181
Visit Number	4-5	6	7	8	9	10	11	12	13	14	15	16		17	18
3 Month Challenge Study Days (Post-Vaccination)	89-90	91	92	93	94	95	96	97	98	99	100	101		119	181
Visit Number	6-7	8	9	10	11	12	13	14	15	16	17	18		19	20
Continuing informed consent (written exam)	X														
Admission/acclimation	X														
Physical Exam	X	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a		X ^a	X ^a
Vital Signs	X	X ^b	X ^b	X ^b	X ^b	X ^b	X ^b	X ^b	X ^b	X ^b	X ^b	X ^b		X ^a	X ^a
EKG		X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a			
Safety Labs	X ^d	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a			
Urine Pregnancy Test, if required	X														
Challenge		X													
Antibiotic Treatment (if severe diarrhea or ≥ 96 hours post-challenge)						X	X	X	X	X	X				
Oral or IV Rehydration		X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a	X ^a			
Solicited Adverse Reactions		X	X	X	X	X	X	X	X	X	X	X			
Adverse Event Evaluation	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Prior/Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Serology - Vibriocidal Assay		X ^c										X		X	X
Serology - Anti-CT (b) (4)		X ^c													X
Stool Collection		X	X	X	X	X	X	X	X	X ^a	X ^a	X ^a			
PBMC: B-cell memory ^f		X													X ^g

^a Determined as needed by the Investigator.

^b Taken every 8 hours unless more frequent monitoring was needed.

^c Collected before challenge.

^d Abbreviated safety labs taken prior to vaccination for 10 day challenge group and within 1 week prior to admission into the in-patient unit for the 3 month challenge group. Results obtained before challenge strain inoculum.

^e If a subject met discharge criteria prior to Day 21 (or Day 101), performed procedures listed for Day 21 (or Day 101) (±3 days).

^f A subset of subjects provided whole blood for B cell memory assessment measured at Day 1 pre-vaccination, at Day 91 in subjects challenged in the 3-month challenge group, and at Day 181 in non-challenged vaccine and challenged placebo recipients in the 10-day challenge group.

^g 10 day challenge group.

Source: STN 125597_0. Module 5.3.5.1. Study PXVX-VC-200-003 Clinical Study Report, Table 6.

6.1.8 Endpoints and Criteria for Study Success

Primary Endpoints

Two primary analyses were performed. Please refer to Section 6.1.1 for details. The null hypothesis states that vaccine efficacy is < 30%. The alternative hypothesis states that vaccine efficacy is ≥ 30%. Success on both primary analyses (at both the 10-day and 3-month challenges) was required to achieve success for the trial.

Secondary Endpoints

The severity of post-challenge disease was assessed based on 2 continuous endpoints: total weight of diarrheal stools and total number of diarrheal stools. The incidence of post-challenge fecal shedding of the challenge strain was evaluated by comparing Vaxchora recipients challenged at 10 days post-vaccination and Vaxchora recipients challenged at 3 months post-vaccination against a pooled group of placebo recipients challenged at either post-vaccination time point.

Tertiary and Exploratory Immunogenicity Endpoints

- Serum vibriocidal antibody measured by a vibriocidal antibody assay, performed at (b) (4) using assays transferred from (b) (4). The LLOQ for each vibriocidal antibody assay (classical Inaba, classical Ogawa, El Tor Inaba and El Tor Ogawa) is (b) (4).
- Anti-CT IgG antibody titers measured by (b) (4), performed at (b) (4) using assays transferred from (b) (4).
- Peripheral blood mononuclear cell (PBMC) specimens were obtained for memory B cells against CT subunit B and cholera LPS, measured by (b) (4) by blinded readers after study unblinding (unlike other testing). This qualified but non-validated method was performed by PaxVax. Exploration of the relationship between 4 types of antigen-specific memory B cells (anti-O1 LPS IgG, anti-O1 LPS IgA, anti-CT IgG and anti-CT IgA) with diarrheal volume was conducted.⁸
- Immune response parameters were assessed as potential immunologic correlates of vaccine-induced protection against moderate/severe diarrhea.

Table 6 shows the timing of immunogenicity analyses for each analysis group.

Table 6. PXVX-VC-200-003. Serum Vibriocidal and Anti-Cholera Toxin Antibody Assays

Group	Classical Inaba (Days)	El Tor Inaba Classical Ogawa El Tor Ogawa (Days)	Anti-Cholera Toxin IgG (b) (4) (Days)	Memory B Cells (Days)
Unchallenged	1, 8, 11, 29, 181 ^a	1, 8, 11, 29, 181 ^a	1, 8, 11, 29, 181 ^a	1, 181 ^a
10-Day Challenge	1, 8, 11, 39, 181 ^b	1, 11, 181 ^b	1, 8, 11, 181 ^b	1, 181 ^b
3-Month Challenge	1, 8, 11, 29, 91 ^c , 119, 181	1, 11, 29, 91 ^c , 181	1, 8, 11, 29, 91 ^c , 181	1, 91 ^c

Note: Vaccination = Day 1.

⁷ Module 5.3.1.4 Validation Report for Serum Vibriocidal Assays.

⁸ The primary outcome variable for these analyses was total diarrheal volume rather than moderate/severe cholera since memory B cell percentages are continuous and are most efficiently analyzed using a continuous endpoint.

Note: Pre-challenge immunogenicity endpoints were analyzed by combining all pre-challenge data (*italics*) across groups. Post-challenge immunogenicity (**bold**) endpoints were reported separately by group for the 10-day, 3-month and placebo groups.

^a Performed in vaccine recipients to determine Day 181 post-vaccine levels.

^b Performed in challenged placebo recipients to determine Day 181 post-challenge levels.

^c Performed in vaccine recipients prior to challenge to determine Day 91 post-vaccine/pre-challenge levels. This analysis was added for placebo recipients after initial data analysis was performed in order to gain a better understanding of the uniqueness of the vaccine-induced response.

Source: STN 125597_0. Module 5.3.5.1, PXVX-VC-200-003 Clinical Study Report, Table 10.

Clinical Reviewer Note: Vibriocidal antibodies directed against heterologous cholera strains of *V. cholerae* O1 were measured in order to assess the level of cross-reactive antibody response induced by the classical Inaba vaccine strain. Cross-reactive antibody responses were also assessed in study -005.

6.1.9 Statistical Considerations & Statistical Analysis Plan

- Once all subjects in the 10-day challenge group completed the clinic visit 28 days after challenge, an interim analysis was conducted to evaluate the 10-day challenge primary endpoint and to identify potential immunological correlates of protection. A 99.9% CI on vaccine efficacy was constructed at the interim analysis. A small alpha penalty ($\alpha=0.001$ of the overall α of 0.05 for the 10-day challenge) was taken to maintain the overall Type 1 error at $\leq 5\%$ across both co-primary objective analyses. A small alpha penalty ($\alpha=0.001$), of the overall alpha was allocated to the 3-month challenge, because the attack rate of half of the pooled placebo group was revealed at the interim analysis. A 95.1% 2-sided CI was used to assess the primary analysis of the 10-day and 3-month challenges.
- Sample size calculation:
 - Efficacy was estimated at 90% based on results of a challenge trial similar to the current study.²⁵ The expected attack rate of moderate to severe diarrhea was estimated from two prior trials involving 63 placebo recipients challenged with the same dose and strain of virulent *V. cholera* used in PXVX-VC-200-003.^{25,46}
 - The 10 day and 3 month challenge sample sizes provide 95% and 93% power respectively for meeting the associated co-primary objectives. If analyses of the two challenge groups were completely independent, which is a conservative assumption, the power for achieving both co-primary objectives would be $95\% \times 93\%=88\%$.
- No adjustments for covariates were made in any analyses.
- Estimates of efficacy against moderate to severe diarrhea and efficacy against mild or worse diarrhea were provided for sex, race and blood type subgroups. Analyses were not broken down by age due to the narrow age range of subjects enrolled in the trial nor by ethnicity due to the small number of Hispanic or Latino subjects enrolled.

Analysis Populations:

Five analysis populations were delineated: the randomized population, the intent-to-treat (ITT) population, the safety population, the immunogenicity evaluable population, and the memory B cell analysis population. All efficacy analyses, except for the immunogenicity analyses, were based on the ITT population unless otherwise stated. Immunogenicity analyses were based on the Immunogenicity Evaluable Population.

1. The *Randomized Population* includes all subjects randomized to the study; this population was used in all demographics and baseline summaries.
2. The *ITT Population* includes all randomized subjects who received treatment (based on subject's randomized treatment); this population was used in all efficacy analyses.
3. The *Safety Population* includes all subjects who received treatment; this population was used in all safety analyses.
4. The *Immunogenicity Evaluable Population* includes all subjects who received treatment and had evaluable classical Inaba vibriocidal antibody results from Day 1 and from at least one post-vaccination time point prior to challenge; this population was used for immunogenicity analyses.
5. The *Memory B cell Analysis Population* includes all subjects in the ITT population who received vaccine and were not challenged or who received placebo and were challenged at 10 days post-vaccination and had evaluable anti-O1 LPS IgA memory B cell results at Day 1 and Day 91. The same analyses were added for subjects who received placebo and were challenged at 3 months post-vaccination after initial data analysis was performed.

6.1.10 Study Population and Disposition

6.1.10.1 Populations Enrolled/Analyzed

See Section 6.1.10.1.3.

6.1.10.1.1 Demographics

Among all randomized subjects, the mean age was 31.0 yrs. More males were in the vaccine group (71.6%) compared to the placebo group (54.9%). Overall by race, 67.5% of randomized subjects were Black, 29.4% were White, 0.5% were American Indian/Alaskan Native, 0.5% were Asian, and 2.0% were other. By ethnicity, 4.6% were Hispanic or Latino. Overall 50.3% had blood type O.

Among subjects selected for either challenge cohort, the mean age of the challenge population was 31.4 years. More males were challenged in the vaccine group (76.5%) compared to the placebo group (57.6%). Overall by race, 70.9% of the challenge population was Black, 25.4% were White, 0.7% were American Indian/Alaskan Native, 0.7% were Asian, and 2.2% were other. By ethnicity, there were 3.7% Hispanic or Latino participants. Overall, 56.0% of challenged subjects had blood type O.

Baseline demographics and blood type for vaccine and placebo recipients by challenge status are shown in Table 7. There was a higher proportion of subjects with Type O blood in the challenged groups compared to the unchallenged groups, because the target was that 60% of subjects in each challenge group would have blood type O. Enrichment for blood type O may have also led to a slight racial imbalance between challenged and unchallenged subjects, since the prevalence of Type O blood is higher in black adults than in white adults.⁴⁵

Demographics of the Immunogenicity Evaluable Population and Intent-to-Treat (ITT) Populations (data not shown) were similar to the demographics of the PXVX0200 subjects in the 10-day challenge; however, 50% of PXVX0200 and 50% of placebo recipients in the Immunogenicity Evaluable and ITT Populations had Type O blood and 67% of PXVX0200 subjects and 68% of placebo subjects were Black.

Table 7. PXVX-VC-200-003. Demographics – Randomized Population.

Baseline Characteristics	10 Day Challenged		3 Month Challenge		Unchallenged	
	PXVX0200 N=35	Placebo N=33	PXVX0200 N=33	Placebo N=33	PXVX0200 N=27	Placebo N=36
Age in years						
Mean±SD	30.5±6.7	31.6±8.4	33.1±8.2	30.3±7.7	30.8±8.3	29.8±7.5
Gender, n(%)						
Male	25 (71.4)	18 (54.5)	27 (81.8)	20 (60.6)	16 (59.3)	18 (50.0)
Female	10 (28.6)	15 (45.5)	6 (18.2)	13 (39.4)	11 (40.7)	18 (50.0)
Race, n(%)						
American Indian or Alaskan Native	1 (2.9)	0	0	0	0	0
Asian	1 (2.9)	0	0	0	0	0
Black or African American	21 (60.0)	21 (63.6)	27 (81.8)	26 (78.8)	16 (59.3)	22 (61.1)
White	10 (28.6)	11 (33.3)	6 (18.2)	7 (21.1)	10 (37.0)	14 (38.9)
Other	2 (5.7)	1 (3.0)	0	0	1 (3.7)	0
Ethnicity, n(%)						
Hispanic or Latino	2 (5.9)	1 (3.0)	1 (3.0)	1 (3.1)	2 (7.4)	2 (5.6)
Not Hispanic or Latino	32 (94.1)	32 (97.0)	32 (97.0)	31 (96.9)	25 (92.6)	34 (94.4)
ABO Blood Type, n(%)						
Type O	19 (54.3)	19 (57.6)	20 (60.6)	17 (51.5)	9 (33.3)	15 (41.7)
Not Type O	16 (45.7)	14 (42.4)	13 (39.4)	16 (48.5)	18 (66.7)	21 (58.3)

Note: Percentages were based on the number of randomized subjects who had non-missing values in each treatment arm.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-003 Clinical Study Report, Table 13

6.1.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

At least one pre-existing medical history was reported by 89.5% of PXVX0200 recipients, 92.2% of placebo recipients, 85.3% of challenged PXVX0200 recipients and 90.0% of challenged placebo recipients. The most commonly reported medical history overall included immune system disorders (32.5%), nervous system disorders (22.8%), infections and infestations (17.3%) and tobacco user (16.8%). Gastrointestinal disorders were reported by 11.6% and 11.8% of PXVX0200 and placebo recipients respectively; they were reported by 10.3% and 10.6% of challenged PXVX0200 and placebo recipients respectively. With a few exceptions, the prevalence of medical history was well-balanced between the two vaccine groups and the challenged PXVX0200 recipients vs challenged placebo recipients. A higher proportion of placebo recipients (13.7%) and challenged placebo recipients (12.1%) had a history of asthma compared to PXVX0200 recipients (4.2%) and challenged PXVX0200 recipients (4.4%). Immune system disorders were reported by a slightly higher proportion of challenged PXVX0200 recipients (33.8%) compared to challenged placebo recipients (28.8%). However, there was no consistent trend seen in preferred terms (PTs) terms within the Immune System Disorders system organ class (SOC). There was a slightly higher rate of tobacco use among challenged PXVX0200 recipients (19.1%) compared to challenged placebo recipients (15.2%). There was also a higher rate of skin and subcutaneous tissue disorders among placebo recipients (12.7%) and challenged placebo recipients (15.2%) compared to PXVX0200 recipients (9.5%) and challenged PXVX0200 recipients (8.8%). PXVX0200 recipients (7.4%) and challenged PXVX0200 recipients (5.9%) reported a history of vascular disorders more frequently than the corresponding placebo group (1.0% and 1.5% respectively).

Clinical Reviewer Note:

- Reported medical history included prior procedures and injuries.
- An imbalance in the proportion of males and females in the vaccine and placebo groups was noted. In addition, more males and more black subjects were challenged compared to females and white subjects respectively. The noted imbalances are not suspected to impact the clinical results of this trial, as the subgroup analyses by sex, race, and blood type did not show any clear and consistent differences in effectiveness.

6.1.10.1.3 Subject Disposition

Table 8 below summarizes the disposition of all randomized subjects by study visit number. A total of 197 subjects were randomized in this study; 95 subjects were randomized to PXVX0200 and 102 were randomized to receive placebo. A total of 134 of the 197 subjects were challenged: 68 vaccine recipients and 66 placebo recipients. Thirty-five of the vaccine recipients were challenged at Day 11, and 33 at Day 91: 33 of the placebo recipients were challenged at Day 11 and 33 at Day 91.

Table 8. PXVX-VC-200-003. Subject Disposition – Randomized Population

	PXVX0200 Group			Placebo Group		
	Challenged Day 11 (N=35) n (%)	Challenged Day 91 (N=33) n (%)	Not Challenged (N=27) n (%)	Challenged Day 11 (N=33) n (%)	Challenged Day 91 (N=33) n (%)	Not Challenged (N=36) n (%)
Pre-Challenge Visit Completion ^a						
Day 8	35 (100%)	33 (100%)	26 (96.3%)	33 (100%)	33 (100%)	35 (97.2%)
Day 11	-	33 (100%)	26 (96.3%)	-	33 (100%)	34 (94.4%)
Day 29	-	33	24 (88.9%)	-	32 (97.0%)	36 (100%)
Post-Challenge Visit Completion ^b						
Day 39 ^c	35 (100%) ^c	-	-	32 (97.0%) ^c	-	-
Day 119 ^c	-	33 (100.0%) ^c	-	-	32 (97.0%) ^c	-
Study Completion - Day 181	35 (100.0%)	33 (100.0%)	24 (88.9%)	32 (97.0%)	33 (100%)	34 (94.4%)
Reasons for Early Termination						
Lost to follow-up	0	0	0	0	3 (11.1%)	1 (2.8%)
Noncompliance	0	1 (3%)	0	0	0	0
AE or Lab Abnormality	0	0	0	0	0	0
Other Reason	0	0	0	0	0	1 (2.8%) ^d

^a All subjects completed the Day 1 prechallenge visit.

^b All subjects challenged on Day 11 and Day 91 completed the Day 11 and Day 91 post-challenge visits respectively.

^c 28 days post-challenge.

^d This subject terminated early due to incarceration.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-003 Clinical Study Report, Figure 1 and Table 11.

Subject enrollment by site is shown in Table 9.

Table 9. Study PXVX-VC-200-003, Subject Enrollment by Site – Randomization Population

Site Number	Site	PXVX0200 N=95 n (%)	Placebo N=102 n (%)	Total N=197 n (%)
001	Baltimore, MD	34 (35.8)	31 (30.4)	65 (33.0)
003	Cincinnati, OH	39 (41.1)	44 (43.1)	83 (42.1)
004	Burlington, VT	22 (23.2)	27 (26.5)	49 (24.9)

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-003 Clinical Study Report, Table 4.

Table 10 summarizes the number and proportion of subjects in each analysis population by challenge status.

Table 10. Study PXVX-VC-200-003. Analysis Populations.

Population	PXVX0200 N=95 n (%) ^a	Placebo N=102 n (%) ^a	Total N=197 n (%) ^a
Randomized Subjects	95 (100.0)	102 (100.0)	197 (100.0)
10 Day Challenge Group	35 (36.8)	33 (32.4)	68 (34.5)
3 Month Challenge Group	33 (34.7)	33 (32.4)	66 (33.5)
Unchallenged Subjects	27 (28.4)	36 (35.3)	63 (32.0)
ITT Population	95 (100.0)	102 (100.0)	197 (100.0)
10 Day Challenge Group	35 (36.8)	33 (32.4)	68 (34.5)
3 Month Challenge Group	33 (34.7)	33 (32.4)	66 (33.5)
Unchallenged Subjects	27 (28.4)	36 (35.3)	63 (32.0)
Safety Population	95 (100.0)	102 (100.0)	197 (100.0)
10 Day Challenge Group	35 (36.8)	33 (32.4)	68 (34.5)
3 Month Challenge Group	33 (34.7)	33 (32.4)	66 (33.5)
Unchallenged Subjects	27 (28.4)	36 (35.3)	63 (32.0)
Immunogenicity Evaluable Population	94 (98.9)	102 (100.0)	196 (99.5)
10 Day Challenge Group	35 (36.8)	33 (32.4)	68 (34.5)
3 Month Challenge Group	33 (34.7)	33 (32.4)	66 (33.5)
Unchallenged Subjects	26 (27.4)	36 (35.3)	62 (31.5)
Memory B Cell Analysis Population	55 (57.9)	26 (25.5)	81 (41.1)
10 Day Challenge Group	0 (0)	26 (25.5)	26 (13.2)
3 Month Challenge Group	33 (34.7)	0 (0)	33 (16.8)
Unchallenged Subjects	22 (23.2)	0 (0)	22 (11.2)

^a Percentages were based on the number of randomized subjects in each treatment arm.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-003 Clinical Study Report. Table 12.

Protocol Deviations

A total of 47.4% (45/95) of vaccine recipients and 39.2% (40/102) of placebo recipients had protocol deviations. About 6% were related to inclusion/exclusion criteria not being met. Among challenged subjects, 39-40% had protocol deviations. In general, protocol deviations consisted of issues such as post-vaccination vital signs being taken early, out of window visits, and screening lab or vital sign deviations that were not clinically significant (i.e., transient minor out of range values) and pertained to the assessment of eligibility for inclusion in enrollment or in one of the two challenge groups. Other examples of deviations include the following:

- One subject who spilled ~ 2 mL of vaccine during administration was consequently not challenged; one placebo recipient received antibiotics 17 days after placebo injection for a urinary tract infection, chlamydia and upper respiratory infection, and this subject was included in the 3 month challenge (both deviations were assessed as minor without impact on immune response, and the subjects were retained in the Immunogenicity Evaluable Population).
- Five vaccine recipients in the 3 month challenge group received antibiotics 1.5 hours prior to the 96 hours post-challenge time point.⁹
- One vaccine recipient in the 10 day challenge group took acetaminophen < 48 hours prior to receiving vaccination. One vaccine recipient was noted 1 day post-vaccination to be taking ibuprofen every 6-8 hours for back pain, which had not been previously disclosed. Neither subject was challenged as planned at 3 months post-vaccination. Both subjects were retained in the immunogenicity evaluable population.
- One possibly related grade 3 AE was not reported in real time.
- One subject with probable Gilbert's Syndrome, a benign condition, obtained an enrollment waiver and was included in the 3 month challenge group.
- Two deviations included two subjects who had no records of receiving replacement fluids that were indicated.

Clinical Reviewer Note: Although rates of protocol deviations are noted to be high in both the vaccine (47.4%) and placebo (39.2%) groups, most were considered minor by the applicant and by the FDA clinical reviewer and did not result in a need for exclusion from immunogenicity and/or efficacy analyses.

6.1.11 Efficacy Analyses

6.1.11.1 Analyses of Primary Endpoint(s)

Table 11 presents the results of the primary analysis of vaccine efficacy for the 10-day and 3-month challenge groups in the ITT population. Both co-primary objectives of the study were met, as the lower bound of the 95.1% CI of vaccine efficacy was $\geq 30\%$ at both time points. Ten-day efficacy was 90.3% (95.1% CI 62.7%, 100.0%) and a 3-month efficacy was 79.5% (95.1% CI 49.9%, 100.0%).

Table 11. Study PXVX-VC-200-003. Vaccine Efficacy Against Moderate to Severe Diarrhea for the 10 Day and 3 Month Challenge Groups, Intent-to-Treat Population.

Parameter	PXVX0200 10 Day Challenge N=35 n (%)	PXVX0200 3 Month Challenge N=33 n (%)	Combined Placebo Challenges N=66 n (%)
Overall Severity			
No qualifying diarrhea	30 (85.7)	18 (54.5)	5 (7.6)
Mild: < 3 L - L of diarrhea	3 (8.6)	11 (33.3)	22 (33.3)
Moderate: ≥ 3 L - 5 L of diarrhea	1 (2.9)	2 (6.1)	11 (16.7)
Severe: > 5 L of diarrhea	1 (2.9)	2 (6.1)	28 (42.4)
Attack Rate ^a	2 (5.7)	4 (12.1)	39 (59.1)

⁹ Antimicrobials were to be administrated for severe diarrhea. All other subjects who did not receive antimicrobials were to be given antimicrobials starting 96 hours after challenge.

Parameter	PXVX0200 10 Day Challenge N=35 n (%)	PXVX0200 3 Month Challenge N=33 n (%)	Combined Placebo Challenges N=66 n (%)
Vaccine Efficacy (95% CI) ^b	90.3 (62.7, 100.0)	79.5 (49.9, 100.0)	-

Note: Percentages were based on the number of subjects with a non-missing value within each treatment group.

Note: Mild diarrhea was defined as the passage of ≥ 2 unformed stools (grade 3 to 5) over a 48 hour period ≥ 200 mL or a single unformed stool ≥ 300 mL and < 3 L total diarrhea.

^a Attack rate: Moderate or severe diarrhea (≥ 3 L overall diarrheal purge).

^b Vaccine efficacy = [(Attack Rate in Placebo Group – Attack Rate in Vaccine Group)/Attack Rate in Placebo Group]*100.

Source: STN 125597_0. Module 5.3.5.1, PXVX-VC-200-003 Clinical Study Report, Table 14 and STN 125597_17. Module 1.11.3 (Efficacy Information Amendment).

Clinical Reviewer Comment: Although the study did not pre-specify comparisons between the 10 day and 3 month challenge groups, the data suggest that vaccine efficacy is lower at 3-months post-vaccination compared to 10-days post-vaccination.

6.1.11.2 Analyses of Secondary Endpoints

Efficacy Against Diarrhea of Any Severity

Vaccine efficacy against diarrhea of any severity, a secondary endpoint, was 84.5% (95% CI, 67.0%-100.0%) in the 10-day challenge group and 50.8% (95% CI, 33.6%-66.8%) in the 3 month challenge group (Table 13). Vaccine efficacy against severe diarrhea was 93.3% (95% CI, 56.2%-100.0%) in the 10 day challenge group and 85.7% (95% CI, 46.2%-100.0%) in the 3 month challenge group. The incidence of diarrhea of any severity among vaccine recipients in the 10-Day and 3-Month challenge groups and among combined (10-day and 3 month) placebo recipient challenge groups is described in Table 12.

Table 12. Study PXVX-VC-200-003. Vaccine Efficacy Against Secondary Study Endpoints for the 10 Day and 3 Month Challenge Groups, Intent-to-Treat Population.

Parameter	PXVX0200 10 Day Challenge N=35 n (%)	PXVX0200 3 Month Challenge N=33 n (%)	Combined Placebo Challenges N=66 n (%)
Attack Rate of Mild or Worse Diarrhea	5 (14.3)	15 (45.5)	61 (92.4)
Vaccine Efficacy (95% CI) Against Mild or Worse Diarrhea	84.5 (67.0, 100.0)	50.8 (33.6, 66.8)	
Attack Rate of Severe Diarrhea	1 (2.9)	2 (6.1)	28 (42.4)
Vaccine Efficacy (95% CI) Against Severe Diarrhea	93.3 (56.2, 100.0)	85.7 (46.2, 100.0)	

Note: Percentages were based on the number of subjects with a non-missing value within each treatment group.

Note: Mild diarrhea was defined as the passage of ≥ 2 unformed stools (grade 3 to 5) over a 48 hour period ≥ 200 mL or a single unformed stool ≥ 300 mL and < 3 L total diarrhea.

Note: Severe diarrhea was defined as the passage of > 5 L of unformed stools (grade 3 to 5) over a 48 hour period.

^a Vaccine efficacy = [(Attack Rate in Placebo Group – Attack Rate in Vaccine Group)/Attack Rate in Placebo Group]*100.

Source: STN 125597_0. Module 5.3.5.1, PXVX-VC-200-003 Clinical Study Report, Table 15.

Clinical Reviewer Comment: Within each challenge group, there was a trend toward higher efficacy against severe diarrhea compared to efficacy against diarrhea of any severity.

Clinical Reviewer Comment: The number of days with grade 3+ stools and with positive stool cultures shown in Tables 52 and 53 in Appendix 1 suggest waning of protection by 3 months post-vaccination. The 3-Month Challenge group appeared to have more days with diarrhea or positive stool cultures than the 10 day challenge group but fewer than the combined placebo challenge group.

6.1.11.3 Subpopulation Analyses

Subgroup analysis did not reveal a clear impact of sex, race (black and white), or blood type (O and non-O) on efficacy (Table 13). Point estimates of efficacy for all subgroups were ≥ 70%. Subsets were generally small and confidence intervals are noted to be wide.

Table 13. PXVX-VC-200-003. Vaccine Efficacy - Subgroup Analyses, ITT Population

	PXVX0200 10-day Challenge	PXVX0200 3-Month Challenge	Combined Placebo Challenges
All Subjects	N=35	N=33	N=66
Attack Rate ^a (%)	5.7	12.1	59.1
VE ^b [% (95% CI)] ^c	90.3 (62.7, 100.0)	79.5 (49.9, 100.0)	-
Blood Type O	N=19	N=20	N=36
Attack Rate ^a (%)	10.5	15.0	69.4
VE ^b [% (95% CI)] ^c	84.8 (50.2, 100.0)	78.4 (44.0, 100.0)	-
Non-O Blood Types	N=16	N=13	N=30
Attack Rate ^a (%)	0	7.7	46.7
VE ^b [% (95% CI)] ^c	100.0 (48.1, 100.0)	83.5 (20.5, 100.0)	-
Females	N=10	N=6	N=28
Attack Rate ^a (%)	10.0	16.7	57.1
VE ^b [% (95% CI)] ^c	82.5 (22.8, 100.0)	70.8 (-7.8, 100.0)	-
Males	N=25	N=27	N=38
Attack Rate ^a (%)	4.0	11.1	60.5
VE ^b [% (95% CI)] ^c	93.4 (60.2, 100.0)	81.6 (48.2, 100.0)	-
Black	N=21	N=27	N=47
Attack Rate ^a (%)	4.8	14.8	61.7
VE ^b [% (95% CI)] ^c	92.3 (56.2, 100.0)	76.0 (42.7, 100.0)	-
White	N=10	N=6	N=18
Attack Rate ^a (%)	10.0	0	50.0
VE ^b [% (95% CI)] ^c	80.0 (7.5, 100.0)	100.0 (12.8, 100.0)	-

^a Attack rate: moderate or severe diarrhea (≥ 3L overall diarrheal purge)

^b Vaccine Efficacy (VE) = [(Attack rate in placebo group – Attack rate in vaccine group) / Attack rate in placebo group] * 100

^c Confidence intervals only provided for VE.

Note: Percentages based on number of subjects with non-missing value in each treatment group.
Source: STN 125597_0, Module 5.3.5.1, PXVX-VC-200-003 Section 14.2 (Efficacy Results), Tables 14.2.1.1 – 14.2.1.14.

Clinical Reviewer Note: As expected, the attack rate is noted to be higher among placebo recipients with blood type O compared to placebo recipients of non-O blood type. The attack rate among white placebo recipients is slightly lower compared to the attack rate among black placebo recipients; this may be because type O blood is more prevalent in black adults than in white adults.⁴⁵

Moderate to Severe Diarrhea Attack Rates By Site

Differences in the attack rate of moderate to severe diarrhea after the 10 day and 3 month challenge cohorts are shown by site in Table 14. It is noted that at the University of Vermont, the 3 month challenge attack rate is 0% among both PXVX0200 and placebo recipients. The reasons for this low attack rate in the placebo group are unclear.

Table 14. PXVX-VC-200-003. Attack Rate of Post-Challenge Moderate to Severe Diarrhea by Clinical Site

	PXVX0200 Group		Placebo Group	
	10 Day Challenge n/N (%) [95% CI]	3 Month Challenge n/N (%) [95% CI]	10 Day Challenge n/N (%) [95% CI]	3 Month Challenge n/N (%) [95% CI]
Baltimore, MD (01)	1/12 (8%) [0%, 38%]	3/13 (23%) [5%, 54%]	6/12 (50%) [21%, 79%]	7/11 (64%) [31%, 89%]
Cincinnati, OH (03)	0/12 (0%) [0%, 26%]	1/15 (7%) [0%, 32%]	9/12 (75%) [43%, 95%]	12/17 (71%) [44%, 90%]
Burlington, VT (04)	1/11 (9%) [0%, 41%]	0/5 (0%) [0%, 52%]	5/9 (56%) [21%, 86%]	0/5 (0%) [0%, 52%]

Note: n= Number of subjects with moderate or worse diarrhea; N=number of subjects challenged.

Note: Moderate or severe diarrhea severity = ≥3 L of overall diarrheal purge.

Source: STN 125597_0. Module 5.3.5.1. PXVX-VC-200-003 Clinical Study Report. Table 29.

Clinical Reviewer Comment: The 0% attack rate may partially explain the lower vaccine efficacy at 3 months post-vaccination compared to 10 days post-vaccination. However, this finding does not significantly impact the study's primary results, as the low 3 month challenge attack rate in Vermont would not overestimate vaccine efficacy.

6.1.11.4 Dropouts and/or Discontinuations

Missing values were left out of analyses.

6.1.11.5 Exploratory and Post Hoc Analyses

Pre-Vaccination Anti-Vibriocidal Antibody Titers

Pre-vaccination anti-vibriocidal GMTs are shown in Table 15.

Clinical Reviewer Comment: There was no pre-specified or post-hoc analysis of pre-immunization antibody titers. Most subjects had low levels of pre-existing baseline anti-vibriocidal antibody titers to each of the *V. cholerae* O1 strains evaluated in this study, as

reflected by the GMTs and median (range) titers in Table 16 below. A few subjects had higher titers which are reflected in the range of baseline titers. The reason for the pre-existing antibody titers is unclear. Pre-existing antibodies, however, do not impact the study's main findings, as seroconversion was defined as a 4-fold rise in antibody titers from baseline levels.

Table 15. PXVX-VC-200-003. Vibriocidal Geometric Mean Titer (GMT) Prior to Vaccination, Classical Inaba *V. Cholerae* – Immunogenicity Evaluable Population

Cholera Strain	PXVX0200 N=94		Placebo N=102	
	GMT (95% CI)	Median, (Range)	GMT (95% CI)	Median (Range)
Classical Inaba	46 (37, 58)	20 (20, 2560)	63 (48, 84)	40 (20, 5120)
El Tor Inaba	43 (33, 56)	20 (20, 5120)	67 (47, 94)	20 (20, 10240)
Classical Ogawa	67 (49, 91)	40 (20, 10240)	90 (65, 124)	40 (20, 10240)
El Tor Ogawa	53 (39, 70)	20 (20, 10240)	63 (46, 87)	40 (20, 10240)

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-003 Clinical Study Report, Tables 14.4.1, 14.4.4, 14.4.7 and 14.4.10.

Post-vaccination, Pre-challenge Immune Responses

Overall, the immune response post-vaccination, pre-challenge was higher in vaccine recipients than placebo recipients at all time points [Tables 6 and 17]. This is in contrast to the immune response after challenge, which was higher in placebo recipients than vaccine recipients at all time points.

Immune response was assessed against the homologous Classical Inaba strain of *V. cholerae* and against three heterologous *V. cholerae* O1 subtypes: El Tor Inaba, classical Ogawa, and El Tor Ogawa (Table 16). Anti-vibriocidal antibody GMTs peaked on Day 11, and the peak percentage of subjects with a 4-fold rise in vibriocidal antibodies occurred on Day 29. Serum vibriocidal titers for the 33 vaccine recipients in the 90-day challenge declined from a peak GMT of 3294 on Day 11 to a GMT of 271 on Day 91 [Data not shown]. Serum vibriocidal antibody levels immediately preceding challenge were lower in the 3-Month Challenge group than in the 10-Day Challenge group: that is, the Day 91 GMT in the 3-Month Challenge group was 271 [95% CI, 158-462], compared with 5999 [95% CI, 3169-11355] in the Day 11 GMT in the 10-Day Challenge Group [data not shown].

Pre-challenge anti-cholera toxin antibody GMT peaked on Day 29 (rather than Day 11 as seen with vibriocidal immune responses) and the cumulative percentage of subjects with a 4-fold rise in anti-cholera toxin antibody reached a maximum by Day 181 (Table 17). This suggests a slower rise in anti-body response compared to vibriocidal antibody response.

Table 16. PXVX-VC-200-003. Post-Vaccination, Pre-Challenge Vibriocidal Antibody Response Against Homologous Classical Inaba *V. cholerae* and against Three Heterologous *V. cholera* Serotypes and Biotypes (El Tor Inaba, Classical Ogawa, and El Tor Ogawa). Immunogenicity Evaluable Population.

Cholera Strain	Peak (Day11) Vibriocidal Antibody GMT (95% CI)		Peak (Day 29) Cumulative % with 4-fold rise ^a in Vibriocidal Antibody		Peak % with Vibriocidal Antibody Titer ≥ 2560 (at any pre-challenge time point) ^b	
	PXVX0200 N=93	Placebo N=99	PXVX0200 N=94	Placebo N=102	PXVX0200 N=94	Placebo N=102
Classical Inaba	4313 (2873, 6476)	65 (48, 88)	90.4	2.0	76.6% (Day 91)	3.9
El Tor Inaba	6898 (4370, 10889)	63.1 (45, 89)	91.5	4.9	85.1 (Day 91)	10.8
Classical Ogawa	2324 (1519, 3554)	94.0 (67, 131)	87.2	4.9	64.9 (Day 181)	10.8
El Tor Ogawa	2239 (1492, 3358)	71.5 (51, 101)	89.4	5.9	63.8 (Day 11)	10.8

^a The highest post-vaccination seroconversion rate is reported. This describes the cumulative percentage of subjects who had at least a 4-fold rise in titer over the titer measured by Day 1. Peak conversion typically occurred by Day 29 and was maintained through Days 91 and 181. Seroconversion rates approached 90% by Day 11 for all 4 cholera strains: 89.4% (95% CI 81.3, 94.8) for Classical Inaba, 90.4% (95% CI 82.6, 95.5) for El Tor Inaba, 86.2% (95% CI 77.5, 92.4) for Classical Ogawa and 88.3% (95% CI 80.0, 94.0) for El Tor Ogawa.

^b A large majority of subjects who achieved a vibriocidal antibody titer ≥ 2560 did so by Day 11: 73.4% for Classical Inaba, 80.9% for El Tor Inaba, 62.8% for Classical Ogawa and 63.8% for El Tor Ogawa.

Source: STN 125597, Module 5.3.5.1 PXVX-VC-200-003 Clinical Study Report Tables 21-22, and Tables 14.4.5 – 14.4.12.

Clinical Reviewer Note: Seroconversion rates against each of the four O1 subtypes presented in the Vaxchora package insert reflect data from the integrated summary of effectiveness (ISE). The ISE analyses dropped one subject from study -003 because the subject did not have a Day 11 measurement (STN 125597_46, module 1.11.3). Therefore, 84/93 subjects [90.3% (95% CI 82.4, 95.5)] seroconverted against classical Inaba, 85/93 subjects [91.4% (95% CI 83.8, 96.2)] seroconverted against El Tor Inaba, 81/93 subjects [87.1% (95% CI 78.5, 93.2)] seroconverted against classical Ogawa, and 83/93 subjects [89.2% (95% CI 81.1, 94.7)] seroconverted against El Tor Ogawa.

Table 17. PXVX-VC-200-003. Pre-Challenge and Post-Vaccination Anti-Cholera Toxin (CT) Antibody Responses. Immunogenicity Evaluable Population.

Peak (Day 29) Anti-CT Antibody GMT (95% CI)		Peak (Day 181) ^a Cumulative % with 4-fold rise ^b in anti-CT Antibody	
PXVX0200 N=57	Placebo N=68	PXVX0200 N=94	Placebo N=102
1609.8 (1102.9, 2349.6)	535 (443, 646)	38.3	0

Source: STN 125597, Module 5.3.5.1 PXVX-VC-200-003 Clinical Study Report Tables 14.4.13 – 14.4.14.

Clinical Reviewer Comment:

Overall, immune responses (anti-vibriocidal antibody GMTs and seroconversion rates against each of the 4 *V. cholerae* O1 strains and anti-CT antibody GMTs and seroconversion rates) post-challenge were higher in placebo compared to vaccine recipients. This may be due to limited replication of the challenge strain in vaccinated subjects resulting in a limited immune response (Tables 54-55 in Appendix 1).

Relationship Between Vibriocidal Antibody and Primary Endpoint

In exploratory analyses, the relationship between the concentration of vibriocidal antibody against the vaccine strain and the incidence of moderate/severe diarrhea or any diarrhea were evaluated as potential correlates of vaccine-induced protection. Serum vibriocidal titer (GMT), fold rise, seroconversion, and a range of vibriocidal titer cutoffs (including ≥ 2560 , i.e., potential seroprotection cutoffs) at Days 11 and 91 post-vaccination and pre-challenge were each assessed.

Of the 68 challenged vaccine recipients, 62 (91%) seroconverted prior to challenge. A total of 97% (60/62) of vaccine recipients who seroconverted by Day 11 were protected at the time of challenge. Rates of protection among subjects who seroconverted by Day 11 were similar at 10 days post-vaccination and 3 months post-vaccination (Table 18). Among 66 placebo recipients, 1 subject seroconverted

Similarly, the relationship between seroconversion and a continuous measure of diarrhea, total stool volume, was evaluated. For the 68 vaccine recipients in the 10-Day and 3-Month challenge studies, total post-challenge diarrheal volume was significantly greater in vibriocidal non-converters at Day 11 than in seroconverters ($p=0.001$; median volume=6.8 L, $n=6$ for non-converters; median volume=0.0 L, $n=62$ for seroconverters; Wilcoxon Rank Sum Test).

Table 18. PXVX-VC-200-003. Rates of Protection Against Moderate/Severe Diarrhea in Seroconverting Vaccinees.

	PXVX0200 10 Day Challenge N=35	PXVX0200 3 Month Challenge N=33
Seroconverters at Day 11	33/35 (94.3)	29 (87.9%)
Moderate/Severe Diarrhea in Seroconverters	1/33 (3.0)	1/29 (3.4)
Moderate/Severe Diarrhea in Non-Seroconverters	1/2 (50%)	3/4 (75%)
Seroconverters Protected Against Moderate/Severe Diarrhea	32/33 (96.7)	28/29 (96.6)
Rate of Protection Against Moderate/Severe Diarrhea Among Seroconverters	97% (95% CI 84, 100)	97% (95% CI 82, 100)

Note: Of the 66 placebo recipients challenged at 10 days and 3 months, 1 placebo recipient seroconverted at Day 11 (2%), and this subject did not develop moderate/severe diarrhea. Source: STN 125597_0. Module 5.3.5.1. PXVX-VC-200-003 Clinical Study Report. Table 28 and PXVX-STAT-VIB-003. Table 2.

Associations between vibriocidal antibody response and moderate/severe cholera were evaluated in exploratory analyses. Odd ratios, adjusted using multivariate logistic regression for age, sex, race and blood group as covariates) were calculated using conditional maximum likelihood, where an odds ratio of 1 represents no association

between immune response and moderate/severe cholera, and the extent to which the observed odds ratio differs from 1 provides a descriptive measure of the strength of the pairwise association.

Tables 22 and 23 show the odds ratio calculations for associations between vibriocidal antibody seroconversion (defined using various fold rises from baseline to 10 days post-vaccination) against the vaccine strain and moderate to severe diarrhea at 10 days and 3 months post-vaccination, respectively.

In the evaluations of Day 11 vibriocidal antibody levels using “seroprotective” cutoffs, it was determined that higher titer cutoffs resulted in higher odds ratio [data not shown]. In the 10 day challenge, the odds ratios ranged from 4.6 (titer ≥ 40 , $p=0.02$) to 41.0 (titer ≥ 2560 , $p<0.001$). These high titer cutoffs would characterize 10-25% of protected vaccines as non-responders. In addition, a couple of placebo recipients had titers at or above these cutoffs, suggesting a high titer can occur independent of vaccination. Corresponding evaluations following the 3 month challenge were similar; the odds ratios ranged from 6.4 (titer ≥ 40 , $p=0.02$) to > 39 (titer ≥ 2560 , $p<0.001$). Therefore, seroprotective cutoff values were not considered ideal for assessing the associations between vibriocidal antibody levels and moderate/severe diarrhea.

In the evaluations of vibriocidal antibody levels using seroconversion rates at Day 11 (Tables 19 and 20), 4-fold and 8-fold rises in antibody titer resulted in an odds ratio of 50 ($p<0.001$) in the 10 day challenge. In the 3 month challenge, a 4-fold and 8-fold rise in antibody titer resulted in an odds ratio of 39 and 35 respectively (both p -values were < 0.001). Based on the strength of the association between vibriocidal seroconversion at Day 11 and protection against moderate/severe diarrhea in both the day 11 and day 91 challenge cohorts, seroconversion was selected as the immunogenicity endpoint to be used in the immunogenicity bridging analysis (bridging effectiveness to 46-64 year old subjects in study PVXV-VC-200-005). The strength of the association between fold-rise and outcome was noted to be similar for 4-fold to 16-fold rises in vibriocidal antibody responses in both the 10 day and 3 month challenge groups. A 4-fold increase was selected because it has been well-studied and understood in the literature.

Clinical Reviewer Comment: CBER agreed with using 4-fold rise (seroconversion) as an immunogenicity bridge for effectiveness in adults over 45 years of age.

Table 19. PXVX-VC-200-003. Fold-Increase in Anti-Vibriocidal Antibody Against Vaccine Strain (Baseline to 10 Days Post-Vaccination) versus Moderate to Severe Diarrhea Among PXVX0200 and Placebo Recipients Challenged at 10 Days Post-Vaccination.

		Moderate to Severe Diarrhea		p-value	Odds Ratio
		Yes	No		
Fold-Increase ≥ 2 at Day 11	Yes	7	34	0.001 ^b	6
	No	15	12		
Fold-Increase ≥ 4 at Day 11	Yes	1	33	< 0.001	50
	No	21	13		
Fold-Increase ≥ 8 at Day 11	Yes	1	33	< 0.001	50
	No	21	13		
Fold-Increase ≥ 16 at Day 11	Yes	0	32	< 0.001	> 45
	No	22	14		

		Moderate to Severe Diarrhea		p-value	Odds Ratio
		Yes	No		
Fold-Increase \geq 32 at Day 11	Yes	0	28	< 0.001	> 31
	No	22	18		
Fold-Increase \geq 64 at Day 11	Yes	0	23	< 0.001	> 20
	No	22	23		
Fold-Increase \geq 128 at Day 11	Yes	0	19	< 0.001	> 14
	No	22	27		

^a This table shows data for the 68 subjects who were challenged at 10 days post-vaccination (35 PXVX0200 recipients and 33 placebo recipients).

^b Fisher's exact test.

Source: STN 125597_0. Module 5.3.5.1. PXVX-STAT-VIB-003. Table 5.

Table 20. PXVX-VC-200-003. Fold-Increase in Anti-Vibriocidal Antibody Against Vaccine Strain (Baseline to 10 Days Post-Vaccination) versus Moderate to Severe Diarrhea Among PXVX0200 and Placebo Recipients Challenged at 3 Months Post-Vaccination.

		Moderate to Severe Diarrhea		p-value	Odds Ratio
		Yes	No		
Fold-Increase \geq 2 at Day 11	Yes	5	30	< 0.001 ^b	8
	No	18	13		
Fold-Increase \geq 4 at Day 11	Yes	1	28	< 0.001	39
	No	22	15		
Fold-Increase \geq 8 at Day 11	Yes	1	27	< 0.001	35
	No	22	16		
Fold-Increase \geq 16 at Day 11	Yes	1	27	< 0.001	35
	No	22	16		
Fold-Increase \geq 32 at Day 11	Yes	0	27	< 0.001	> 35
	No	23	16		
Fold-Increase \geq 64 at Day 11	Yes	0	25	< 0.001	> 29
	No	23	18		
Fold-Increase \geq 128 at Day 11	Yes	0	19	< 0.001	> 17
	No	23	24		

^a This table shows data for the 68 subjects who were challenged at 10 days post-vaccination (35 PXVX0200 recipients and 33 placebo recipients).

^b Fisher's exact test.

Source: STN 125597_0. Module 5.3.5.1. PXVX-STAT-VIB-003. Table 9.

Clinical Reviewer Note:

- Odds ratios calculated from logistic regressions must be interpreted with caution, as values differ from relative risk ratios when the disease is not rare, with a tendency toward more extreme values. Spearman rank correlation coefficients are provided below as supportive data.
- In the combined population of 134 challenged vaccine and placebo recipients, there was a statistically significant association between serum vibriocidal titer on Day 11 and total post-challenge diarrheal volume (Spearman's rank correlation $r = -0.74$; $p < 0.001$). However, moderately high titers were observed on Day 11 for both vaccine and placebo recipients, and among subjects with identical Day 11 titers, vaccine recipients tended to be protected while placebo recipients were not. The potential for non-specific elevation of serum vibriocidal titer suggests that reliance on titer at a

single post-vaccination time point (ie, Day 11) as a bridging criterion would add noise and decrease the statistical power of evaluations of equivalence across populations.

- There was also a statistically significant association between fold-rise in serum vibriocidal antibody titer from Day 1 to Day 11 and total post-challenge diarrheal volume (Spearman's $r=-0.72$; $p<0.001$). The usage of fold-rise neutralizes the effect of nonspecific elevation in vibriocidal titer; fold-rises in titer at Day 11 were observed frequently in vaccine recipients and extremely rarely in placebo recipients. Furthermore, seroconversion, defined as a ≥ 4 -fold rise in vibriocidal titer from pre-vaccination to post-vaccination levels, identified "vaccine take" such that only 2 of 62 (3%) of seroconverting vaccine recipients developed moderate/severe cholera after challenge. Based on this relationship between seroconversion and protection, along with the fact that approximately 90% of vaccine recipients seroconverted, vibriocidal seroconversion at Day 11 was used as an immunologic measure for bridging effectiveness from 18 through 45 year olds to older adults.

Anti-CT Antibody as an Immune Correlate of Duration of Protection

Serum anti-CT antibody titers on Days 1, 8, and 11 as well as fold rises from Day 1 to Days 8 and 11 were assessed in the 10-Day Challenge Group while titers at Days 1, 8, 11, 29, and 91 as well as fold rises from Day 1 to Days 8, 11, 29, and 91 were assessed in the 3-Month Challenge Group to allow measurements of association with diarrheal outcomes post-challenge.

Anti-CT antibody titer was not strongly associated with outcome. Overall, anti-CT antibody titer increased post-vaccination in only a subset. Only slightly less than half (16/33) of the 3 month challenge vaccine recipients had seroconverted prior to challenge. In the 33 vaccine recipients in the 3-Month Challenge study, there was no significant difference in total post-challenge diarrheal volume between cumulative CT seroconverters at Day 91 and non-converters ($p=0.18$; median volume=0.5 L, $n=17$ for non-converters; median volume=0.2 L, $n=16$ for seroconverters; Wilcoxon Rank Sum Test).

Exploratory Evaluation of Antigen-specific Memory B Cell Response as an Immune Correlate of Duration of Protection (Data not shown)

There was no correlation between anti-O1 LPS IgG, anti-CT IgG, and anti-CT IgA memory B cells at Day 91 and total stool volume post-challenge [data not shown]. However, fold-increases ≥ 1 and ≥ 1.5 in anti-LPS IgA on day 91 were significantly associated with moderate/severe cholera (odds ratio range 3.4-4.0). Similar to analyses of anti-vibriocidal antibody responses, the fold-increase from Day 1 to Day 91 in anti-LPS IgA memory B cells appeared to be more strongly associated with *total diarrheal volume*¹⁰ than to the percentage above a pre-defined threshold value (Spearman correlation=-0.56, $p<0.001$, $n=33$). In order to explore duration of protection, the Applicant plans to conduct studies in the future to evaluate whether memory B cells persist beyond the time points evaluated in study 003.

10 Total diarrheal volume was defined as a continuous variable that included the cumulative volume of any stools grade 3-5 (regardless of mild, moderate or severe diarrhea grading). So a subject with a single unformed stool of 100g would be included in this value. Mild diarrhea was defined as ≥ 2 unformed stools (Grade 3-5) over a 48 hour period that was ≥ 200 grams or a single unformed stool that totaled ≥ 300 g but less than 3 liters total diarrheal volume.

Comparison of Vaccine- and Challenge-Induced Memory B Cell Response

Comparison of the vaccine-induced versus challenge-induced fold-rise in anti-O1 LPS IgA memory B cells by Day 181 did not reach statistical significance ($p=0.1564$; Wilcoxon rank test comparing values between treatment groups) (data not shown, Table 14.4.25.2 in CSR erratum).

6.1.12 Safety Analyses

6.1.12.1 Methods

The *Safety Population* includes all subjects who received treatment; this population was used in all safety analyses. Safety was evaluated after vaccination and after challenge as described in section 6.1.7. Vaccine and placebo recipients were compared based on the frequency of each adverse reaction (and by the highest reported severity level) using individual Fisher's exact tests. No adjustments were made for multiplicity. Post-challenge adverse reactions were described by highest reported severity for the 10-day and 3-month challenge groups. Solicited adverse reactions were analyzed using the same subgroups described in section 6.1.9.

6.1.12.2 Overview of Adverse Events

A total of 197 subjects were enrolled in this study and included in the safety population; 95 subjects received a single vaccination of PXVX0200 and 102 subjects received placebo.

Post-Vaccination Solicited Adverse Reactions

Solicited adverse reactions after study product administration were reported by 49.5% of vaccine recipients and 50.0% of placebo recipients (Table 21). The most commonly reported adverse reactions among vaccine and placebo recipients were tiredness (33.7%), headache (28.0%), abdominal pain (20.7%), lack of appetite (20.7%), and nausea/ vomiting (18.1%). The rates of solicited adverse reactions were similar between the vaccine and placebo groups, and there were no statistically significant differences between the groups. Diarrhea defined as ≥ 4 loose stools per 24 hours was reported in 1.1% of vaccine recipients compared with 3.0% of placebo recipients. Most solicited adverse reactions were graded as mild or moderate.

Subgroup analyses by blood type, sex, and race did not reveal clear trends in solicited adverse reactions [data now shown].

Table 21. PXVX-VC-200-003. Solicited Adverse Reactions Post-Vaccination

Solicited Adverse Reaction	PXVX0200 N=93 ^a n (%)	Placebo N=100 ^a n (%)
Any Solicited Adverse Reaction	46 (49.5)	50 (50.0)
Tiredness ^b	32 (34.4)	33 (33.0)
Mild	22 (23.7)	17 (17.0)
Moderate	8 (8.6)	13 (13.0)
Severe	2 (2.2)	3 (3.0)
Potentially Life-Threatening	0	0

Solicited Adverse Reaction	PXVX0200 N=93 ^a n (%)	Placebo N=100 ^a n (%)
Headache ^b	23 (24.7)	31 (31.0)
Mild	15 (16.1)	16 (16.0)
Moderate	8 (8.6)	12 (12.0)
Severe	0	3 (3.0)
Potentially Life-Threatening	0	0
Abdominal Pain ^b	20 (21.5)	20 (20.0)
Mild	12 (12.9)	11 (11.0)
Moderate	8 (8.6)	8 (8.0)
Severe	0	1 (1.0)
Potentially Life-Threatening	0	0
Lack of Appetite ^b	17 (18.3)	23 (23.0)
Mild	10 (10.8)	17 (17.0)
Moderate	6 (6.5)	3 (3.0)
Severe	1 (1.1)	3 (3.0)
Potentially Life-Threatening	0	0
Nausea/Vomiting ^c	14 (15.1)	21 (21.0)
Mild	10 (10.8)	13 (13.0)
Moderate	4 (4.3)	6 (6.0)
Severe	0	2 (2.0)
Potentially Life-Threatening	0	0
Fever ^d	2 (2.2)	1 (1.0)
Mild	1 (1.1)	1 (1.0)
Moderate	1 (1.1)	0
Severe	0	0
Potentially Life-Threatening	0	0
Diarrhea ^e	1 (1.1)	3 (3.0)
Mild	0	1 (1.0)
Moderate	1 (1.1)	1 (1.0)
Severe	0	1 (1.0)
Potentially Life-Threatening	0	0

^a N=number of subjects who completed a diary card following vaccination.

^b Headache, fatigue, myalgia, abdominal pain, and lack of appetite grading: 1 (mild): mild, no interference with activity; 2 (moderate): some interference with activity; 3 (severe): significant, prevents daily activity; 4 (potentially life threatening): ER visit or hospitalization.

^c Vomiting grading scale: 1 (mild): 1-2 episodes/24 hours; 2 (moderate): > 2 episodes/24 hours; 3 (severe): requires IV hydration; 4 (potentially life threatening): ER visit or hospitalization for hypotensive shock.

^d Fever grading scale: 1(mild): 38.0-38.4°C; 2 (moderate): 38.5-38.9°C; 3 (severe): 39-40°C; 4 (potentially life threatening) > 40°C.

^e Diarrhea grading: 1 (mild): 4 loose stools/24 hours; 2 (moderate): 5 loose stools/24 hours; 3 (severe): ≥ 6 loose stools/24 hours; 4 (potentially life threatening): emergency room (ER) visit or hospitalization.

Source: STN 125597_0. Module 5.3.5.1. PXVX-VC-200-003 Clinical Study Report. Table 30.

Unsolicited Adverse Events and Serious Adverse Events Following Vaccination

Adverse events, excluding those occurring following challenge, were reported by 17.9% of vaccine and 16.7% of placebo recipients and were mostly mild in severity. There were no clear differences in the percentage of vaccine recipients reporting individual AEs or AEs grouped by SOC compared with placebo recipients. The most common AEs reported among vaccine recipients were upper respiratory tract infection (3.2%), flatulence (2.1%), and diarrhea (2.1%), while among placebo recipients the most common AEs were fatigue (3.9%), rhinorrhea (2.9%), upper respiratory tract infection (2.0%), musculoskeletal pain (2.0%), cough (2.0%), and sneezing (2.0%). Other AEs reported by 1 subject (1.1% of all vaccine recipients) each included fatigue, musculoskeletal pain, decreased appetite, dizziness, gastrointestinal sounds abnormal, hypertension, oropharyngeal pain, abdominal pain, arthralgia, eye pain, feeling hot, pyrexia, sinus congestion, sports injury, and toothache. Statistical comparisons were not planned or performed comparing AEs in vaccine and placebo recipients due to the small numbers of AEs in each category.

Diarrhea was reported as an AE in 2 vaccine recipients only, and it was considered related to vaccine administration. One subject reported mild diarrhea 8-10 days post-vaccination and another subject reported mild diarrhea 3-10 days post-vaccination. These events were recorded as AEs and as solicited AEs as per protocol, because they extended beyond the 7-day post-vaccination period. All other types of AEs that occurred in vaccine recipients and not in placebo recipients involved 1 subject each.

The most commonly reported adverse events by SOC among PXVX0200 and Placebo group recipients respectively were Gastrointestinal disorders (6.3% vs 2.9%), General Disorders and Administration Site Conditions (3.2% vs 5.9%), Infections and Infestations (3.2% vs 2.0%), Musculoskeletal and Connective Tissue Disorders (2.1% vs 4.9%), and Respiratory, Thoracic and Mediastinal Disorders (2.1% vs 3.9%).

There was 1 SAE reported in a placebo recipient who was hospitalized for orthopedic surgery. There was one potentially life threatening event post-challenge in a placebo recipient who developed hyperkalemia 3 days post-challenge. These events were considered unrelated to study product administration. Subject narratives are below.

Post-vaccination vital signs that were considered by site investigators to be clinically significant include a mild increase in systolic blood pressure (SBP: 140s) in three vaccine and one placebo recipient.

Post-Challenge Solicited Adverse Reactions

Solicited adverse reactions were reported within approximately 10 days post-challenge (or until discharge) by 65.7% of vaccine recipients in the 10-Day Challenge group and by 51.5% of vaccine recipients in the 3-Month Challenge group, compared with 87.9% of combined placebo group post-challenge. The most common solicited reactions among vaccine and placebo recipients were malaise, abdominal cramps, headache, and nausea/vomiting (Table 22). Compared to placebo recipients in the combined placebo groups, vaccine recipients in the 10-day and 3-month challenge groups had lower rates of any solicited adverse reaction, malaise, abdominal cramps, nausea/vomiting, and fever and vaccine recipients in the 3-month challenge group had a statistically significantly lower rate of headache compared to the combined placebo group. A few subjects reported severe malaise, headache, abdominal cramps, and nausea/vomiting in

all challenge groups (including placebo), however the remaining subjects reported mostly mild adverse reactions.

Table 22. PXVX-VC-200-003. Solicited Adverse Reactions Post-Challenge During Approximate 10 day In-Patient Stay^a

Solicited Adverse Reaction	10-Day Challenge PXVX0200 N=35 n (%)	3-Month Challenge PXVX0200 N=33 n (%)	Combined Placebo Challenge N=66 n (%)
Any Solicited Adverse Reaction	23 (65.7)	17 (51.5)	58 (87.9)
Fever	1 (2.9)	2 (6.1)	18 (27.3)
Mild	0 (0.0)	2 (6.1)	15 (22.7)
Moderate	1 (2.9)	0 (0.0)	3 (4.5)
Severe	0 (0.0)	0 (0.0)	0 (0.0)
Malaise	8 (22.9)	8 (24.2)	41 (62.1)
Mild	7 (20.0)	4 (12.1)	20 (30.3)
Moderate	0 (0.0)	2 (6.1)	14 (21.2)
Severe	1 (2.9)	2 (6.1)	7 (10.6)
Headache	12 (34.3)	9 (27.3)	35 (53.0)
Mild	9 (25.7)	5 (15.2)	21 (31.8)
Moderate	1 (2.9)	4 (12.1)	13 (19.7)
Severe	2 (5.7)	0 (0.0)	1 (1.5)
Abdominal Cramps	8 (22.9)	10 (30.3)	45 (68.2)
Mild	8 (22.9)	6 (18.2)	27 (40.9)
Moderate	0 (0.0)	2 (6.1)	13 (19.7)
Severe	0 (0.0)	2 (6.1)	5 (7.6)
Nausea/Vomiting	9 (25.7)	7 (21.2)	38 (57.6)
Mild	9 (25.7)	4 (12.1)	16 (24.2)
Moderate	0 (0.0)	2 (6.1)	16 (24.2)
Severe	0 (0.0)	1 (3.0)	6 (9.1)

^a Mean number of days in in-patient facility was 11.4 overall (range 9-13 days) for the 10-day challenge group and 11.2 days (range 10-12 days) for the 3-month challenge group.

Source: STN 125597_0, Module 5.3.5.2, PXVX-VC-200-003 Clinical Study Report, Tables 14.5.3.1 and 14.5.3.2 and STN 125597_17, Module 1.11.3 Efficacy Information Amendment, Tables 14.1.3.1.1 and 14.1.3.2.1.

6.1.12.3 Deaths

No subjects died during the study.

6.1.12.4 Nonfatal Serious Adverse Events

One SAE was reported 57 days after challenge. A placebo recipient was in a motor vehicle accident 57 days after challenge. He sustained a right lateral tibial plateau fracture with split depression and right medial lateral meniscal tear. He was hospitalized

for orthopedic surgery (open reduction and internal fixation and repair of lateral meniscus).

Clinical Reviewer Comment: This SAE was considered unrelated to vaccination, and the FDA medical officer agrees.

6.1.12.6 Clinical Test Results

Not applicable.

6.1.12.7 Dropouts and/or Discontinuations

Among subjects who were challenged, 1 terminated prior to completing the Day 181 visit; this subject was a challenged placebo recipient who was withdrawn due to relocation and noncompliance after completing the Day 39 visit. Among unchallenged groups, 3 vaccine recipients and 1 placebo recipient were lost to follow-up. Additionally, one unchallenged placebo recipient terminated early due to incarceration. No subject terminated early due to an adverse event.

6.1.13 Study Summary and Conclusions

Efficacy Conclusions

This study demonstrated that one oral dose of PXVX0200 confers efficacy against moderate or severe diarrhea at 10 days [90.3% (95% CI, 62.7%-100.0%)] and 3 months [79.5% (95% CI, 49.9%-100.0%)] following challenge with 1×10^5 CFU of heterologous *Vibrio cholerae* O1 El Tor Inaba. Both co-primary objectives were met since the lower 95% confidence bound on efficacy exceeded 30% in both challenges. Subgroup analyses by blood type, sex, and race did not reveal differences in efficacy results between subgroups.

The vaccine appeared to result in a decrease in the following secondary endpoints evaluating disease severity post-challenge: diarrhea of any severity, volume of diarrhea, number of stools, number of days with grade 3+ stools, days of fecal shedding and peak fecal *V. cholerae* concentration in the vaccine recipients in the 10 day and 3-month challenge groups compared to the combined placebo challenge groups.

Although the study did not pre-specify comparisons between the 10 day and 3 month challenge groups, the efficacy and secondary endpoint data appear to suggest that vaccine efficacy may be lower among vaccine recipients challenged at 3-months than in vaccine recipients challenged at 10-days post-vaccination.

Immunogenicity Conclusions

Vaxchora was immunogenic against the classical Inaba vaccine strain. A total of 89.4% of vaccine recipients seroconverted (≥ 4 -fold rise in vibriocidal antibody titer) against the vaccine strain by 10 days post-vaccination (day 11). Among vaccine recipients, vibriocidal GMTs against the vaccine strain started out at 46 at baseline, peaked on day 11 at 4313 and then diminished rapidly to 155 on Day 181. Seroconversion rates against the *V. cholerae* O1 classical Inaba strain at 10 days post-vaccination correlated with protection from direct challenge with an El Tor Inaba strain at 10 days and 3 months post-vaccination.

Seroconversion rates against *V. cholerae* O1 El Tor Ogawa and classical Ogawa strains (86.2%-88.3%) were similar to seroconversion rates against the classical Inaba and El Tor Inaba strains (89.4-90.4%). In the context of existing scientific literature demonstrating cross-protection across the four major *V. cholerae* O1 subtypes following natural infection or vaccination with another CVD 103-HgR live oral cholera vaccine, the immunogenicity data described above support the effectiveness of Vaxchora against disease caused by El Tor Ogawa, classical Ogawa and classical Inaba strains of *V. cholerae* O1 (also see section 2.3).^{5,6,7,8}

Safety Conclusions

A total of 197 subjects were enrolled in this study and included in the safety population; 95 subjects received a single vaccination of PXVX0200 and 102 subjects received placebo. PXVX0200 was well tolerated. The rates of solicited adverse reactions and unsolicited adverse events were similar between the vaccine and placebo groups. Most solicited adverse reactions were graded as mild or moderate. Compared to placebo, a higher rate of diarrhea was observed in vaccine recipients (3.6% vs 1.6%). There were no serious adverse events that were considered related to the study product. Subgroup analyses by blood type, sex, and race did not reveal clear trends in solicited adverse reactions.

6.2 Study PXVX-VC-200-004 (NCT02094586)

Study PXVX-VC-200-400 was entitled “A Phase III Randomized, Double-blind, Placebo-Controlled Three-Lot Consistency Study in Healthy Adult Volunteers to Assess Immunogenicity, and Clinical Acceptability of a Single-dose of the Live Oral Cholera Vaccine Candidate PXVX0200, *Vibrio cholerae* O1 Serotype Inaba Vaccine Strain CVD 103-HgR.” The study began enrollment on May 12, 2014, and the last subject completed the last study visit on February 23, 2015.

6.2.1 Objectives

Primary Immunogenicity Objective

The primary immunogenicity objective of this study was to demonstrate immunologic equivalence of three different production lots of PXVX0200.

- Primary endpoint: Serum vibriocidal antibody measured at Day 11
- Equivalence criteria: The GMT of each lot must be within $\pm 50\%$ of each other lot with 95% confidence. Specifically, the 95% CI around each pairwise ratio of GMTs must be within [0.67, 1.5].

Secondary Immunogenicity Objectives

1. To estimate the seroconversion rate (≥ 4 -fold rise) by serum vibriocidal antibody by Day 11.
2. Estimate the antibody response profile up to 6 months post-vaccination in an Immune Sub-study Population. The associated endpoints included:
 - Serum vibriocidal antibody and serum IgG anti-cholera toxin GMT at Days 1, 11, 29, 91, and 181.
 - Seroconversion rate by serum vibriocidal antibody and serum anti-CT antibody at Days 11, 29, 91, and 181.

Exploratory Objective

The exploratory objective was to evaluate the activity of anti-O1 lipopolysaccharide (LPS) immunoglobulin A (IgA) memory B cell response up to 6 months post-vaccination (Immune Sub-study Population) based on mean and median percentage of anti-O1 LPS IgA memory B cells among all IgA memory B cells.

Clinical Reviewer Note: Because the memory B cell (b) (4) assay was not validated, results are not included in the review of this study. However, results of exploratory analyses evaluating antigen-specific memory B cell response as an immune correlate of duration of protection from study -003 are presented in section 6.1.11.5.

Safety Objective

To evaluate the safety of PXVX0200. The associated endpoints included the incidence and severity of solicited adverse reactions and the incidence and severity of unsolicited adverse events (AEs).

6.2.2 Design Overview

This study was a Phase 3 randomized (8:1), double-blind, placebo-controlled lot consistency study in 18 through 45 year olds (inclusive) at 25 sites (19 sites in the U.S. and 6 sites in Australia). A total of 3146 subjects with no prior history of cholera infection or travel to a cholera-endemic area in the previous 5 years were randomly assigned to receive a single dose of one of three lots of PXVX0200 (N=2795) or placebo (N=351) according to randomization blocks that used an 8:8:8:3 ratio. The randomization schedule was created using randomized permuted blocks of 27, and randomized subjects were assigned the treatment (PXVX0200 (one of 3 blocks) or placebo). Subject eligibility was determined during an up-to-45-day screening period and subjects were vaccinated on Day 1. Each subject was scheduled for at least three visits: the vaccination visit (could be combined with Screening visit) on Day 1, and post-vaccination visits on Day 11 and Day 29. A phone follow-up was scheduled for Day 181.

Thirty-one subjects were enrolled in the Immune sub-study that required additional blood sample collection and visits (carried out at a subset of 6 clinical sites) to enable longitudinal behavior. Each participating site was given a target number of subjects to enroll, and they offered enrollment in the sub-study to each subject enrolling in the overall study until the target number of subjects had agreed to participate. Randomization in the sub study followed the same scheme as the rest of the study.

The primary objective of this study was to demonstrate immunologic equivalence of three different production lots of PXVX0200. The control group consisted of subjects who received placebo (physiological saline). Other immunologic objectives included assessment of the seroconversion rate by serum vibriocidal antibody by Day 11 and the antibody response profile up to 6 months post-vaccination. The sample size of this study generated a large portion of the PXVX0200 safety database.

Blinding:

The study was conducted in a double-blind manner. The investigational vaccine was administered by unblinded personnel in the clinic that was not involved in other aspects of the study, including safety monitoring.

6.2.3 Population

Inclusion Criteria

1. Able to understand the study, give written consent, and comply with the study requirements and procedures.
2. Healthy males and females 18 to 45 years of age (inclusive) without significant medical history and physical examination findings at screening.
3. Women of childbearing potential must have a negative urine pregnancy test at screening prior to vaccination. Female subjects must be of non-childbearing potential (defined as surgically sterile or postmenopausal for more than 1 year), or if of childbearing potential must be practicing abstinence or using an effective licensed method of birth control within 2 months of vaccination and must agree to continue such precautions during the study.

Exclusion Criteria

1. Currently active unstable or undiagnosed medical conditions including immunodeficiency, cardiovascular disease, respiratory disease, endocrine disorder, liver disease, renal disease, gastrointestinal disease, neurologic illness, psychiatric disorder requiring hospitalization, or current drug or alcohol abuse. Examples of unstable or undiagnosed medical conditions include unstable angina pectoris, shortness of breath on exertion without clear etiology, and chronic renal failure requiring dialysis. Examples of conditions that do not meet exclusion criteria include mild controlled hypertension, mild controlled asthma, and treated depression without hospitalization.
2. Abnormal stool pattern defined as < 3 stools per week or > 2 stools per day in past 6 months.
3. Regular use of laxatives in the past 6 months.
4. Previously received a licensed or investigational cholera vaccine.
5. History of cholera or enterotoxigenic *Escherichia coli* infection (natural infection or experimental challenge).
6. Travel to a cholera-endemic area in the previous 5 years.
7. Received or plans to receive any other licensed vaccines, except for seasonal influenza vaccine, from 14 days prior to the study vaccination through 11 days after vaccination.
8. Received or plans to receive antibiotics or chloroquine within 14 days prior to the study vaccination through 11 days after vaccination.
9. Recipient of bone marrow or solid organ transplant.
10. Use of systemic chemotherapy in the previous 5 years prior to the study.
11. Malignancy (excluding non-melanotic skin cancers) or lymphoproliferative disorders diagnosed or treated during the past 5 years.
12. Received or plans to receive systemic immunosuppressive therapy, radiation therapy, parenteral or high-dosage inhaled steroids (> 800 µg/day of beclomethasone dipropionate or equivalent) within 6 months prior to the study vaccination through Day 11.
13. History of Guillain-Barré Syndrome.
14. Pregnant or nursing.

Note: Site personnel were cautious to avoid enrolling subjects who cohabited, worked, or studied in the same facility with another study subject to ensure individual blinding was maintained.

6.2.4 Study Treatments or Agents Mandated by the Protocol

Study products administered included PXVX0200 (Vaxchora) and placebo. Three lots of vaccine were used in this study (Lot A: P700.550-1CA03, Lot B: P700.550-3CA03 and Lot C: P700.550-6BA03). Each active component packet contained 1×10^9 CFU/dose at release. A single lot of buffer component was used in this study (Lot No. C1111013). Clinical sites used either sterile Water for Irrigation USP or purified, spring, distilled bottled water for reconstitution of the buffer component packet. The final vaccine suspension was administered orally at a dose of 1×10^9 CFU in an opaque cup.

Please see section 6.1.4, for other details regarding the description of the vaccine and placebo and administration.

6.2.5 Directions for Use

Directions for use were the same as those described in section 6.1.5, except that sites used sterile Water for Irrigation USP, purified bottled water, spring bottled water, or distilled bottled water for reconstitution of the buffer component packet.

6.2.6 Sites and Centers

This study was conducted at 19 sites in the United States and 6 sites in Australia.

6.2.7 Surveillance/Monitoring

- There was no monitoring immediately post-vaccination for acute side effects.
- Please see section 6.1.7 for a description of safety monitoring post-vaccination, including solicited adverse reaction, unsolicited adverse events, serious adverse events and medically attended adverse events. With the exception of no monitoring immediately post-vaccination for acute side effects, all other aspects of post-vaccination monitoring of adverse reactions/events were identical to methods used in study -003.

Table 23. PXVX-VC-200-004 Study Flowchart

Study Days	Screen (-45 days to -0 days)	Baseline Day 1	Day 11 (± 1 day)	Day 29 (± 3 days)	Day 181 Phone follow-up (± 7 days)
Visit Number	1	2	3	4	5
Informed consent	X				
Medical History	X	X			
Physical Examination	X	X ^a			
Vital Signs	X	X			
ABO Blood Typing		X			
Urine Pregnancy Test, if required ^b	X	X			
Vaccination		X			
Adverse Event Evaluation		X ^c	X	X	X ^d
Prior/Concomitant Medications	X	X	X	X	X ^e
Memory Aid: Solicited Adverse Reactions		Distribute	Collect		
Serology: Vibriocidal Assay		X	X		

^a If indicated by updated medical history.

^b Conduct or collect before vaccination.

^c After vaccination.

^d Serious adverse events (SAE) only.

^e Associated with Day 181 reported SAEs.

Source: STN 125597_0. Module 5.3.5.1. PXVX-VC-200-004 Clinical Study Report, Table 3.

Serologic Immunogenicity Laboratory Assessments

- A validated vibriocidal assay was used for the assessment of vibriocidal antibodies against classical Inaba, classical Ogawa, El Tor Inaba, and El Tor Ogawa strains. A validated anti-CT IgG antibody (b) (4) was used to measure serum anti-CT antibody response. These assays were performed at (b) (4) using assays transferred from (b) (4).
- PBMC samples were analyzed by an (b) (4) assay to assess for anti-O1 LPS IgA memory B cell responses. The (b) (4), developed in collaboration with (b) (4), was performed by PaxVax. No inter-laboratory standardization was required since only one lab was used for each immunogenicity assessment.
- The time points for immunogenicity assessments are shown in Tabel 24 below.

Table 24. PXVX-VC-200-004. Immunogenicity Assessments – Study Days for Vibriocidal Antibody Assay

Subject Group	Vibriocidal Assessment with classical Inaba (Study Days)	Anti-Cholera Toxin Antibody (Study Days)	Memory B Cells
All Subjects	1, 11	-	-
Immune Sub-study	1, 11, 29, 91, 181	1, 11, 29, 91, 181	1, 91, 181

Source: STN 125597_0. Module 5.3.5.1. PXVX-VC-200-004 Clinical Study Report, Table 7.

6.2.8 Endpoints and Criteria for Study Success

The primary immunogenicity endpoint and criteria for success and the secondary and exploratory immunogenicity endpoints are described in section 6.2.1.

6.2.9 Statistical Considerations & Statistical Analysis Plan

Unless otherwise specified, all statistical tests were two-sided with a significance level of $\alpha=0.05$, and 95% confidence intervals (CIs) were constructed. Subgroup analyses by age, sex and blood type were performed for the primary endpoint.

The primary analysis consisted of three between-lot equivalence tests of serum vibriocidal antibody GMT measured at Day 11. The relationship between any two lots of vaccine was characterized by the geometric mean ratio (GMT_A/GMT_B , GMT_A/GMT_C and GMT_B/GMT_C) and it's CI. The p-values for each ratio were constructed using three separate two one-sided test procedures at the 0.025 level of significance. The lots were considered equivalent if the 95% CI of each GMT ratio was within [0.67, 1.50]. The null (H_0) and alternative (H_1) hypotheses are as follows:

H_0 : ($\mu_A/\mu_B \leq 0.67$ or $\mu_A/\mu_B \geq 1.50$) or ($\mu_A/\mu_C \leq 0.67$ or $\mu_A/\mu_C \geq 1.50$) or ($\mu_B/\mu_C \leq 0.67$ or $\mu_B/\mu_C \geq 1.50$)

H_1 : $0.67 < \mu_A/\mu_B < 1.50$ and $0.67 < \mu_A/\mu_C < 1.50$ and $0.67 < \mu_B/\mu_C < 1.50$

Analysis Populations

1. Intent-to-Treat (ITT) Population: randomized subjects who received study treatment. It was analyzed according to the randomized treatment.
2. Immunogenicity Evaluable Population: subset of subjects in the ITT Population who were randomized to receive PXVX0200 and met the following criteria:
 - no major protocol deviations that affected immunogenicity.
 - a valid post-vaccination vibriocidal antibody assay result for Day 11.Analyses of the primary study endpoint and the secondary seroconversion rate endpoint were conducted with the Immunogenicity Evaluable Population.
3. Immune Sub-study Population: the subset of vaccine and placebo recipients who were selected at the time of randomization for memory B-cell evaluations as well as the additional vibriocidal and anti-CT antibody sample collection. Given that the sub-study entailed more visits, It was carried out at a subset of planned study sites. Each participating site offered enrollment in the Immune Sub-Study to each subject enrolling in the overall study until the target number of subjects had agreed to participate. Randomization of Immune-Sub-Study subjects followed the same scheme as the rest of the study. Analyses of the secondary antibody response profile endpoint and exploratory endpoint was conducted with this population.

Determination of sample size:

The sample size of 878 subjects per vaccine lot was chosen to yield 80% power for meeting the study's primary objective. The calculation allowed for a 5% dropout rate. Assuming independence among the comparisons, 93% power in each comparison was sufficient to yield 80% overall power. Calculations assumed that the GMT of each lot was no more than 10% greater than any other lot.

If a particular adverse reaction was expected to occur in 5% of placebo recipients, there was 86% power to detect a significant difference between treatment groups if the true frequency of the symptom among vaccine recipients was 10% (assuming a dropout rate of 5% in both groups). If an adverse reaction was expected to occur in 1% of placebo recipients – then there was 87% power to detect a significant difference between groups if the true frequency among vaccine recipients was 4%. The study population was large enough that an AE expected to occur in 1 in every 1000 vaccine recipients was likely to be observed in the study; specifically, there was about a 92% chance that such an event would be observed at least once during the study.

Sensitivity Analyses for the Primary Endpoint

The study included the following two prospectively designed sensitivity analyses 1) an analysis of vibriocidal equivalence (the primary study objective) adjusting for covariates including blood type, sex, and age to evaluate for any impact on Day 11 GMT ratio and 95% CI; and 2) an analysis of the GMT ratio and 95% CI adjusted for a single covariate, baseline vibriocidal titer. The variability of the GMT ratio used in the primary analysis could have been inflated by titers that were below the lower limit of quantification (LLOQ), which were assigned to the value of the LLOQ itself. To assess the impact of low titers, the primary analysis was repeated excluding any titer below the LLOQ. In addition, lots were compared based on the proportion of subjects with titers below the LLOQ on Day 11. A final sensitivity analysis compared ratios of the proportion of subjects in each lot who seroconverted by vibriocidal titer.

6.2.10 Study Population and Disposition

6.2.10.1 Populations Enrolled/Analyzed

See Section 6.2.10.1.3.

6.2.10.1.1 Demographics

Baseline demographics and blood type for vaccine and placebo recipients are shown in Table 25. The mean age of vaccine recipients was 29.9 years and of placebo recipients was 29.5 years. A larger percentage of subjects were women than men in both groups, 54.6% female in the vaccine group and 55.8% female in the placebo group. Most subjects were white, 69.1% of vaccine recipients and 62.1% of placebo recipients; black or African American subjects accounted for most of the remaining subjects, 24.7% of vaccine and 32.8% of placebo recipients. Overall, 10.0% of the subject population was Hispanic or Latino. The percentages of subjects with non-Type O blood were similar in the vaccine and placebo groups, 51.4% and 54.6%, respectively. Across individual vaccine lots, subjects were evenly distributed by age, sex, race, and ethnicity as well as blood type (data not shown). There were no obvious disparities likely to affect study results. The baseline characteristics in the Immune Sub-study Population followed a similar pattern to those in the full Randomized population except that no men and no subjects with Type O blood were included in the Immune Sub-study placebo group [Data not shown].

Table 25. PXVX-VC-200-004. Demographics – Randomized Population.

Baseline Characteristics	PXVX0200 All Lots N=2795	Placebo N=351	Total N=3146
Age in years			
Mean±SD	29.9±7.8	29.5±7.5	29.9±7.8
Gender, n(%)			
Male	1268 (45.4)	155 (44.2)	1423 (45.2)
Female	1527 (54.6)	196 (55.8)	1723 (54.8)
Race, n(%)			
American Indian or Alaskan Native	12 (0.4)	1 (0.3)	13 (0.4)
Asian	59 (2.1)	5 (1.4)	64 (2.0)
Native Hawaiian or Other Pacific Islander	8 (0.3)	1 (0.3)	9 (0.3)
Black or African American	6914 (24.7)	115 (32.8)	806 (25.6)
White	1930 (69.1)	218 (62.1)	2148 (68.3)
Multiracial	54 (1.9)	7 (2.0)	61 (1.9)
Other	41 (1.5)	4 (1.1)	45 (1.4)
Ethnicity, n(%)			
Hispanic or Latino	284 (10.2)	31 (8.8)	315 (10.0)
Not Hispanic or Latino	2511 (89.8)	320 (91.2)	2831 (90.0)
ABO Blood Type, n(%)			
Type O	1357 (48.6)	159 (45.4)	1516 (48.3)
Not Type O	1433 (51.4)	191 (54.6)	1624 (51.7)
Body Mass Index			
Mean±SD	28.2±7.0	28.3±7.5	28.2±7.0

Note: Percentages were based on the number of randomized subjects who had non-missing values in each treatment arm.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-004 Clinical Study Report, Table 15.

6.2.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

At least one pre-existing medical condition/medical history was reported by 76.9% of PXVX0200 recipients and 73.1% of placebo recipients. The most commonly reported medical conditions overall included surgical and medical procedures (34.8%), immune system disorders (28.4%), nervous system disorders (20.9%), psychiatric disorders (17.3%), and infections and infestations (15.8%). A medical history of gastrointestinal disorder was reported by 11.0% of PXVX0200 recipients and 11.1% of placebo recipients. The prevalence of medical conditions was well-balanced between the two vaccine groups and the challenged PXVX0200 vs placebo recipients.

6.2.10.1.3 Subject Disposition

Tables 26 and 27 below summarize the disposition of all randomized subjects through Day 181 by study group and by PXVX0200 lot number respectively. A total of 3146 subjects were randomized, 2795 to one of three lots of PXVX0200 and 352 to placebo. Overall, 92.7% of vaccine recipients and 94.3% of placebo recipients completed the study. There was one death due to suicide 85 days after receipt of PXVX0200 which was not considered related to study product. Two PXVX0200 recipients discontinued early due to serious adverse events that were not related to PXVX0200 administration. One subject had an exacerbation of depression and another subject had a fracture of the left patella. One subject was discontinued by the physician due to noncompliance. Narratives are provided in sections 6.2.12.2, 6.2.12.3 and 6.2.12.4.

Table 26. PXVX-VC-200-004. Subject Disposition – Randomized Population

Disposition	PXVX0200 All Lots N=2795	Placebo N=351	Total N=3146
Study Completion (Day 181)	2590 (92.7)	331 (94.3)	2921 (92.8)
Study Visit Completion			
Day 1	2788 (99.7)	351 (100.0)	3139 (99.8)
Day 11	2737 (97.9)	345 (98.3)	3082 (98.0)
Day 29	2694 (96.4)	341 (97.2)	3035 (96.5)
Reasons for Early Termination			
Withdrawal by Subject	26 (0.9)	3 (0.9)	29 (0.9)
Failure to Meet Randomization Criteria	1 (< 0.1)	1 (0.3)	2 (< 0.1)
Lost to Follow-up	168 (6.0)	16 (4.6)	184 (5.8)
Physician Decision	1 (< 0.1)	0	1 (< 0.1)
Protocol Deviation	0	0	0
Adverse Event	2 (< 0.1)	0	2 (< 0.1)
Death	1 (< 0.1)	0	1 (< 0.1)
Other	6 (0.2)	0	6 (0.2)

Note: Percentages were based on the number of randomized subjects who had non-missing values in each treatment arm.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-004 Clinical Study Report, Table 9.

Table 27. PXVX-VC-200-004. Subject Disposition: Individual PXVX0200 Lots – Randomized Population.

Disposition	PXVX0200 Lot A N=927	PXVX0200 Lot B N=933	PXVX0200 Lot C N=935
Study Completion (Day 181)	848 (91.5)	866 (92.8)	876 (93.7)

Disposition	PXVX0200 Lot A N=927	PXVX0200 Lot B N=933	PXVX0200 Lot C N=935
Study Visit Completion			
Day 1	925 (99.8)	929 (99.6)	934 (99.9)
Day 11	904 (97.5)	910 (97.5)	923 (98.7)
Day 29	885 (95.5)	898 (96.2)	911 (97.4)
Reasons for Early Termination			
Withdrawal by Subject	10 (1.1)	9 (1.0)	7 (0.7)
Failure to Meet Randomization Criteria	0	1 (0.1)	0
Lost to Follow-up	65 (7.0)	53 (5.7)	50 (5.3)
Physician Decision	0	0	1 (0.1)
Adverse Event	1 (0.1)	1 (0.1)	0
Death	1 (0.1)	0	0
Other	2 (0.2)	3 (0.3)	1 (0.1)

Note: Percentages are based on the total number of subjects treated with each vaccine lot.
Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-004 Clinical Study Report, Table 10.

Table 28 below summarizes the number and proportion of subjects in each analysis population.

Table 28. PXVX-VC-200-004. Analysis Populations - Randomized Population.

Population	PXVX0200 All Lots N=2795	Placebo N=351	Total N=3146
Randomized Subjects	2795 (100.0)	351 (100.0)	3146 (100.0)
Intent-to-Treat (ITT)	2788 (99.7)	351 (100.0)	3139 (99.8)
Safety	2789 (99.8)	350 (99.7)	3139 (99.8)
Immunogenicity Evaluable	2688 (96.2)	334 (95.2)	3022 (96.1)
Reasons not Included in Immunogenicity Evaluable	107 (3.8%)	17 (4.8%)	124 (3.9)
Randomized and Not Vaccinated ^a	7 (0.3)	0	7 (0.2)
Major Protocol Deviation that Affected Immunogenicity (other than Randomized and Not Vaccinated) ^a	43 (1.5)	8 (2.3)	51 (1.6)
No Day 11 Vibriocidal (classical Inaba) Result	57 (2.0)	9 (2.6)	66 (2.1)
Immune Sub-Study	26 (0.9)	6 (1.7)	32 (1.0)

Note: Percentages are based on the the number of randomized subjects in each treatment arm.

^aThe 58 subjects excluded from the Immunogenicity Evaluable Population were associated with 61 protocol deviations.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-004 Clinical Study Report, Table 12.

Protocol Deviations

A total of 18.4% of vaccine recipients and 19.1% of placebo recipients had any protocol deviation respectively. Table 29 below gives the breakdown of protocol deviations by study group. The most common deviation for both the combined vaccine group and the

placebo group was visits occurring outside of the protocol-specified window, 11.2% for vaccine recipients and 12.5% for placebo recipients. Protocol deviations in the 'Other' category were reported at a similar frequency, 4.3% for vaccine recipients and 4.6% for placebo recipients and included: laboratory processing errors, subjects randomized and not vaccinated, investigational product randomization errors, subjects cohabiting or co-working, and other administrative deviations from the protocol. Across the three vaccine lots, 17.7%, 19.8% and 17.8% of Lot A, B, and C recipients had protocol deviations respectively. The incidence of protocol deviations by category was similar across the individual vaccine lots (data not shown).

Table 29. PXVX-VC-200-004. Protocol Deviations – Randomized Population

Protocol Deviations	PXVX0200 All Lots N=2795	Placebo N=351	Total N=3146
Number of Subjects with Any Protocol Deviations	515 (18.4)	67 (19.1)	582 (18.5)
Assessment out of window	61 (2.2)	2 (0.6)	63 (2.0)
Inclusion/Exclusion criteria not met	2 (< 0.1)	0	2 (< 0.1)
Informed consent deviations	13 (0.5)	1 (0.3)	14 (0.4)
Protocol required assessment not done	57 (2.0)	6 (1.7)	63 (2.0)
Received excluded concomitant treatment	8 (0.3)	2 (0.6)	10 (0.3)
SAE reporting deviation	1 (< 0.1)	0	1 (< 0.1)
Subject ate or drank within 60 minutes before or after vaccination	1 (< 0.1)	0	1 (< 0.1)
Visit out of window	313 (11.2)	44 (12.5)	357 (11.3)
Wrong dose	3 (0.1)	0	3 (< 0.1)
Other ^a	121 (4.3)	16 (4.6)	137 (4.4)

Note: Percentages are based on the the number of subjects in each treatment arm.

Note: Subjects could have more than 1 protocol deviation.

^a "Other" includes laboratory procedure errors, subjects randomized and not vaccinated, investigational product randomization errors, subjects cohabiting or co-working, and other administrative deviations from the protocol.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-004 Clinical Study Report, Table 13.

Protocol deviations that led to exclusion from the Immunogenicity Evaluable Population were Major Protocol Deviations and included subjects:

- Who were randomized and did not receive vaccine
- Who received treatment different from what they were randomized to receive.
- Who received antibiotics 14 days prior to vaccination through 7 days after vaccination.
- From whom specimens were obtained outside of -3 to +5 days for Day 11 and ± 7 days for Day 29.

A total of 58 subjects (1.8% of vaccine recipients and 2.3% of placebo recipients) were excluded from the Immunogenicity Evaluable Population due to one of the aforementioned types of protocol deviations. In addition, 66 subjects (2.0% of vaccine recipients and 2.6% of placebo recipients) did not have a valid Day 11 vibriocidal antibody result due to inadvertent sample shipping and handling errors (13 subjects) and subjects missing their Day 11 visit (53 subjects).

One subject was reenrolled in error and received a second dose of PXVX0200 2 months after receiving the initial dose. The subject did not report any solicited adverse reaction or adverse event with either administration and was analyzed once as treated with the second dose considered as a concomitant medication.

Clinical Reviewer Comment: Overall, the proportion of subjects with deviations that led to exclusion from the Immunogenicity evaluable population was similar in the two treatment groups (3.8% of vaccine recipients, 4.8% of placebo recipients), which suggests that randomization was maintained.

6.2.11 Efficacy Analyses

6.2.11.1 Analyses of Primary Endpoint(s)

Table 30 presents the results of the primary analysis, evaluating immunologic equivalence of the three different production lots of PXVX0200 at Day 11. The primary objective was met, as the 95% CI for each GMT ratio was within (0.67, 1.5).

Table 30. PXVX-VC-200-004. Vibriocidal Geometric Mean Titer at Day 11 – Immunogenicity Evaluable Population.

	Lot A (P700-1CA03) N=892	Lot B (P700-3CA03) N=887	Lot C (P700-6BA03) N=909
Day 11 GMT (95% CI)^a	9220 (8219, 10343)	10034 (8942, 11260)	9827 (8770, 11012)

Note: The primary objective of the study was met if the CIs for all three lot geometric mean ratios were within [0.67, 1.5]. Assay results assessed vibriocidal activity against the classical Inaba biotype of *V. cholerae*. Values below the LLOQ ^{(b) (4)} were assigned the value of the LLOQ.

^aConfidence interval estimated directly from an ANOVA model with log-transformed vibriocidal titer at Day 11 as the outcome and lot as the single explanatory factor.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-004 Clinical Study Report, Table 18.

Table 31. PXVX-VC-200-004. Primary Analysis: Test for Equivalence of Vibriocidal Geometric Mean Titer (GMT) at Day 11 Across Three Lots – Immunogenicity Evaluable Population.

	Lot A:B	Lot B:C	Lot A:C
Day 11 GMT Ratio (95% CI)^b	0.92 (0.78, 1.08)	1.02 (0.87, 1.20)	0.94 (0.80, 1.10)

Note: Assay results assessed vibriocidal activity against the classical Inaba biotype of *V. cholerae*. Values below the LLOQ ^{(b) (4)} were assigned the value of the LLOQ.

^aConfidence intervals for geometric mean ratios were estimated using a common standard error derived from an ANOVA model with log-transformed vibriocidal titer at Day 11 as the outcome and lot as the single explanatory factor.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-004 Clinical Study Report, Table 18.

Sensitivity Analysis for Primary Objective: Sex, Race, Age and Titer Subgroups (data not shown)

A sensitivity analysis performed for the primary objective adjusting for covariates including blood type, sex, and age demonstrated that there was no significant association between blood type and titer or sex and titer; but age had a statistically significant effect on GMT ($p < 0.0001$). After adjusting for age, sex, and blood type, the three GMT ratios and associated 95% CIs were very similar (nearly identical) to the

corresponding calculations in the primary analysis. Adjusted Day 11 serum vibriocidal GMTs for antibodies against homologous classical Inaba were 9225 (95% CI 8230, 10341), 10053 (8967, 11271), and 9939 (8877, 11127) for Lots A, B, and C, respectively.

Additional sensitivity analyses comprised assessments that adjusted for baseline vibriocidal titer, included all vaccine recipients as treated, excluded titers less than the lower limit of quantitation (LLOQ), only included titers <LLOQ, and only included subjects who seroconverted. Baseline titers were significantly associated with GMT ($p < 0.0001$). After adjusting for baseline titer, GMT ratios were similar to those from the primary analysis, except in the analyses that included only samples with titers < LLOQ, which had very small sample sizes of 5 (0.6%), 7 (0.8%), and 9 (1.0%) for Lots A, B, and C, respectively.

6.2.11.2 Analyses of Secondary Endpoints

Estimation of the Seroconversion Rate of Vibriocidal Antibody By Day 11

Seroconversion, defined as a ≥ 4 -fold rise in vibriocidal antibody against homologous classical Inaba, was 94% [95% CI 93%, 94%] in vaccine recipients and 4% [95% CI 2%, 7%] in placebo recipients ($p < 0.0001$) at Day 11 (Table 32). GMTs at baseline and 10 days post-vaccination showed similar kinetics compared to those described in section 6.1.11.5 (study PXVX-VC-200-003) [data not shown].

Table 32. PXVX-VC-200-004. Vibriocidal Seroconversion Rate Against classical Inaba Vaxchora strain at 10 Days Postvaccination (Day 11) - Immunogenicity Evaluable Population

	PXVX0200 N=2687 ^a n (%)	Placebo N=334 ^a n (%)	p-value ^b
n ^c	2513	14	<0.0001
Seroconversion Rate ^d (95% CI)	94 (93, 94)	4 (2, 7)	<0.0001

^a Number of subjects with analyzable samples available from both baseline and 10 days postvaccination.

^b Fisher's exact test.

^c Number who seroconverted at 10 days postvaccination.

^d Seroconversion was defined as ≥ 4 -fold rise in anti-vibriocidal antibody titer from baseline to 10 days postvaccination.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-004 Clinical Study Report, Table 20.

Clinical Reviewer Comment: Anti-vibriocidal GMTs against the vaccine strain at 10 days post-vaccination appeared higher in study -004 compared to study 003 (Tables 30 and 16 respectively). This may be due to the higher Vaxchora dose administered in study -004 compared to study -003. GMTs for serum vibriocidal antibody against the classical Inaba vaccine strain on Days 1, 11, 29, 91, and 181 in the Immune Sub-Study Population in all lots were similar to the kinetics described in section 6. 1.11.5 for study -003 (Appendix 2).

6.2.11.3 Subpopulation Analyses

Subgroup analyses were performed by age, race, sex and blood type. Subanalyses by ethnicity are not shown because numbers of Hispanic or Latino subjects were too small for meaningful interpretation.

Table 33 below shows seroconversion rates across four non-overlapping age ranges of adults enrolled in studies PXVX-VC-200-004 and PXVX-VC-200-005. Seroconversion appears similar across the four age groups ranging from 18 through 45.

Table 33. PXVX-VC-200-004. Classical Inaba Seroconversion at 10 Days Post-Vaccination by Age – All PXVX0200 Recipients in Study PXVX-VC-200-004, Immunogenicity Evaluable Population

	Age (Years)			
	18-24	25-31	32-38	39-45 ^a
n	828	775	574	510
Seroconversion % (95% CI)^b	95 (93, 96)	93 (91, 95)	94 (91, 95)	92 (90, 95)

^a Two subjects were 46 years old and included in the 39-45 age range.

^b Includes subjects with non-missing vibriocidal antibody titers at baseline and at 10 days post-vaccination

Source: STN 125597_0, Module 2.7.3, Integrated Summary of Effectiveness, Table 24 and PXVX-Stat-Hoc-ISE (Post Hoc Analysis 5).

No clear differences were noted in serum classical Inaba vibriocidal seroconversion rate by race, sex, or blood type at Day 11 (Table 34).

Table 34. PXVX-VC-200-004. Classical Inaba Seroconversion at 10 Days Post-Vaccination by Race, Sex and Blood Type – All PXVX0200 Recipients in Study PXVX-VC-200-004, Immunogenicity Evaluable Population

	Black N=671	White N=1855	Male N=1206	Female N=1482	Type O Blood N=1299	Type Non-O Blood N=1386
Seroconversion % (95% CI)^a	94.0 (92.0, 95.7)	93.3 (92.1, 94.4)	94.4 (92.9, 95.6)	92.8 (91.4, 94.1)	94.1 (92.7, 95.4)	92.9 (91.5, 94.2)

^a Values < the LLOQ were assigned the value of the LLOQ.

Source: STN 125597_0, Module 2.7.3, Integrated Summary of Effectiveness, Tables 25,27 and Module 11.3.1.1.

6.2.11.4 Dropouts and/or Discontinuations

Missing values were left out of analyses.

6.2.12 Safety Analyses

6.2.12.1 Methods

The Safety Population was defined as the population of randomized subjects who received study treatment and was analyzed according to the treatment actually received. The Safety Population was used in all safety reporting and analyses.

Safety was assessed as described in section 6.2.7. Fisher's exact tests were used to compare vaccine and placebo groups for solicited adverse reactions. Comparisons were repeated for subgroups defined by sex, race, and blood type. No statistical inference was performed based on AE data.

6.2.12.2 Overview of Adverse Events

A total of 3139 subjects were enrolled in this study and included in the safety population; 2789 subjects received a single vaccination of PXVX0200 and 350 subjects received placebo.

Solicited Adverse Reactions

After vaccine administration, solicited adverse reactions were reported by 51.9% of vaccine recipients and 43.2% of placebo recipients (Table 35). The most common reactions among vaccine and placebo recipients were tiredness, headache, and abdominal pain. With the exception of lack of appetite and fever, there was a trend toward higher rates of solicited adverse reactions among vaccine recipients compared to placebo recipients. . Diarrhea (defined as ≥ 4 loose stools per 24 hours) was also reported in higher proportion of vaccine recipients (3.9%) compared to placebo recipients (1.2%). Most solicited adverse reactions were mild.

There appeared to be a greater frequency of combined moderate (defined as 5 loose stools per 24 hours) and severe (defined as ≥ 6 loose stools per 24 hours) diarrhea in vaccine recipients when compared to placebo recipients [41/2734 (1.5% (95% CI 1.1, 2.0)) vs 1/343 (0.3% (95% C(0.0%, 1.6%))].

Of note, 23 of 2789 (0.8%) vaccine recipients and 0 of 350 (0%) placebo recipients reported severe or worse diarrhea (≥ 6 loose stools per 24 hours after vaccination (Table 37). One vaccine recipient had 18 stools on Day 3 and presented to the emergency room (ER) where cultures revealed *V. cholerae* and enteropathogenic *Escherichia coli*; the narrative for this event is in Section 6.2.12.4.

Of the 106 PXVX0200 recipients that reported diarrhea, 74.5% and 17.9% reported diarrhea for 1 and 2 days respectively (data not shown). Peak diarrhea onset occurred on study day 2. Diarrhea was reported by 3.8% and 2.8% for 3 and 4 days respectively and one subject (0.9%) reported diarrhea for 7 days.

Solicited adverse reactions were reported at very similar rates across the three different vaccine lots (data not shown). Likewise, the frequency of solicited adverse reactions of at least moderate severity was very similar across lots (data not shown).

Table 35. PXVX-VC-200-004. Solicited Adverse Reactions – Safety Population

Solicited Adverse Reaction	PXVX0200 - All Lots N=2734 ^a	Placebo N=343 ^a
Any solicited adverse reaction	1419 (51.9)	148 (43.2)
Tiredness ^b	856 (31.3)	94 (27.4)
Mild	510 (18.7)	56 (16.3)
Moderate	328 (12.0)	34 (9.9)
Severe	18 (0.7)	4 (1.2)
Potentially Life-Threatening	0	0

Solicited Adverse Reaction	PXVX0200 - All Lots	Placebo
Headache ^b	791 (28.9)	81 (23.6)
Mild	516 (18.9)	50 (14.6)
Moderate	261 (9.6)	30 (8.8)
Severe	14 (0.5)	1 (0.3)
Potentially Life-Threatening	0	0
Abdominal Pain ^b	510 (18.7)	58 (16.9)
Mild	330 (12.1)	41 (12.0)
Moderate	169 (6.2)	17 (5.0)
Severe	11 (0.4)	0
Potentially Life-Threatening	0	0
Nausea/Vomiting ^c	501 (18.3)	52 (15.2)
Mild	364 (13.3)	39 (11.4)
Moderate	129 (4.7)	13 (3.8)
Severe	8 (2.9)	0
Potentially Life-Threatening	0	0
Lack of Appetite ^b	451 (16.5)	57 (16.6)
Mild	321 (22.7)	42 (12.2)
Moderate	121 (4.4)	15 (4.4)
Severe	9 (0.3)	0
Potentially Life-Threatening	0	0
Diarrhea ^d	106 (3.9)	4 (1.2)
Mild	65 (2.4)	3 (0.9)
Moderate	18 (0.7)	1 (0.3)
Severe	22 (0.8)	0
Potentially Life-Threatening	1 (0.04)	0
Fever ^e	17 (0.6)	4 (1.2)
Mild	6 (0.2)	1 (0.3)
Moderate	8 (0.3)	3 (0.9)
Severe	2 (0.07)	0
Potentially Life-Threatening	1 (0.04)	0

^a N=number of subjects who completed a memory aid following vaccination.

^b Headache, fatigue, myalgia, abdominal pain, and lack of appetite grading: 1 (mild): mild, no interference with activity; 2 (moderate): some interference with activity; 3 (severe): significant, prevents daily activity; 4 (potentially life threatening): ER visit or hospitalization.

^c Vomiting grading scale: 1 (mild): 1-2 episodes/24 hours; 2 (moderate): > 2 episodes/24 hours; 3 (severe): requires IV hydration; 4 (potentially life threatening): ER visit or hospitalization for hypotensive shock.

^d Diarrhea grading: 1 (mild): 4 loose stools/24 hours; 2 (moderate): 5 loose stools/24 hours; 3 (severe): ≥ 6 loose stools/24 hours; 4 (potentially life threatening): emergency room (ER) visit or hospitalization

^e Fever grading scale: 1(mild): 38.0-38.4°C; 2 (moderate): 38.5-38.9°C; 3 (severe): 39-40°C; 4 (potentially life threatening) > 40°C.

Source: STN 125597_0, Module 5.3.5.2, PXVX-VC-200-004 Clinical Study Report, Table 24.

Clinical Reviewer Comment: Rates of diarrhea are noted to be higher among vaccine recipients (3.9%) compared to placebo recipients (1.2%) in study -004. In study -003, which also enrolled 18-49 year old adults, 2.4% of vaccine recipients and 2.0% of placebo recipients reported any diarrhea within 7 days after vaccination. The difference in diarrhea rates between studies -003 and -004 may be due to the larger sample size enrolled into study -004 resulting in a more precise measurement of solicited adverse reactions. The difference in results between the two studies may also be explained by the higher Vaxchora dose administered in study -004.

Solicited Adverse Reactions by Subgroups

The frequencies of solicited adverse reactions were evaluated by sex, race (black and white), and blood type (O and non-O) for vaccine and placebo groups.

Women vaccine recipients reported solicited reactions slightly more frequently than men; specifically, 57.23% [54.69%, 59.74%] of women vaccine recipients reported at least one sign or symptom compared to 45.35% [42.54%, 48.19%] of men. The difference between sexes was observed among vaccines for every solicited reaction except fever. A similar pattern was observed between men and women in the placebo group, except for diarrhea; numbers were smaller among placebo recipients and confidence intervals are noted to be wider.

Differences in frequency of solicited adverse reactions were also noted between races. White vaccine recipients reported them more frequently than African-American or black vaccine recipients: 54.37% [52.09%, 56.64%] of white vaccine recipients reported at least one solicited adverse reaction compared to 43.24% [39.47%, 47.05%] of African-American or black vaccine recipients. Higher rates among whites were observed for each individual sign or symptom except fever and lack of appetite. In the placebo group, smaller differences were noted for 3 solicited reactions (headache, nausea/vomiting, and tiredness). Overall, 45.07% [38.26%, 52.02%] of white placebo recipients reported solicited adverse reactions compared to 39.82% [30.73%, 49.46%] of African-American or blacks.

Small differences were noted in solicited reactions between vaccine recipients of different blood types. Blood type O vaccine recipients had slightly higher rates of headache, lack of appetite, nausea/vomiting and tiredness. A similar trend was not observed among placebo recipients; slightly higher rates were observed among placebo recipients compared to vaccine recipients for 2 reactions (lack of appetite and nausea/vomiting). Numbers were noted to be smaller among placebo recipients and CIs were wider compared to vaccine recipients.

Clinical Reviewer Note: The noted differences in solicited adverse reactions based on sex and race are not likely to reflect differences in safety of the study product, since similar trends were observed in both the study vaccine and placebo groups. It is possible that there are differences in reporting by men and women which explain the sex and race subgroup analysis results. The differences noted based on blood type, although small, are more likely to reflect a possible impact of blood type on safety profile, since similar results were not observed in the vaccine and placebo group.

Unsolicited Adverse Events

Adverse events post-vaccination through Day 29 were reported by 23.0% of vaccine recipients and 24.0% of placebo recipients and were mostly mild in severity. The most common AEs reported among both vaccine and placebo recipients were headache (2.74%), fatigue (2.13%), and upper respiratory tract infection (1.98%). There were no trends demonstrating a higher proportion of vaccine recipients reporting individual AEs or AEs grouped by SOC compared with placebo groups. Adverse events in the Gastrointestinal Disorders SOC were most common in both vaccine and placebo recipients, 6.17% and 6.00% of subjects, respectively.

Each individual AE was reported by $\leq 3.14\%$ of vaccine or placebo recipients. Statistical analyses were not performed comparing AEs in the vaccine and placebo groups due to the small numbers of AEs in each category, but point estimates were similar in the two groups. Diarrhea was recorded as an AE in 0.36% of vaccine recipients and 0.29% placebo recipients, not contributing additional meaningful information to the discussion of diarrhea as a solicited adverse reaction above.

For AEs of moderate or higher severity, the most common AEs among both vaccine and placebo subjects were those also reported most commonly for all AEs, fatigue (vaccine 1.18%; placebo 0.86%), headache (vaccine 1.08%; placebo 0.86%), and upper respiratory tract infection (vaccine 0.65%; placebo 0.86%).

Narratives

A narrative is provided in section 6.2.12.3 for the one death in the study. Narratives are provided in section 6.2.12.4 for 23 other serious adverse events reported among 19 subjects. Narratives for two subjects who reported grade 4 events that did not meet the definition of a SAE but were considered probably related to the study product (fever of 104.7°F and diarrhea that resulted in an ER visit) are provided below.

Narratives of Other Non-Serious Significant Adverse Events

1. Fever of 104.7°F; Onset Day 1; Related; Resolved

Subject: a 25-year-old white female with history of erythromycin and penicillin allergy since 1996, Caesarean section in 2009, chronic anemia since 2010, back pain and heartburn since 2010, cholecystectomy in 2012, and anxiety, depression, and insomnia since 2013. The screening physical exam and vital signs were normal. The subject was randomized on 6/24/14 and received vaccine. On the day of vaccination, the subject developed grade 3 tiredness and on 6/25/14, she developed an isolated fever to 104.7°F. The tiredness was moderate by 6/26/14, mild by 6/27/14, and resolved by 7/10/14. The event is considered probably related to study vaccine.

2. Diarrhea that resulted in an ER visit; Onset Day 2; Related; Resolved

Subject: a 25-year-old white female with history of allergy to penicillin since 1990, exercise induced asthma from 2003-2006, seasonal allergic rhinitis since 2003, depression since January 2012, and a bulging cervical disk from a neck injury since July 2012. The screening physical exam and vital signs were normal. The subject was randomized on 6/17/14 and she received vaccine. On 6/18/14, the subject developed nausea and had 1 episode of diarrhea. On 6/19/14, she developed a total of 18 bowel movements and sought medical care in the ER. She was not hypotensive, tachycardic, or orthostatic and received 1 liter of IVF prior to discharge. Stool cultures identified both *Vibrio cholerae* and enteropathogenic *Escherichia coli*. Diarrhea decreased to 5 stools Day 4, 3 stools Day 5, and no diarrhea by Day 6. The

event is considered probably related to the study product and a concomitant infection with wild-type enteropathogenic *Escherichia coli*.

Clinical Reviewer Comment: The two non-serious significant AEs summarized above are considered probably related to the study vaccine by the FDA clinical reviewer.

6.2.12.3 Deaths

There was one death during the study due to suicide; this event is considered not related to study product by the study investigator, medical monitor and the FDA clinical reviewer. The narrative is provided below.

Narrative of Death

Suicide; Onset Day 85; Not Related; Resolved

Subject: a 38-year-old white male with a history of seasonal allergies and occasional headaches since 1985. The screening physical exam and vital signs were normal. The subject was randomized on (b) (6) at which time he received PXVX0200. Follow-up assessment revealed no solicited signs and symptoms and the subject did not report any AEs until this SAE occurred. Concomitant medications included only occasional use of acetaminophen for headache. After receiving a letter stating that the subject did not complete his last phone contact for the study, the subject's ex-wife called the site stating that she was unaware of his participation in the study. She reported his death by suicide and stated that she did not know any specific details. She did mention that "he had been a little depressed and under stress." She did not have the death certificate. This event is considered not related to the study vaccine.

6.2.12.4 Nonfatal Serious Adverse Events

The incidence of SAEs through Day 181 was similar in vaccine and placebo groups, 0.61% and 0.86%, respectively. Narratives for 23 SAEs, which occurred among 19 subjects, are provided below. No SAEs were considered related to the study product by the study investigator, an independent medical monitor at the site, and by the FDA clinical reviewer. All SAEs resolved by Day 181 except for the two SAEs that resulted in early withdrawal (exacerbation of depression and fracture of left patella).

Narratives of Serious Adverse Events

1. Acute colitis; Onset Day 100; Not Related; Resolved

A 31-year-old white male placebo recipient with a history of intermittent occasional rectal pain of unknown etiology developed acute colitis about 3 months after receipt of the placebo. He was admitted to the hospital and received IV fluids and antibiotics. The event is considered not related to the study vaccine.

2. Diabetic Ketoacidosis; Onset Day 71; Not Related; Resolved

A 21-year-old white female Vaxchora recipient with history of type 1 diabetes mellitus and celiac disease developed diabetic ketoacidosis about 2 months after vaccination. The event is considered not related to study vaccine.

3. Fracture of left ankle; Onset Day 148; Not Related; Resolved

A 33-year-old white female Vaxchora recipient fell down a hill while jogging and broke her left ankle about 2.5 months post-vaccination that required surgical repair. This event is considered not related to the study vaccine.

4. Renal Calculi; Onset Day 148; Not Related; Resolved
A 23-year-old male Vaxchora recipient with a prior family history of renal calculi was hospitalized about 5 months post-vaccination for renal calculi per CT scan. This event is considered not related to the study vaccine.
5. Pyelonephritis; Onset Day 7; Not Related; Resolved
A 35-year-old white female Vaxchora recipient reported blood in her urine on the day of vaccination and nausea, emesis, chills, fever, lower abdominal pain and bilateral flank pain on the day after vaccination. Five days after vaccination, she had a fever (102.5°F). She was hospitalized the same day for pyelonephritis and treated with antibiotics. This event is considered not related to the study vaccine.
6. Left Rib Fracture from Assault and Small Right Apical Pneumothorax; Onset Day 19; Not Related; Resolving
A 43-year-old black female Vaxchora recipient was assaulted about 2 weeks after vaccination. This event is considered not related to the study vaccine.

Jaw fracture; Onset Day 164; Not Related; Resolved
The same subject was involved in a motor vehicle accident and was hospitalized for a jaw fracture. This event is considered not related to the study vaccine.
7. Kidney infection; Onset Day 141; Not Related; Resolved
A 24-year-old white female placebo recipient with a history of juvenile rheumatoid arthritis and pancreatitis was admitted to the hospital for a kidney infection and treated with antibiotics and pain medications. This event is considered not related to the study vaccine.
8. Pneumothorax; Onset Day 3; Not Related; Resolved
A 41-year-old white male Vaxchora recipient was jailed after an altercation with the police during which he was tasered. Two days post-vaccination, he experienced chest pain. A chest CT confirmed rib fractures and a moderate pneumothorax. This event is considered not related to study vaccine. The subject was terminated early on 6/27/14 per principal investigator discretion due to protocol noncompliance.
9. Seizures; Onset Day 7; Not Related; Resolved
A 25-year-old white female Vaxchora recipient with a history of occasional tension headaches experienced a generalized seizure about 1 week after enrollment following a domestic trip by airplane. She was taken to a local ER where she had a second seizure. She was hospitalized and treated with Keppra. She reported having a normal CT and MRI. She denied head trauma, recent illness or fever, alcohol use, new medications, history of seizures, or solicited adverse reactions post-vaccination. She did report being sleep deprived. The subject was lost to follow-up. This event was considered not related to the study vaccine due to the absence of significant solicited adverse reactions that would afford a biological mechanism for relatedness.
10. Cholecystitis; Onset Day 92; Not Related; Resolved
A 43-year-old black female Vaxchora recipient with a history of high cholesterol developed acute cholecystitis about 3 months after vaccination. She was found to have gall stones and underwent a laparoscopy and cholecystectomy. This event is considered not related to the study vaccine.

11. Kidney Infection; Onset Day 177; Not Related; Resolved
A 21-year-old white female Vaxchora presented to the ER with chills, fever, and right upper quadrant and right costovertebral angle tenderness about 6 months after vaccination. She was 16 weeks pregnant at this time and had recently been treated for a urinary tract infection with Bactrim and Macrobid. Principal diagnosis was pyelonephritis and the subject was treated with antibiotics, including Rocephin and Cefalexin. This event is considered not related to the study vaccine. Follow-up of the pregnancy revealed a healthy full term female infant born (b) (6).
12. Cholelithiasis Onset Day 92 and Appendicitis Onset Day 96 Not Related; Resolved
A 31-year-old white female Vaxchora recipient reported abdominal pain and was admitted to the hospital about 3 months after vaccination. She was treated with IV antibiotics, a cholecystectomy was performed, and she was discharged the same day. The abdominal pain continued and the subject was admitted again 4 days later. An appendectomy was performed, and the abdominal pain resolved on the same day. This event is considered not related to the study.
13. Ileus; Onset Day 59; Not Related; Resolved
A 28-year-old white female Vaxchora recipient with a history of gastric bypass surgery, cholecystectomy and obesity started to experience abdominal pain and vomiting about 2 months after vaccination. She presented to the ER and was admitted to the hospital for ileus. This event is considered not related to the study vaccine.
14. Left ankle stress fracture; Onset Day 66; Not Related; Resolved
A 21-year-old white male Vaxchora recipient sustained a left ankle fracture during military training 2.5 months after vaccination. This event is considered not related to the study vaccine.
15. Recurrent tonsillitis; Onset Day 34; Not Related; Resolved
A 20-year-old white female placebo recipient developed tonsillitis about one month after vaccination. She was admitted to the hospital for a scheduled bilateral tonsillectomy. Per the histology report, tonsillar tissue showed reactive follicular hyperplasia. This event is considered not related to the study vaccine.
- Post-tonsillectomy bleed;
Five days post-tonsillectomy, the subject had a blood clot in her left tonsil fossa and was hospitalized. This event is considered not related to the study vaccine.
16. Acute appendicitis; Onset Day 107; Not Related; Resolved
A 19-year-old male Vaxchora recipient was admitted to the hospital with acute appendicitis about 3.5 months after vaccination. The subject underwent a laparoscopic appendectomy with no complications. This event is considered not related to the study vaccine
17. Exacerbation of Depression; Onset Day 8; Not Related; Lost to Follow-Up (LTFU) Day 1
A 31-year-old white male with history of depression that was reportedly stable on Zoloft at time of screening was randomized to receive Vaxchora. One and a half months after vaccination, the site was informed that the subject was admitted to a psychiatric facility due to exacerbation of depression. The subject did not consent to

more information being released. This event is considered unlikely to be related to the study vaccine.

18. Fracture of Left Patella; Onset Day 10; Not Related; LTFU Day 86

A 26-year-old white female Vaxchora recipient sustained a fracture of the left patella about 1.5 weeks after vaccination and was having surgery that day. She reported no AEs on Days 1 through 8. This event is considered not related to the study vaccine.

19. Thyroid cancer; Onset Day 96; Not Related; Resolved

A 32-year-old white female Vaxchora recipient was diagnosed with grade 3 trigeminal neuralgia about 2 weeks after vaccination; the event was considered not related, and it was treated with amoxicillin. About 3 months later, the fine needle biopsy results revealed an encapsulated papillary thyroid carcinoma. The subject underwent a hemi-thyroidectomy followed by a complete thyroidectomy. This event is considered not related to the study vaccine.

6.2.12.6 Clinical Test Results

Not applicable.

6.2.12.7 Dropouts and/or Discontinuations

There were 2 SAEs that resulted in early withdrawal (exacerbation of depression and fracture of left patella). See narratives in section 6.2.12.4.

6.2.13 Study Summary and Conclusions

The primary objectives of this Phase 3 study were to evaluate safety and immunogenicity of a single oral dose of PXVX0200 in adults aged 18 to 45 as well as to compare consistency of manufacture of three production lots with respect to immunogenicity. The objective of demonstrating consistency of manufacture was met since the three lots met the pre-specified criterion of immunologic equivalence: the 95% CI around each pairwise GMT ratio comparing Lots A:B [0.78, 1.08], Lots B:C [0.87, 1.20], and Lots A:C [0.80, 1.10] fell within the interval [0.67, 1.5].

A total of 3139 subjects were included in the safety population; 2789 subjects received a single vaccination of PXVX0200 and 350 subjects received placebo. Solicited adverse reactions were reported more commonly among vaccine recipients (51.90%) than placebo recipients (43.15%) ($p=0.0024$). The incidence of solicited adverse reactions did not reveal significant differences between vaccine and placebo recipients except for headache, reported in 28.93% of vaccine recipients and 23.62% of placebo recipients ($p=0.0419$), and diarrhea, reported in 3.88% of vaccine recipients and 1.17% of placebo recipients ($p=0.0079$). There were no serious adverse events that were considered related to the study product. Subgroup analyses by blood type, sex, and race did not reveal clear trends in solicited adverse reactions.

6.3 Study PXVX-VC-200-005 (NCT02100631): Immunogenicity Bridging to 46-64 Year Olds (Inclusive)

Study PXVX-VC-200-500 was entitled “A Phase III Randomized, Double-blind, Placebo controlled Study in Older Adults to Assess Immunogenicity and Clinical Acceptability of a Single Dose of the Live Oral Cholera Vaccine Candidate PXVX0200 *Vibrio cholerae* O1

Serotype Inaba Vaccine Strain CVD 103-HgR.” The study began enrollment on May 19, 2014, and the last subject completed the last study visit on January 15, 2015.

6.3.1 Objectives (Primary, Secondary, etc)

Primary Bridging Objectives

1. Demonstrate that seroconversion (defined as a ≥ 4 -fold rise over baseline) by classical Inaba vibriocidal antibody at Day 11 in older adults ages 46-64 years was non-inferior to seroconversion at Day 11 in younger adults ages 18-45 years following vaccination with PXVX0200.
 - Primary endpoint: seroconversion (defined as a ≥ 4 -fold rise over baseline) by classical Inaba vibriocidal antibody at 10 days post-vaccination.
 - Non-inferiority margin: The lower bound of the two-sided 95% CI on the difference in seroconversion rate between older and younger adults must be greater than -10 percentage points.
2. Demonstrate that the lower bound of the two-sided 95% CI on seroconversion by classical Inaba vibriocidal antibody at Day 11 is greater than 70% in older adults ages 46-64 years following vaccination with PXVX0200.

Secondary Bridging Objectives

Compare the classical Inaba vibriocidal geometric mean titer (GMT) attained by older adults ages 46-64 years to the GMT of younger adults ages 18-45 years following vaccination with PXVX0200.

- Secondary endpoint: ratio of the geometric mean of the vibriocidal antibody titer against the classical Inaba biotype of *V. cholerae* measured at Day 11 in 46 through 64 year olds to the analogous quantity measured at Day 11 in 18 through 45 year olds with corresponding 2-sided 95% CI on the geometric mean ratio [46-64 yr olds/18-45 yr olds]. Antibody titer values less than the LLOQ were assigned the same value as the LLOQ when calculating GMTs.

Exploratory Immunogenicity Objectives

1. Characterize the magnitude and kinetics of the vibriocidal antibody response against 4 *V. cholerae* biotypes and serotypes – classical Inaba, El Tor Inaba, classical Ogawa, and El Tor Ogawa – in older adults ages 46-64 years following vaccination with PXVX0200.
2. Characterize the magnitude and kinetics of the anti-CT antibody response in older adults ages 46-64 years following vaccination with PXVX0200.
3. Characterize the memory B cell response in older adults ages 46-64 years following vaccination with PXVX0200 based on the percentage of anti-O1 LPS IgA memory B cells of total IgA memory B cells at Days 1, 91, and 181 in the Immune Sub-study Population.

Clinical Reviewer Note: Because the memory B cell (b) (4) assay was not validated, results are not included in the review of this study. However, results of exploratory analyses evaluating antigen-specific memory B cell response as an immune correlate of duration of protection from study -003 are presented in section 6.1.11.5.

Safety Objective

Evaluate the safety of PXVX0200 in older adults ages 46-64 years.

6.3.2 Design Overview

The study was a Phase 3 randomized, double-blind, placebo-controlled study conducted to evaluate the safety and immunogenicity of PXVX0200 in older adults 46 through 64 years of age with no prior history of cholera infection or travel to a cholera-endemic area in the previous 5 years. A total of 398 subjects were randomized in a 3:1 ratio to receive one oral dose of either vaccine (N=299) or placebo (N=99); the placebo control was physiological saline. The randomization schedule used randomized permuted blocks of 8. The primary bridging objectives were to demonstrate that 1) seroconversion by classical Inaba vibriocidal antibody at Day 11 in older adults ages 46 through 64 years was non-inferior to seroconversion at Day 11 in younger adults 18 through 45 years of age (historical comparator group, from study 004) and 2) the lower bound of the 2-sided 95% CI on seroconversion by classical Inaba vibriocidal antibody at Day 11 is greater than 70% in older adults aged 46 through 64 years. The former objective serves to bridge to the efficacy established in 18 through 45 year old adults (in study PXVX-VC-200-003) by demonstrating that PXVX0200 generates an immune response in older adults that is non-inferior to the response in younger adults in the lot consistency study. The younger adult comparator group used for the bridging analysis consisted of the Immunogenicity Evaluable Population from study PXVX-VC-200-004 lot consistency study. Forty-five subjects from 6 sites were planned to be included in an Immune Sub-study to evaluate memory B cell activity and kinetics of vibriocidal and anti-CT antibody responses. Subjects enrolling in the overall study were offered enrollment into the Sub-study until the target number of subjects agreed to participate. Randomization of the Immune Sub-study subjects followed the same scheme as the rest of the study.

Subject eligibility was determined during a 45-day screening period and subjects were vaccinated on Day 1. Each subject was scheduled for at least four visits: a Screening Visit, a vaccination visit on Day 1 and post-vaccination visits on Day 11 and Day 29. Telephone follow-up contacts were scheduled for Day 91 and Day 181. Subjects in the sub-study returned to the clinic on Days 91 and 181 for assessments and blood draw. Subjects in the sub-study had blood samples drawn for memory B cell analysis at Days 1, 91, and 181. Samples for vibriocidal antibody and anti-CT antibody were also collected on Days 1, 11, 29, 91, and 181. The maximum total study duration for a given subject was approximately 226 days, including the screening period.

Blinding:

The study was conducted in a double-blind manner. An unblinded site pharmacist received the randomization form, determined the assigned treatment based upon the randomization schedule provided, and dispensed the PXVX0200 vaccine or placebo as appropriate. The study product was administered by unblinded personnel in the clinic. Subjects, clinical site personnel, principal investigators, and the sponsor remained blinded to individual treatment assignments until agreement had been obtained with the FDA on the primary endpoints and all subjects had completed their Day 181 visit. Biostatistics and data management personnel were unblinded (and portions of the database were locked) to subjects' treatment assignments after all subjects had completed their Day 29 visit in order to begin work on analyses. The remainder of the database was locked after all Day 181 visits had been completed. The primary analysis was conducted after all Day 181 data were available.

Clinical Reviewer Note:

1. The target concentration of 1×10^9 was selected to correspond to the upper end of the concentration range of 2×10^8 to 1×10^9 CFU/dose that was used in the previously marketed Orochol/Mutachol vaccine for the traveler's indication. Lot No. P700.550-6BA03 was the sole lot used in this study. It was also one of the three lots used in the lot-consistency study (PXVX-VC-002-004).
2. We agreed with PaxVax's approach to use the lot consistency study population (PXVX-VC-200-004) to bridge to the older adults (PXVX-VC-200-005) based on the following rationale:
 - a. As documented in our December 18, 2014 communication, we agreed with the use of vibriocidal antibody seroconversion (≥ 4 -fold rise above baseline) at 10 days post-vaccination as the primary endpoint to bridge vaccine efficacy to older adults. This was based on evidence provided from study PXVX-VC-200-003 that support its association between seroconversion and protection against moderate/severe diarrhea in both the 10 day and 3 month challenge cohorts.
 - b. The sample size of vaccinees in study PXVX-VC-200-004 (N=2687) was larger than the sample size in study PXVX-VC-200-003 (N=94). Thus, there was sufficient statistical power to conduct the primary non-inferiority comparison using the agreed up pre-specified non-inferiority criterion.
 - c. The two studies were very similar in design. They both evaluated the same Vaxchora dose (1×10^9 CFU) and had similar eligibility criteria. They also both used the same validated vibriocidal antibody assay.

6.3.3 Population

With two exceptions, study PXVX-VC-200-005 inclusion criteria were identical to those described in study PXVX-VC-200-004: 1) study -005 enrolled adults 46 through 64 years old (inclusive); and 2) no abnormal screening laboratory test results were allowed in study -005.

With one exception, study PXVC-VC-200-005 exclusion criteria were identical to those described in study PXVX-VC-200-004. In study -005, three categories of concomitant vaccines/medications were not allowed after vaccination until 29 days post-vaccination. This included any licensed vaccine (other than influenza vaccine), any antibiotic or chloroquine and any systemic immunosuppressive therapy (including radiation therapy or parenteral or high-dosage inhaled steroids). In contrast, study -004 did not allow receipt of these vaccines/ medications through 11 days post-vaccination.

6.3.4 Study Treatments or Agents Mandated by the Protocol

Study products administered included PXVX0200 (Vaxchora) and placebo. One lot of vaccine, Lot No. P700.550-6BA03, was used in this study. Each active component packet contained 1×10^9 CFU/dose at release. A single lot of buffer component was used in this study (Lot No. C1111013). Clinical sites used either sterile Water for Irrigation USP or purified, spring, distilled bottled water for reconstitution of the buffer component packet. The final vaccine suspension was administered orally at a dose of 1×10^9 CFU in an opaque cup.

Please see section 6.1.4, for other details regarding the description of the vaccine and placebo and administration.

6.3.5 Directions for Use

Directions for use were the same as those described in section 6.2.5.

6.3.6 Sites and Centers

The trial was conducted at 16 sites in the United States.

6.3.7 Surveillance/Monitoring

Safety surveillance for study PXVX-VC-200-005 was identical to the safety surveillance of study PXVX-VC-200-004 (see section 6.2.7).

Table 36. PXVX-VC-200-005 Study Flowchart.

Study Days	Screen (-45 days to -1 day)	Baseline Day 1	Day 11 (± 1 day)	Day 29 (± 3 days)	Telephone Contact Day 91 (± 7 days)	Telephone Contact Day 181 (± 7 days)
Visit Number	1	2	3	4	5	6
Informed consent	X					
Medical History	X	X				
Physical Examination	X	X ^a				
Vital Signs	X	X				
ABO Blood Typing	X					
Screening Labs	X					
Urinalysis	X					
Urine Pregnancy Test, if required	X	X ^b				
Vaccination		X				
Adverse Event Evaluation		Distributed	Collected	X	X ^c	X ^c
Prior/Concomitant Medications	X	X	X	X	X	X ^d
Memory Aid: Solicited Adverse Reactions		X	X			
Serology: Vibriocidal Assay		X	X	X		

^a If indicated by updated medical history.

^b Conducted or collected before vaccination.

^c Including Serious Adverse Events (SAEs).

^d Associated with Day 181 reported SAEs.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-005 Clinical Study Report, Table 3.

6.3.8 Endpoints and Criteria for Study Success

The primary immunogenicity endpoint and criteria for success and the secondary and exploratory immunogenicity endpoints are described in section 6.2.1.

The complete set of immunogenicity variables used to characterize the immune response in older adults ages 46-64 years were:

- Vibriocidal endpoints assessed in all subjects in the main study:
 - Seroconversion by vibriocidal antibody against the classical Inaba biotype of *V. cholerae* at Days 11 and 29.
 - Cumulative seroconversion by vibriocidal antibody against the classical Inaba biotype of *V. cholerae* through Days 11 and 29.
 - GMT of vibriocidal antibody against the classical Inaba biotype of *V. cholerae* at Days 1, 11, and 29.
 - Seroconversion by vibriocidal antibody against each of three other biotypes of *V. cholerae* (El Tor Inaba, classical Ogawa, and El Tor Ogawa) at Day 11.
 - GMT of vibriocidal antibody against each of 3 other biotypes of *V. cholerae* at Days 1 and 11.
- Vibriocidal endpoints assessed in the Immune Sub-study subjects:
 - Seroconversion by vibriocidal antibody against the classical Inaba biotype of *V. cholerae* at Days 11, 29, 91, and 181.
 - Cumulative seroconversion by vibriocidal antibody against the classical Inaba biotype of *V. cholerae* through Days 11, 29, 91, and 181.
 - GMT of vibriocidal antibody against the classical Inaba biotype of *V. cholerae* at Days 1, 11, 29, 91, and 181.
- Anti-CT endpoints assessed in the Immune Sub-study:
 - Seroconversion by anti-CT antibody at Days 11, 29, 91, and 181.
 - Cumulative anti-CT antibody seroconversion thru Days 11, 29, 91, and 181.
 - GMT of anti-CT antibody at Days 1, 11, 29, 91, and 181.

Blood samples were collected from all subjects and serum was tested for vibriocidal antibodies and IgG antibodies to CT. PMBCs were also analyzed for anti-O1 LPS and anti-CT IgG and IgA memory B cell responses, but results are reported for the anti-O1 LPS IgA response only (See explanation provided in section 6.1.8 under Tertiary and Exploratory Immunogenicity Endpoints as well as section 6.1.11.5 under Results Pertaining to Antigen-specific Memory B Cell Response as an Immune Correlate of Duration of Protection). Table 37 outlines the time points for immunogenicity assessments for all subjects and for subjects in the Immune Sub-study.

Table 37. PXVX-VC-200-005. Study PXVX-VC-200-005 Schedule of Immunogenicity Assessments

	Vibriocidal Assessment with classical Inaba (Study Days)	Vibriocidal Assessment with 3 other O1 Subtypes (Study Days)	Anti-CT Antibody (Study Days)	Memory B Cells (Study Days)
All Subjects	1, 11, 29	1, 11	None	None
Immune Sub-study, n=45	1, 11, 29, 91, 181	None	1, 11, 29, 91, 181	1, 91, 181 ^a

^a Only samples from vaccine recipients were analyzed.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-005 Clinical Study Report, Table 7.

6.3.9 Statistical Considerations & Statistical Analysis Plan

All statistical tests were two-sided with a significance level of $\alpha=0.05$, unless specified otherwise. Confidence intervals were constructed at the level of 95% unless specified otherwise. Immunogenicity values below the lower limit of quantification (LLOQ) were assigned the same value as the LLOQ.

Analysis Populations

1. Bridging Analysis Population: All subjects in the study PXVX-VC-200-005 Immunogenicity Evaluable Population (age 46-64) who received PXVX0200 were used for the primary, secondary, and additional bridging analyses. All subjects in the study PXVX-VC-200-004 Immunogenicity Evaluable Population (age 18-45) who received PXVX0200 were used as the comparator group for the primary, secondary, and additional bridging analyses.
2. Immunogenicity Analysis Populations
 - a. Intent-to-Treat (ITT) Population
The ITT population was defined as the population of randomized subjects who received study treatment. The ITT population was analyzed according to the treatment to which they were randomized.
 - b. Immunogenicity Evaluable Population: The Immunogenicity Evaluable Population was defined as the subset of subjects in the Intent-to-Treat population who had:
 - no major protocol deviations that affected immunogenicity
 - valid classical Inaba vibriocidal antibody results at both baseline and Day 11All immunogenicity analyses for the main study were conducted with the Immunogenicity Evaluable Population.
3. Immune Sub-study Population: The Immune Sub-study Population was defined as the subset of vaccine and placebo recipients who were selected at the time of randomization for memory B-cell evaluations as well as additional vibriocidal and anti-CT antibody sample collections. Given that the sub-study entailed more visits, it was carried out at a subset of planned study sites. Each participating site offered enrollment in the Immune Sub-Study to each subject enrolling in the overall study until the target number of subjects had agreed to participate. Randomization of Immune-Sub-Study subjects followed the same scheme as the rest of the study. All immunogenicity analyses for the sub-study were conducted with the Immune Sub-study Population.
4. Randomized Population: The Randomized Population included all randomized subjects and was used in all analyses of demographics and baseline summaries.

Determination of Sample Size

For the primary objective, the necessary sample size was calculated under the assumption that the true seroconversion rate is no more than 3.5 percentage points lower in older adults than in younger adults. In addition, based on earlier studies of CVD 103-HgR, the rate in younger adults is about 90%. Under these assumptions and using a Z-test with pooled variance, a sample of 280 vaccinated older adults and 2500 younger adults yields approximately 90% power to show that the lower limit of the 95% CI on the difference in the seroconversion rate is greater than the non-inferiority margin of -10 percentage points. Assuming 5% dropouts, the sample size was increased to 300

vaccine recipients. Given the 3:1 ratio of vaccine to placebo recipients, the total required sample size was estimated to be 400 older adults.

To meet the second primary objective, the lower bound of the 95% CI on Day 11 seroconversion rate is required to exceed 70%. The lower limit of the 95% CI on seroconversion in one challenge study conducted by Tacket et al²⁵ was 78%. With an overall sample size of 300 vaccinees, and assuming a true seroconversion rate of 80% among older adults, power was estimated to be 97% power to show that the lower 95% confidence bound on seroconversion is greater than 70%. Given the close correlation between the two primary endpoints, power estimates were not adjusted to account for multiple comparisons.

There was 95% power to detect significance if the true incidence of a particular symptom was 20% among vaccine recipients but expected to occur in only 5% or fewer placebo recipients. There was about a 95% chance that such an event expected to arise in 1 out of every 100 vaccinees would be observed at least once among the 300 vaccine recipients.

Sensitivity Analysis for the Primary Endpoint

A sensitivity analysis was conducted using logistic regression, with age group, baseline titer, sex and blood type as predictors and Day 11 seroconversion as a binary outcome. The Day 11 seroconversion rates and the difference between the seroconversion rates of older and younger adults was estimated from the logistic regression together with their 95% CIs.

6.3.10 Study Population and Disposition

6.3.10.1 Populations Enrolled/Analyzed

See section 6.3.10.1.3.

6.3.10.1.1 Demographics

In the Randomized Population (data not shown), vaccine and placebo recipients were similar in the breakdown by mean and median age, sex, race ethnicity and BMI; there was a smaller proportion of PXVX0200 recipients that had type O blood (37.1%) compared to placebo recipients (47.5%). Overall, the mean age at enrollment was 53.8 years, 54.3% of subjects were female, 74.9% were White and 21.9% were Black, 7.5% were Hispanic or Latino, and the mean BMI was 29.4.

The Bridging Analysis Population (Table 38), which includes 18-45 year old subjects in the Immunogenicity Evaluable Population from the lot consistency study and 46-64 year old subjects in the Immunogenicity Evaluable Population from the current study, differed in mean age (53.8 years vs 30.0 years); in addition, a smaller proportion of the 46-64 year old subjects had Type O blood (37.5%) compared to the 18-45 year old subjects (48.3%). The two populations were otherwise similar with regards to the breakdown of subjects by sex, race, and ethnicity.

Table 38. PXVX-VC-200-005. Demographics – Bridging Analysis Population

Baseline Characteristics	46-64 Yr Olds Study -005 N=291	18-45 Yr Olds Study -004 N=2688	Total N=2979
Age in years			
Mean±SD	53.8±5.0	30.0±7.8	32.3±10.4
Gender, n(%)			
Male	133 (45.7)	1206 (44.9)	1339 (44.9)
Female	159 (54.3)	1482 (55.1)	1640 (55.1)
Race, n(%)			
American Indian or Alaskan Native	6 (2.1)	11 (0.4)	17 (0.6)
Asian	0	56 (2.1)	56 (1.9)
Native Hawaiian or Other Pacific Islander	1 (0.3)	8 (0.3)	9 (0.3)
Black or African American	65 (22.3)	671 (25.0)	736 (24.7)
White	216 (74.2)	1855 (69.0)	2071 (69.5)
Multiracial	2 (0.7)	50 (1.9)	52 (1.7)
Other	1 (0.3)	37 (1.4)	38 (1.3)
Ethnicity, n(%)			
Hispanic or Latino	24 (8.2)	268 (10.0)	292 (9.8)
Not Hispanic or Latino	266 (91.4)	2420 (90.0)	2686 (90.2)
ABO Blood Type, n(%)			
Type O	109 (37.5)	1299 (48.3)	1408 (47.3)
Not Type O	182 (62.5)	1387 (51.6)	1569 (52.7)
Body Mass Index			
Mean±SD	29.8±6.7	28.2±7.0	28.4±7.0

Note: Percentages based on the number of subjects who had non-missing values in each group.

Note: The 46 thru 64 year group of the Bridging Analysis Population comprised all subjects in the Immunogenicity Evaluable Population of study PXVX-VC-200-005 while the 18 thru 45 year group comprised all subjects in the Immunogenicity Evaluable Population of study PXVX-VC-200-004.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-005 Clinical Study Report, Table 13.

6.3.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

At least one pre-existing medical condition/medical history was reported by 94.9% of PXVX0200 recipients and 93.9% of placebo recipients. The most commonly reported medical conditions overall include surgical and medical procedures (63.0%), musculoskeletal and connective tissue disorders (39.0%), immune system disorders (35.4%), metabolism and nutrition disorders (27.1%), psychiatric disorders (26.3%), vascular disorders (25.6%), gastrointestinal disorders (25.3%), and nervous system disorders (24.3%). A higher proportion of placebo recipients reported musculoskeletal and connective tissue disorders (48.5% vs 35.8%), metabolism and nutrition disorders (31.3% vs 25.7%) and psychiatric disorders (34.3% vs 23.6%) compared to PXVX0200 recipients. PXVX0200 recipients reported gastrointestinal disorders more frequently (27.7%) than placebo recipients (18.2%); PTs reported by > 1 subject and reported more frequently among PXVX0200 recipients compared to placebo recipients included dyspepsia (6.1% vs 2.0%), haemorrhoids (4.1% vs 1.0%), tooth development disorder (1.7% vs 1.0%), and constipation (1.4% vs 1.0%).

6.3.10.1.3 Subject Disposition

A total of 398 subjects were randomized, 299 to vaccine and 99 to placebo. In the randomized population (Table 39), 97.7% of vaccine recipients and 96% of placebo recipients completed the study. Of the 11 subjects who terminated early, 7 were lost to follow up, 3 did not receive study product because it was not available, and one

withdrew because of a death in the family. In the Immune Sub-study Population [Data not shown], 97.2% of vaccine recipients and 100% of placebo recipients completed the study.

Table 39. PXVX-VC-200-005. Subject Disposition - Randomized Population

Disposition	PXVX0200 N=299	Placebo N=99	Total N=398
Study Completion (Day 181)	292 (97.7)	95 (96.0)	387 (97.2)
Study Visit Completion			
Day 1	296 (99.0)	99 (100.0)	395 (99.2)
Day 11	296 (99.0)	99 (100.0)	395 (99.2)
Day 29	294 (98.3)	99 (100.0)	393 (98.7)
Day 91	293 (98.0)	97 (98.0)	390 (98.0)
Reasons for Early Termination			
Withdrawal by Subject	1 (0.3)	0	1 (0.3)
Lost to Follow-up	3 (1.0)	4 (4.0)	7 (1.8)
Other ^a	3 (1.)	0	3 (1.0)

Note: Percentages were based on the total number of subjects in each treatment arm.

^a "Other" includes 3 vaccine recipients randomized but not vaccinated because study product was not available.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-005 Clinical Study Report, Table 8.

Analysis Populations

Table 40 below summarizes the number and proportion of subjects in each analysis population. Three subjects were excluded from the ITT, Safety and Immunogenicity Evaluable Populations because they did not receive study treatment after they were randomized to PXVX0200 because the investigational vaccine was not available. An additional 5 subjects were excluded from the Immunogenicity Evaluable Population; of the 5 subjects, three had major protocol deviations and 2 had sample handling errors as follows:

- 1 subject who was administered 57 mL (instead of 100 mL) of vaccine prior to emesis.
- 1 subject who received antibiotics for diarrhea 6 days after vaccination
- 1 subject who had the Day 11 visit occur on Day 21
- 1 subject who had no Day 1 vibriocidal (classical Inaba) result due to sample handling error
- 1 subject who had not Day 11 vibriocidal (classical Inaba) result due to sample handling error.

Protocol Deviations

A total of 17.4% of vaccine recipients and 22.2% of placebo recipients had a protocol deviation. Most protocol deviations were considered minor and without meaningful impact on study results. Protocol deviations that led to exclusion from the Immunogenicity Evaluable Population were Major Protocol Deviations and included subjects:

- Who were randomized and did not receive vaccine
- Who received treatment different from what they were randomized to receive.

- Who received antibiotics 14 days prior to vaccination through 7 days after vaccination.
- From whom specimens were obtained outside of -3 to +5 days for Day 11 and \pm 7 days for Day 29.

Major protocol deviations that could affect immunogenicity resulted in 8 subjects being excluded from the Immunogenicity Evaluable Population, as summarized above.

Table 40. PXVX-VC-200-005. Study PXVX-VC-200-005 Subject Population and Groups – Randomized Population

Population	PXVX0200 N=299 n (%)	Placebo N=99 n (%)	Total N=398 n (%)
Randomized	299 (100.0)	99 (100.0)	398 (100.0)
Intent-to-Treat ^a	296 (99.0)	99 (100.0)	395 (99.2)
Safety ^b	296 (99.0)	99 (100.0)	395 (99.2)
Immunogenicity Evaluable ^c	291 (97.3)	99 (100.0)	390 (98.0)
Reasons not included in Immunogenicity Evaluable			
Randomized and not vaccinated	3 (1.0)	0 (0)	3 (0.8)
Major Protocol Deviations that Affected Immunogenicity (other than Randomized and not Vaccinated)	3 (1.0)	0 (0)	3 (0.8)
No Day 1 Vibriocidal (classical Inaba) Result	1 (0.3)	0 (0)	1 (0.3)
No Day 11 Vibriocidal (classical Inaba) Result	1 (0.3)	0 (0)	1 (0.3)
Immune Sub-study ^d	36 (12.0)	9 (9.1)	45 (11.3)

^a ITT=Randomized subjects who received vaccine or placebo analyzed according to randomized treatment.

^b Safety = Subjects who received vaccine or placebo analyzed by treatment actually received.

^c Immunogenicity Evaluable = Treated subjects with evaluable vibriocidal results from Day 1 and Day 11 and who had no major protocol deviations that affect immunogenicity.

^d Immune Sub-study = Subjects selected for the Immune Sub-study.

Source: STN 125597_0. Module 5.3.5.1. PXVX-VC-200-005 Clinical Study Report, Table 10.

6.3.11 Efficacy Analyses

6.3.11.1 Analyses of Primary Endpoint(s)

Table 41 presents the results of the primary objectives. By 10 days post-vaccination (Day 11), 90.4% (95% CI 86.4, 93.5) of 46-64 year old PXVX0200 recipients (study 005) and 93.5% (95% CI 92.5, 94.4) of 18-45 year old PXVX0200 recipients (study 004) seroconverted by classical Inaba vibriocidal antibody. The two primary objectives were met, as the lower limit of the 2-sided 95% CI on the difference in the seroconversion rate between the two age groups was greater than -10% (lower limit was -6.7%), and the lower limit of the 2-sided 95% CI on the seroconversion rate in the 46-64 year old subjects was > 70% (lower limit was 86.4%).

Similar results were obtained from a sensitivity analysis that used logistic regression to estimate the difference between seroconversion rates among the 46-64 year old subjects and the 18-45 year old subjects while adjusting for the effects of sex, blood type, and baseline titer (data not shown). The logistic regression yielded a lower limit of

the 2-sided 95% CI on the difference in seroconversion rate of -6.9%; and, the lower limit of the 2-sided 95% CI on seroconversion in the 46-64 year age group was 86.5% from the logistic regression model.

Table 41. PXVX-VC-200-005. Primary Bridging Analysis: Vibriocidal Antibody Seroconversion Against Classical Inaba *V. cholerae* at 10 Days Post-Vaccination – Bridging Analysis Population

Study Day Statistic	46 thru 64 Year Olds Study -005 N=291 ^a			15 thru 45 Year Olds Study -004 N=2687 ^a			Difference ^b	
	n ^c	%	95% CI	n	%	95% CI	%	95% CI
Seroconversion	263	90.4	86.4, 93.5	2513	93.5	92.5, 94.4	-3.1	-6.7, 0.4

^a N=number of subjects with any analyzable samples available at both Day 1 and Day 11.

^b Difference in seroconversion rate (46 thru 64 year olds – 18 thru 45 year olds)

^c n=number of subjects who seroconverted, defined as a ≥ 4 -fold rise in serum vibriocidal antibody titer against classical Inaba *V. cholerae* at 10 days post-vaccination.

Source: STN 125597_0. Module 5.3.5.1. PXVX-VC-200-005 Clinical Study Report, Table 15.

6.3.11.2 Analyses of Secondary Endpoints

Table 42 presents the results of the secondary bridging objective, which was to compare the classical Inaba vibriocidal GMT attained by 46-64 year olds to the GMT of 18-45 year olds (i.e., via a GMT ratio). By 10 days post-vaccination (Day 11), the classical Inaba vibriocidal GMT among 46-64 year old PXVX0200 recipients was significantly lower than the GMT among 18-45 year old PXVX0200 recipients. The GMT among older adults was 4282 (95% CI 3344, 5484) compared with a GMT of 9688 (95% CI 9067, 10351) among younger adults ($p < 0.0001$). The geometric mean ratio (46-64 yrs/18-45 yrs) was 0.44 (95% CI 0.34, 0.57).

Table 42. PXVX-VC-200-005. Secondary Bridging Analysis: Vibriocidal Geometric Mean Titer Against Classical Inaba *V. cholerae* at 10 Days Post-Vaccination – Bridging Analysis Population

Study Day Statistic	46 thru 64 Year Olds Study -005 N=291 ^a		15 thru 45 Year Olds Study -004 N=2688 ^a		GMT Ratio ^b	
	GMT	95% CI	GMT	95% CI	%	95% CI
GMT^b	4282	3344, 5484	9688	9067, 10351	0.44	0.34, 0.57
Mean, Max Titers	20, 163840		20, 327680			

^a N=number of subjects with an analyzable sample available at the indicated visit.

^b The geometric mean and geometric mean ratio, together with their 95% CIs, were based on t-statistics assuming normal distribution of the log titer.

Source: STN 125597_0. Module 5.3.5.1. PXVX-VC-200-005 Clinical Study Report, Table 16.

6.3.11.3 Subpopulation Analyses

Subgroup analyses were performed by age, race, sex and blood type. Subanalyses by ethnicity are not shown because numbers of Hispanic or Latino subjects were too small for meaningful interpretation.

Table 43 below shows seroconversion rates across four non-overlapping age ranges. Seroconversion appears to decrease by about 2 to 3% with each higher age group.

Table 43. PXVX-VC-200-005. Classical Inaba Seroconversion at 10 Days Post-Vaccination by Age – All PXVX0200 Recipients in Study PXVX-VC-200-005, Immunogenicity Evaluable Population

	Age (Years)		
	46-52	53-59	60-64
n	135	109	47
Seroconversion % (95% CI)^a	92 (86, 96)	90 (83, 95)	87 (74, 95)

^a Includes subjects with non-missing vibriocidal antibody titers at baseline and at 10 days post-vaccination

Source: STN 125597_0, Module 2.7.3, Integrated Summary of Effectiveness, Table 24 and PXVX-Stat-Hoc-ISE (Post Hoc Analysis 5).

No clear differences were noted in serum classical Inaba vibriocidal seroconversion rate by race, sex, or blood type at Day 11 (Table 44).

Table 44. PXVX-VC-200-005. Classical Inaba Seroconversion at 10 Days Post-Vaccination by Race, Sex and Blood Type – All PXVX0200 Recipients in Study PXVX-VC-200-005, Immunogenicity Evaluable Population

	Black N=65	White N=216	Male N=133	Female N=158	Type O Blood N=109	Type Non-O Blood N=182
Seroconversion % (95% CI)^a	90.8 (81.0, 96.5)	90.7 (86.1, 94.3)	91.7 (85.7, 95.8)	89.2 (83.3, 93.6)	92.7 (86.0, 96.8)	89.0 (83.5, 93.2)

^a Values < the LLOQ were assigned the value of the LLOQ.

Source: STN 125597_0, Module 2.7.3, Integrated Summary of Effectiveness, Tables 25,27 and 11.3.1.1.

6.3.11.4 Dropouts and/or Discontinuations

Missing values were left out of analyses.

6.3.11.5 Exploratory and Post Hoc Analyses

Additional Immunogenicity Objectives in 46-64 Year Olds

Additional immunogenicity objectives included characterizing 1) the magnitude and kinetics of the vibriocidal antibody response against 4 *V. cholerae* biotypes/serotypes – classical Inaba, El Tor Inaba, classical Ogawa, and El Tor Ogawa, and 2) the magnitude and kinetics of the anti-CT antibody response in older adults. For immune responses against Classical Inaba, cumulative seroconversion rates through 29 were provided, since few subjects were expected to seroconvert > 30 days after vaccination. For other biotypes and serotypes, seroconversion was characterized by Day 11 seroconversion rates and GMTs, as vibriocidal assessments for the other non-Classical Inaba biotypes and serotypes were only assessed on study days 1 and 11.

Immunologic Response Against Homologous Classical Inaba

1. *Cumulative Seroconversion in the Immunogenicity Evaluable Population (data not shown)*

By day 11, 90.4% (95% CI 86.4, 93.5) of 291 older PXVX0200 recipients in the Immunogenicity Evaluable Population seroconverted compared with 0% (95% CI 0,

3.7) of 99 placebo recipients. By Day 29, 94.2% (95% CI 90.8, 96.6) had seroconverted compared with 1% (95% CI 0.0, 5.5) of placebo recipients.

In the Sub-study Population, 97.2% of 36 older vaccine recipients had seroconverted by Day 11 compared with no placebo recipients, and by Day 29, 100.0% had seroconverted compared with no placebo recipients.

2. *GMTs of vibriocidal antibody against classical Inaba biotype of V. cholerae (data not shown)*
 - a. Immunogenicity Evaluable Population: From a Day 1 GMT for vaccine recipients and placebo recipients of 44 and 43, respectively, the GMT for vaccine recipients increased to 4282 (95% CI 3344, 5484) on Day 11 and was 2206 (95% CI 1780, 2735) on Day 29, while no change was seen for placebo recipients.
 - b. Immune Sub-Study Population: From a Day 1 GMT for vaccine recipients and placebo recipients of 39 and 54, respectively, the GMT for vaccine recipients increased to a peak of 4740 (95% CI 2541, 8845) on Day 11 and steadily decreased to 2412 (95% CI 1410, 4126) on Day 29, 177 (95% CI 114, 273) on Day 91, and 124 (95% CI 85, 179) on Day 181. No change over time was seen for placebo recipients).

Immunologic Response Against Other Biotypes and Serotypes in Immunogenicity Evaluable Population

Seroconversion rates against each of the O1 subtypes evaluated were higher among vaccine recipients compared to placebo recipients. A lower proportion of vaccine recipients seroconverted against the Ogawa serotype strains (71-73%) compared to the Inaba strains (90-91%). See Table 45 below.

Table 45. PXVX-VC-200-005. Seroconversion Rate at Day 11 (Vibriocidal Antibody Titer 4-Fold Rise), All Biotypes and Serotypes – Immunogenicity Evaluable Population

Cholera Strain	PXVX0200 N=291 % (95% CI)	Placebo N=99 % (95% CI)
Classical Inaba	90.4 (86.4, 93.5)	0 (0, 3.7)
El Tor Inaba	91.0 (87.1, 94.1)	5.1 (1.7, 11.4)
Classical Ogawa	73.2 (67.7, 78.2)	2.0 (0.2, 7.1)
El Tor Ogawa	71.4 (65.8, 76.5)	6.1 (2.3, 12.7)

Source: STN 125597_0. Module 5.3.5.1. PXVX-VC-200-005 Clinical Study Report, Table 22.

Immune responses measured by GMT showed a similar trend. On Day 11 the vibriocidal GMT was 4282 (95% CI 3344, 5484) for classical Inaba, 4929 (95% CI 3912, 6209) for El Tor Inaba, 1235 (95% CI 944, 1617) for classical Ogawa, and 1120 (95% CI 863, 1453) for El Tor Ogawa. The corresponding GMTs for placebo recipients were 44, 54, 53, and 50, respectively.

Clinical Reviewer Comment: In contrast, seroconversion rates at 10 days post-vaccination achieved by adults 18 through 45 years of age in study PXVX-VC-200-003 were similar across each of the four O1 subtypes evaluated. See footnote in Table 16.

Anti-Cholera Toxin Immunologic Response in the Immune Sub-study Population

The anti-CT antibody response was characterized by cumulative seroconversion and GMTs in the Immune Sub-Study Population. By Day 11, anti-CT antibody cumulative seroconversion for the 36 older vaccine recipients in the Immune Sub-study Population was 55.6% (95% CI 38.1, 72.1) compared with 0% (95% CI 0, 33.6) for placebo recipients. By Day 29, 69.4% (95% CI 51.9, 83.7) and by Day 91, 72.2% (95% CI 54.8, 85.8) of vaccine recipients had seroconverted, respectively. None of the nine placebo recipients had seroconverted through Day 29, although one did seroconvert by Day 91.

Anti-CT antibody GMT for vaccine recipients reached a peak of 4140 (95% CI 2512, 6822) on Day 29 and decreased to 2870 (95% CI 1985, 4148) on Day 91 and 2049 (95% CI 1380, 3044) on Day 181. GMT for placebo recipients was virtually unchanged across all time points.

6.3.12 Safety Analyses

6.3.12.1 Methods

The Safety Population was defined as the population of randomized subjects who received study treatment and was analyzed according to the treatment actually received. The Safety Population was used in all safety reporting and analyses.

Safety was assessed as described in section 6.3.7.

6.3.12.2 Overview of Adverse Events

A total of 395 subjects were enrolled in this study and included in the safety population; 296 subjects received a single vaccination of PXVX0200 and 99 subjects received placebo.

Solicited Adverse Reactions

Within 7 days after vaccination, solicited adverse reactions were reported by 36.3% of vaccine recipients and 50.5% of placebo recipients (Table 46). The most common solicited reactions among vaccine recipients included headache, tiredness and abdominal pain. A significantly higher proportion of placebo recipients reported tiredness (36.4% vs 20.0%) than vaccine recipients, and the proportion of placebo recipients who reported headache approached statistical significance (30.3% vs. 20.3%). Most adverse reactions were mild and lasted for 1 or 2 days.

When evaluating adverse reactions of at least moderate severity, a statistically significantly higher proportion of placebo recipients (23.2%) reported adverse reaction of moderate or worse intensity compared to vaccine recipients (11.9%) (data not shown).

Evaluation of the frequency of solicited adverse reactions by blood type, sex and race subgroups did not reveal differences between subjects with blood type O vs subjects with non-O blood type. However, females appeared to report more adverse reactions than males and white appeared to report more adverse reactions than blacks in both study groups.

Number of Days with Symptoms for Subjects Who Reported Solicited Adverse Reactions (data not shown, Tables 14.3.5.3.1 and 14.3.5.3.2)

Overall, of the 107 PXVX0200 recipients that reported any solicited reaction, 39.3%, 19.6%, 18.7% and 6.5% reported symptoms for 1, 2, 3 and 4 days respectively (data not shown). Between 2.8% and 5.6% of subjects reported any solicited reaction for a total of 5-8 days. The data were similar between placebo (N=50) and PXVX0200 recipients and for reactions including tiredness, headache, abdominal pain, nausea/vomiting and lack of appetite. Of the 296 PXVX0200 recipients that reported diarrhea, the trend differed in that 85.7% and 14.3% (N=7) reported diarrhea for 1 and 2 days respectively (data not shown); no subject reported diarrhea for more than 2 days. In the placebo group, 100% (N=2) reported diarrhea for one day. Of the 2 subjects that reported fever, both reported fever for 1 day.

Table 46. PXVX-VC-200-005. Solicited Adverse Reactions – Safety Population

Solicited Adverse Reaction	PXVX0200 N=296^a	Placebo N=99^a
Any Solicited Adverse Reaction	107 (36.3)	50 (50.5)
Headache ^b	60 (20.3)	30 (30.3)
Mild	41 (13.9)	19 (19.2)
Moderate	17 (5.8)	11 (11.1)
Severe	2 (0.7)	0
Potentially Life-Threatening	0	0
Tiredness ^b	59 (20.0)	36 (36.4)
Mild	37 (12.5)	20 (20.2)
Moderate	20 (6.8)	16 (16.2)
Severe	2 (0.7)	0
Potentially Life-Threatening	0	0
Abdominal Pain ^b	42 (14.2)	13 (13.1)
Mild	34 (11.5)	10 (10.1)
Moderate	7 (2.4)	3 (3.0)
Severe	1 (0.3)	0
Potentially Life-Threatening	0	0
Nausea/Vomiting ^c	35 (11.9)	12 (12.1)
Mild	27 (9.2)	9 (9.1)
Moderate	7 (2.4)	3 (3.0)
Severe	1 (0.3)	0
Potentially Life-Threatening	0	0
Lack of Appetite ^b	24 (8.1)	12 (12.1)
Mild	14 (4.7)	11 (11.1)
Moderate	8 (2.7)	1 (1.0)
Severe	2 (0.7)	0
Potentially Life-Threatening	0	0
Diarrhea ^d	7 (2.4)	2 (2.0)
Mild	4 (1.4)	1 (1.0)
Moderate	2 (0.7)	0
Severe	1 (0.3)	1 (1.0)
Potentially Life-Threatening	0	0

Solicited Adverse Reaction	PXVX0200	Placebo
Fever ^e	2 (0.7)	0
Mild	2 (0.7)	0
Moderate	0	0
Severe	0	0
Potentially Life-Threatening	0	0

^a N=number of subjects who completed a memory aid following vaccination.

^b Headache, fatigue, myalgia, abdominal pain, and lack of appetite grading: 1 (mild): mild, no interference with activity; 2 (moderate): some interference with activity; 3 (severe): significant, prevents daily activity; 4 (potentially life threatening): ER visit or hospitalization.

^c Vomiting grading scale: 1 (mild): 1-2 episodes/24 hours; 2 (moderate): > 2 episodes/24 hours; 3 (severe): requires IV hydration; 4 (potentially life threatening): ER visit or hospitalization for hypotensive shock.

^d Diarrhea grading: 1 (mild): 4 loose stools/24 hours; 2 (moderate): 5 loose stools/24 hours; 3 (severe): ≥ 6 loose stools/24 hours; 4 (potentially life threatening): emergency room (ER) visit or hospitalization

^e Fever grading scale: 1(mild): 38.0-38.4°C; 2 (moderate): 38.5-38.9°C; 3 (severe): 39-40°C; 4 (potentially life threatening) > 40°C.

Source: STN 125597_0, Module 5.3.5.2, PXVX-VC-200-005 Clinical Study Report, Table 25.

Unsolicited Adverse Events

Unsolicited adverse events were reported by 20.6% of vaccine recipients and 27.3% of placebo recipients through Day 29. Most AEs were mild in severity in both vaccine and placebo groups. There were no trends demonstrating a higher percentage of vaccine recipients reporting individual AEs or AEs grouped by SOC compared with placebo groups. The most common individual AEs reported by vaccine recipients were fatigue (3.7%), back pain (2.0%), and decreased appetite (1.7%). Flatulence, rash, headache, pain in extremity, and nausea were reported by 1% of PXVX0200 recipients. All other AEs were reported by < 1% of vaccine recipients (data not shown, Table 27 in CSR). Among placebo recipients, fatigue, diarrhea, tooth fracture, and upper respiratory tract infection were each reported by 3% of subjects; back pain, flatulence, rash, abnormal feces, sinusitis, and sunburn were each reported by 2.0% of subjects. All other AEs were reported by ≤ 1.0% of placebo recipients (data not shown, Table 27 in CSR). The most common AE SOCs reported among vaccine recipients included General Disorders and Administration Site Conditions (PXVX0200 4.7%, 3.0% placebo), Gastrointestinal Disorders (PXVX0200 4.4%, 9.1% placebo), and Musculoskeletal and connective tissue disorders (PXVX0200 5.4%, 4.0% placebo).

Unsolicited AE Related to Study Product (data not shown, Table 30 in CSR)

Adverse events considered related to study product were reported by 7.1% of vaccine recipients and 9.1% of placebo recipients (data not shown, Table 30 in CSR) and most were mild in severity. Among PXVX0200 recipients, related AEs of mild intensity and not already solicited in the study include flatulence (1.0%), lymph node pain (0.3%), dyspepsia (0.3%), retching (0.3%), pain (0.7%), malaise (0.3%), dizziness (0.3%), dysgeusia (0.3%), and tremor (0.3%). Placebo recipients also reported flatulence and dizziness as related events.

Fatigue of moderate intensity was reported by three (1%) vaccine recipients and one (1%) placebo recipient; two (0.7%) vaccine recipients each reported pain (general) and decreased appetite of moderate intensity; and one (0.3%) vaccine recipient each reported abdominal pain upper, vomiting, malaise, and neck pain of moderate intensity, while 1 (1%) placebo recipient each reported musculoskeletal pain and pain in extremity

of moderate intensity. One vaccine recipient each reported diarrhea and back pain of severe intensity.

6.3.12.3 Deaths

No subjects died during the study.

6.3.12.4 Nonfatal Serious Adverse Events

A total of 3 subjects reported 3 SAEs among 296 PXVX0200 recipients (1.0%); no SAEs were reported among 99 placebo recipients. The 3 SAE reports (atrial fibrillation, myocardial infarction, and spinal compression fracture) occurred among vaccine recipients more than 119 days after vaccination and were considered not related to the study product. Narratives are provided below.

Narratives:

1. Atrial fibrillation; Not Related; Not Resolved

A 57-year-old white male PXVX0200 recipient with a history of hypertension was hospitalized about 5.5 months after vaccination for atrial fibrillation. The subject's history of hypertension likely explains the episode that required hospitalization. This event was considered not related to the study vaccine in the opinion of the investigator, medical monitor and FDA clinical reviewer.

2. Wedge compression fractured T12 vertebrae; Not Related; Resolved

A 58-year-old white male PXVX0200 recipient reported falling out of a tree about 1 month after vaccination, which resulted in a T12 vertebrae fracture. This event was considered not related to the study vaccine in the opinion of the investigator, medical monitor and FDA clinical reviewer.

3. Myocardial Infarction; Not Related; Resolved

A 57-year-old white male PXVX0200 recipient with a history of hypertension, hypercholesterolemia, and sleep apnea taking hydrochlorothiazide, atenolol and aspirin experienced a life-threatening myocardial infarction about 4 months post-vaccination. He was hospitalized and underwent a coronary artery bypass graft. This event was considered not related to the study vaccine in the opinion of the investigator, medical monitor and FDA clinical reviewer.

6.3.12.6 Clinical Test Results

Not applicable.

6.3.12.7 Dropouts and/or Discontinuations

Of the 11 subjects who terminated early, 7 were lost to follow up, 3 did not receive study product because it was not available, and one withdrew because of a death in the family. No subject terminated early due to an adverse event.

6.3.13 Study Summary and Conclusions

Study PXVX-VC-200-005 is the study which supports effectiveness of Vaxchora in adults 46 through 64 years of age. It was designed to 1) evaluate the safety and immunogenicity of a single oral dose of Vaxchora in adults 46 through 64 years of age, and 2) bridge effectiveness in 46 through 64 year olds to 18 through 45 year olds who participated in study PXVX-VC-200-004. Classical Inaba vibriocidal antibody seroconversion at Day 11 was selected as the primary immunogenicity endpoint since it

had been shown to be associated with protection against moderate to severe diarrhea in study PXVX-VC-003.

Immunogenicity Conclusions

The primary objectives of this study included 1) demonstrating that in 46 through 64 year old adults, seroconversion by classical Inaba vibriocidal antibody on Day 11 was non-inferior to seroconversion in 18 through 45 year olds; and 2) that the lower bound of the two-sided 95% CI on seroconversion was greater than 70% in 46 through 64 year old adults. Noninferiority was defined as a lower bound of the two-sided 95% CI on the difference in seroconversion rate being greater than -10 percentage points. Both primary objectives were met. On Day 11 following vaccination, 90.4% [95% CI; 86.4%, 93.5%] of older subjects and 93.5% [95% CI; 92.5%, 94.4%] of younger subjects had seroconverted by classical Inaba vibriocidal antibody and the lower bound of the two-sided 95% CI on the difference in seroconversion between older and younger adults was -6.7%. The lower bound of the two-sided 95% CI on seroconversion in older adults was 86.4%.

A secondary bridging objective compared the classical Inaba vibriocidal GMT attained by older adults to that attained by younger adults. On Day 11 following vaccination, the classical Inaba vibriocidal GMT [95% CI] attained by older adults was 4282 [3344, 5484] compared with the GMT of 9688 [9067, 10351] in younger adults ($p < 0.0001$).

The mean \log_2 fold-increase in classical Inaba vibriocidal antibody titer between Days 1 and 11 was 6.6 [6.2, 7.0] in older adults, which was significantly lower than the mean \log_2 fold-increase of 7.1 [7.0, 7.3] attained by younger adults.

While older adults demonstrated similar seroconversion as younger adults, analyses based on continuous endpoints such as GMT and fold-rise showed that younger adults generate a more robust immune response to vaccination. These findings may be explained by the more sensitive assessment of the magnitude of age-related decreases in immune response afforded by the use of continuous variables such as GMT and fold-rise. Fold-rise, which helps to adjust for variability in baseline titer, yielded a smaller estimate of the difference between age groups compared to GMT. However, the clinical relevance of the noted diminished GMTs and fold-rises are not known.

Vibriocidal immune responses at Day 11 against the four cholera strains tested demonstrated similar results for classical Inaba and El Tor Inaba with a 90.4% [86.4%, 93.5%] seroconversion rate and a 4282 [3344, 5484] GMT and a 91.0% [87.1%, 94.1%] seroconversion rate and a 4929 [3912, 6209] GMT, respectively. Responses for classical Ogawa and El Tor Ogawa vibriocidal antibody were also similar to one another but lower than for the Inaba biotypes with a 73.2% [67.7%, 78.2%] seroconversion rate and a 1235 [944, 1617] GMT and a 71.4% [65.8%, 76.5%] seroconversion rate and a 1120 [863, 1453] GMT, respectively.

The PXVX0200 clinical development program only used the El Tor Inaba strain as a challenge agent in the PXVX-VC-200-003 study, therefore effectiveness against the other three strains can only be inferred. Nonetheless, vibriocidal antibody seroconversion of 90.4%, 73.2% and 71.4% with 95% CI lower bounds of 86.4%, 67.7%, and 65.8% for the classical Inaba, classical Ogawa, and El Tor Ogawa strains, respectively, suggests that protection against these strains in older adults may be expected, which supports the planned indication of immunization against *V. cholerae* serogroup O1.

The anti-CT antibody response resulted in a cumulative seroconversion of 72.2% among vaccine recipients by Day 91 and a GMT peak of 4140 on Day 29, with GMT declining to 2049 on Day 181.

The percentages of anti-O1 LPS IgA memory B-cells increased 2.0-fold [1.0, 4.2] in vaccine recipients from Day 1 to Day 91, and 2.0-fold [1.5, 4.1] from Day 1 to Day 181.

Safety Conclusions

Solicited adverse reactions after vaccine administration were reported by over a third (36.3%) of vaccine recipients and half (50.5%) of placebo recipients. There were no adverse reactions reported in a significantly higher proportion of vaccine recipients compared to placebo recipients. In the subgroup analyses, women reported solicited adverse reactions more frequently than men, and whites reported solicited adverse reactions more frequently than blacks. Similar trends were observed among both vaccine and placebo recipients.

Unsolicited AEs were reported by less than one quarter of vaccine (20.6%) and slightly more than one quarter (27.3%) of placebo recipients with no clinically meaningful differences in the frequency and severity of AEs between vaccine and placebo recipients. Both solicited adverse reactions and unsolicited AEs were mostly mild in severity and most vaccine recipients who reported solicited adverse reactions reported symptoms for 1 or 2 days.

6.4 Study PXVX-VC-200-002 (NCT01585181): Phase 1 Safety and Immunogenicity

Study PXVX-VC-200-002 was a phase 1 randomized (5:1), double-blind, placebo (2 g lactose)-controlled study which evaluated vaccine shedding and the potential for transmission of the vaccine strain from vaccine recipients to close household contacts. The study evaluated a single dose of 4.43×10^8 CFU (lot # PR-1002B) of PXVX0200 in 18 through 50 year old adults with no prior history of cholera or enterotoxigenic *E. coli* infection and who did not live with household contacts under 18 years of age or over 65 years of age. The study was conducted in two U.S. sites. Enrollment began on May 7, 2012, and last subject completed the last study visit on January 7, 2013. In this trial, immunogenicity assays for the trial were performed at a different lab than the one used for the phase 3 trials and relied on methods that were not validated. Therefore, study design and results pertinent to immunogenicity are not presented in this review.

All subjects provided a fresh stool sample or rectal swab before vaccination. Approximately half of subjects provided additional samples or swabs on Days 1, 3, and 7 following vaccination, while the other half provided samples or swabs on Days 2, 4, and 7 following vaccination. Household contacts residing with the study subjects provided a fresh stool sample or rectal swab in clinic on Day 7¹¹ (7 days after vaccination of the study participant in their household) for evaluation of shedding and serum for assessment of seroconversion (≥ 4 fold rise) on Day 28 after vaccination.

¹¹ Baseline was Day 0, and Day 7 was 7 days post-baseline (post-vaccination of randomized study participants).

Solicited reactions were recorded daily by subjects through 7 days post-vaccination using a symptom diary which assessed fever, tiredness, headache, lack of appetite, nausea or vomiting, diarrhea and abdominal pain. Unsolicited adverse events, medically attended events, serious adverse events and new onset chronic medical conditions through 6 months post-vaccination.

A total of 66 subjects (55 vaccine and 11 placebo recipients) and 24 household contacts associated with a vaccine recipient were enrolled. Males comprised 50% of subjects and household contacts; mean age among subjects was 29.9 years and 32.6 years among household contacts; 76%-79% of subjects and household contacts were white and 21% of subjects and household contacts were black; 3% of subjects were Asian.

Shedding of vaccine bacteria was detected overall in 9.6% (95% CI 3.2, 21.0) of vaccine recipients on any day through 7 days post-vaccination. During the 7 days post-vaccination, the proportion of subjects shedding was highest on day 7 [5.8% (95% CI 1.2, 15.9)]. The duration of shedding of the vaccine strain is unknown, as day 7 post-vaccination was the latest time point evaluated. The vaccine strain was not detected in stool samples or rectal swabs collected on day 7 post-vaccination for the 24 household contacts of vaccinees. No household contact seroconverted by Day 28.

Clinical Reviewer Note:

- Antibody responses, especially as assessed by anti-CT(b) (4), appeared higher at one clinical site (CVD at the University of Maryland) than the other (University of Kentucky(UK)). The anti-CT GMT was significantly higher at CVD at each time point (including baseline). The difference between the sites was thought to possibly be due to CVD using sterile, non-bacteriostatic water to reconstitute the lyophilized vaccine prior to vaccination while University of Kentucky used tap water.
- The observation above led to laboratory studies to explore the potential effect of different types of water on vaccine stability. Laboratory studies demonstrated that some types of tap water had a detrimental effect on vaccine stability following reconstitution. Given concerns over whether chlorine or other additives in tap water could affect the dose of Vaxchora, sterile water for irrigation was used in the phase 3 challenge study. Sterile water for irrigation, of purified, spring or distilled bottled water were used in studies -004 and -005. Purified bottled water is specified in the package insert for reconstitution of the vaccine (see section 4.1 for more details regarding specification of purified bottled water in the package insert).

There were no SAEs reported among vaccine or placebo recipients. One household contact reported a SAE of pericarditis 67 days after vaccination of his associated vaccine recipient. This occurred in a 29 year old white male who presented with prostatitis and pericarditis 62 and 67 days, respectively, after vaccination of the associated vaccine recipient. The subject improved after initiation of antibiotic treatment and both conditions resolved within 20 days of the onset of prostatitis. There was no evidence of exposure to vaccine by shedding or seroconversion, and the event was not considered related to the vaccine.

Solicited adverse reactions were reported by 40.0% of vaccine recipients and 45.5% of placebo recipients (Table 47). The most common adverse reactions were abdominal discomfort, headache, and diarrhea. Most solicited adverse reactions were mild. One severe adverse reaction was reported by a placebo recipient (fever between 102° and

104°F). Diarrhea, defined as greater than 2 loose stools per 24 hours, was reported in a higher proportion of vaccine recipients (14.5%) compared to placebo recipients (0.0%). Of the 8 vaccine recipients with diarrhea, 7 reported mild diarrhea and 1 reported moderate diarrhea (defined as ≥ 4 loose stools in 24 hours – the definition of mild diarrhea in study -003).

Table 47. PXVX-VC-200-002. Frequency of Solicited Adverse Reactions

	PXVX0200 N=55 n (%)	Placebo N=11 n (%)
Any Solicited Adverse Reaction	22 (40.0)	5 (45.5)
Abdominal Pain	10 (18.2)	3 (27.3)
Diarrhea	8 (14.5)	0 (0)
Headache	8 (14.5)	2 (18.2)
Tiredness	6 (10.9)	0 (0)
Nausea/Vomiting	4 (7.3)	1 (9.1)
Lack of Appetite	3 (5.5)	1 (9.1)
Fever ^a	1 (1.8)	1 (9.1)

^a Fever is defined as a temperature greater than 100.4°F.

Source: STN 125597_0, Module 5.3.5.2, PXVX-VC-200-002 Clinical Study Report, Table 24

Unsolicited AEs were reported by 30.9% of vaccine recipients and 36.4% of placebo recipients through 28 days post-vaccination. While most AEs were mild or moderate, 3 severe events were reported: a two-day headache beginning 22 days post vaccination in a vaccine recipient; a case of streptococcal pharyngitis in a placebo recipient; and 10 days of diarrhea in a vaccine recipient that began 28 days after vaccination and was accompanied by moderate pharyngitis and moderate pyrexia. None of the severe AEs was considered related to the vaccine by site physicians. Overall, two AEs – one each in the vaccine and placebo groups – were assessed as being possibly related to treatment. One was a mild episode of abdominal discomfort in a vaccine recipient (who also reported severe headache for 2 days noted above) that started on Day 3 after vaccination and lasted for two days and the other was mild abdominal distension in placebo recipient that started on Day 1 of vaccination and was resolved by Day 2.

7. INTEGRATED OVERVIEW OF EFFICACY

This section is not applicable. One phase 3 study evaluated efficacy against moderate to severe diarrhea, and two phase 3 immunogenicity studies were conducted in different age groups. The phase 1 study used a non-validated vibriocidal assay that differed from the validated vibriocidal assays used in the phase 3 studies. Thus, pooling of data is not appropriate.

8. INTEGRATED OVERVIEW OF SAFETY

8.1 Safety Assessment Methods

For all studies, safety evaluations included solicited adverse reactions through 7 days post-vaccination which were recorded daily in a memory aid. Unsolicited adverse events through 28 days post-vaccination and SAEs through 6 months post-vaccination were recorded on the case report form. All summaries of adverse events were based on the

Safety Population, defined as randomized subjects who received study treatment and were analyzed according to the treatment actually received.

8.2 Safety Database

8.2.1 Studies/Clinical Trials Used to Evaluate Safety

The safety of Vaxchora was evaluated in four randomized, placebo-controlled multicenter clinical trials submitted in this BLA. Across the four trials, there were 28 sites: 22 in the U.S. and 6 in Australia.

8.2.2 Overall Exposure, Demographics of Pooled Safety Populations

A total of 3235 adults 18 through 64 years of age received one dose of Vaxchora and 562 adults received one dose of placebo (physiologic saline (N=551) or lactose (N=11)). Of the 3235 adults who received Vaxchora, 3085 subjects received a single dose containing 1×10^9 CFU in the phase 3 lot consistency and older adult immunogenicity studies combined. Of the remaining subjects, 55 subjects received a single dose containing 4.43×10^8 CFU in the phase 1 study and 95 subjects received a single dose containing 5×10^8 CFU in the phase 3 challenge study.

Among the 3235 Vaxchora recipients and 562 placebo recipients 18 through 64 years of age who participated in four clinical trials submitted to this BLA, the mean age was 32.5 years; 53.8% of trial participants were female; by race, 67.1% were white, 27.3% black or African American, 1.8% Asian, 1.7% multiracial, 1.3% other, 0.6% American Indian or Alaskan Native and 0.3% Native Hawaiian or Pacific Islander; by ethnicity, 9.3% were Hispanic or Latino; by blood type, 47.3% had type O blood and 52.6% had non-O type blood.

8.2.3 Categorization of Adverse Events

See section 8.1.

8.3 Caveats Introduced by Pooling of Data Across Studies/Clinical Trials

Pooled safety data should be interpreted with caution, as the studies evaluated varying doses of CVD 103-HgR and different age groups.

8.4 Safety Results

8.4.1 Deaths

There was one death reported in study PXVX-VC-200-004 due to suicide 84 days after receipt of Vaxchora. This event was considered not related to study product. The narrative is provided in section 6.2.12.3.

8.4.2 Nonfatal Serious Adverse Events

Overall, 20/3235 (0.6%) vaccine recipients reported a SAE compared with 3/562 (0.5%) of placebo recipients. Serious adverse events are summarized for each of the phase 3 studies in sections 6.1.12.4, 6.2.12.4, and 6.3.12.4, respectively. None of the SAEs were assessed as related to vaccination. There were no SAEs reported among subjects randomized in the phase 1 study. However, one household contact of a randomized subject reported a SAE of pericarditis 67 days after vaccination of his associated vaccine

recipient; there was no evidence of exposure to the vaccine strain by shedding or seroconversion, and the event was not considered related to the vaccine (see section 6.4).

8.4.3 Study Dropouts/Discontinuations

Overall, 93.7% of vaccine recipients and 95.0% of placebo recipients completed the study. The most frequent reason for early discontinuation was loss to follow up, which occurred in 5.3% of vaccine recipients and 3.9% of placebo recipients. Other reasons for discontinuation occurred in $\leq 0.8\%$ of subjects in either study group (i.e., withdrawal by subject, failure to meet randomization criteria, protocol violation, adverse event, death, physician decision or other).

There were 2 SAEs that resulted in early withdrawal (exacerbation of depression and fracture of left patella). Both occurred in study -004. See narratives in section 6.2.12.4.

8.4.4 Common Adverse Events

Overall, 23.7% of PXVX0200 recipients and 27.4% of placebo recipients reported at least one AE (Table 48). The most commonly reported AEs (Table 51) included headache (2.5-2.7%), fatigue (2.2-3.2%), and upper respiratory tract infection (2.1%).

Table 48. Summary of Most Common Adverse Events After Vaccination with Vaxchora – Integrated Summary of Safety

Adverse Events Within 28 Days of Vaccination	PXVX0200 N=3235	Placebo N=562
Number of Subjects with At Least One Adverse Event	767 (23.7)	154 (27.4)
Headache	80 (2.5)	15 (2.7)
Fatigue	71 (2.2)	18 (3.2)
Upper Respiratory Tract Infection	67 (2.1)	12 (2.1)
Back Pain	45 (1.4)	6 (1.1)
Flatulence	37 (1.1)	7 (1.2)
Abdominal Pain	35 (1.1)	5 (0.9)
Decreased Appetite	29 (0.9)	6 (1.1)
Nausea	28 (0.9)	5 (0.9)
Arthralgia	27 (0.8)	2 (0.4)
Oropharyngeal Pain	24 (0.7)	5 (0.9)
Musculoskeletal Pain	19 (0.6)	5 (0.9)
Constipation	18 (0.6)	5 (0.9)
Dizziness	18 (0.6)	3 (0.5)
Viral Upper Respiratory Tract Infection	18 (0.6)	3 (0.5)
Abnormal Feces	17 (0.5)	2 (0.4)
Diarrhea	16 (0.5)	6 (1.1)
Neck Pain	15 (0.5)	4 (0.7)

Note: The adverse events in this table are those observed at a frequency of $\geq 0.5\%$ in recipients of PXVX0200 in studies PXVX-VC-200-002, -003, -004, and -005.

Source: STN 125597_0, Module 2.7.4, Integrated Summary of Safety, Table 9.

8.4.5 Clinical Test Results

Not applicable.

8.4.6 Systemic and Local Reactogenicity

The most commonly reported solicited adverse reactions (Table 49) included tiredness (29-30%), headache (26-28%), abdominal pain (17-18%), nausea/vomiting (16-17%), lack of appetite (16-17%), and diarrhea (2-4%).

Table 49. Solicited Adverse Reactions After Vaccination with Vaxchora – Integrated Summary of Safety

Solicited Adverse Reactions within 7 Days Post-vaccination	PXVX0200 N=3177 ^a	Placebo N=553 ^a
Tiredness	953 (30.0)	163 (29.5)
Headache	882 (22.8)	144 (26.0)
Abdominal Pain	582 (18.3)	94 (17.0)
Nausea/Vomiting	554 (17.4)	86 (15.6)
Lack of Appetite	495 (15.6)	93 (16.8)
Diarrhea	115 (3.6)	9 (1.6)
Fever	22 (0.7)	6 (1.1)

^a N=Number of subjects who completed a memory aid following vaccination in studies PXVX-VC-200-002, -003, -004, and -005.

Source: STN 125597_0, Module 2.7.4, Integrated Summary of Safety, Table 7.

8.5 Additional Safety Evaluations

8.5.1 Dose Dependency for Adverse Events

Dose dependent adverse events were not formally evaluated in clinical trials with Vaxchora, and no studies have been conducted to examine the safety and effectiveness of re-vaccination.

Clinical Reviewer Note: As previously mentioned, the Vaxchora dose administered in study -003 was lower than the dose administered in studies -004 and -005. However, only study 004 evaluated the same age range of subjects as study -003. Across-study comparisons between studies -003 and -004 show some differences in the reported rates of adverse reactions. Study -004 has a trend towards a higher rate of solicited adverse reactions among vaccine recipients compared to study -003. This observation, however, must be interpreted with caution, as study -004 is a much larger study than study -003. The differences may be due to the larger sample size yielding more precise measurements of solicited adverse reactions. Other factors that might have resulted in different across-study results are differences in the populations reporting practices

8.5.2 Time Dependency for Adverse Events

Not applicable.

8.5.3 Product-Demographic Interactions

In study PXVX-VC-200-003, subgroup analyses by sex, race and blood type did not reveal any clear and consistent differences in safety between subgroups.

In studies PXVX-VC-200-004 and PXVX-VC-200-005, solicited adverse reactions were summarized by trial for subgroups based on sex, age, race, and blood type. Women and White subjects appeared to experience more solicited adverse reactions in study -004, however this was observed in both the Vaxchora and placebo groups. Blood type O vaccine recipients had slightly higher rates of headache, lack of appetite,

nausea/vomiting, and tiredness compared to non-O blood type vaccine recipients; a similar trend was not observed among placebo recipients by blood type.

In study PXVX-VC-200-005, there was no clear trend in solicited adverse reactions by blood type. As in study -004, females and White subjects in study -005 appeared to experience more solicited adverse reactions than males and Black subjects respectively. However, this trend was observed in both Vaxchora and placebo groups.

8.5.4 Product-Disease Interactions

Not evaluated.

8.5.5 Product-Product Interactions

The potential immunologic interference between Vaxchora and concomitant medications was not evaluated in pre-licensure clinical trials. Study eligibility criteria were designed to exclude subjects taking medications that were likely to affect immunogenicity. Because Vaxchora is a live bacterial vaccine, concomitant use of antibiotics is not advised; these agents may be active against the vaccine strain and prevent a sufficient degree of multiplication to occur in order to induce a protective immune response. Immunosuppressive therapies may reduce the immune response to the vaccine.

There are data in the scientific literature demonstrating that the anti-malarial drug chloroquine should not be administered within 1 week of Orochol vaccination since it was shown to diminish the immune response to the vaccine.⁴⁷ A one week time frame was specified because Orochol was reportedly shown to be effective at one week post-vaccination. No interference was seen when Orochol was administered concomitantly with antimalarial mefloquine and proguanil medications or live yellow fever vaccine. Because Vaxchora includes the same active ingredient as Orochol, there is a possibility that chloroquine may interfere with the immune response to Vaxchora. Therefore, the package insert includes a statement in section 7.2 indicating that Vaxchora should be administered at least 10 days before beginning antimalarial prophylaxis with chloroquine. A 10 day timeframe is specified in the Vaxchora package insert, because efficacy was established at 10 days following vaccination.

Likewise, no data are available on concomitant administration of VAXCHORA with other vaccines, including travel vaccines (i.e., other live vaccines such as typhoid vaccine strain Ty21a (Vivotif), oral polio vaccine and yellow fever vaccine).

Each of the Vaxchora clinical studies required that subjects avoid eating or drinking for 60 minutes before and after vaccination to avoid adverse effects on the buffering of gastric acid in the stomach and hence the viability of the vaccine strain. However, immunologic interference due to eating and drinking within an hour before or after vaccine administration has not been studied.

8.5.6 Human Carcinogenicity

VAXCHORA has not been evaluated for the potential to cause carcinogenicity.

8.5.9 Person-to-Person Transmission, Shedding

Please see section 6.4 (Study PXVX-VC-200-002) for more details regarding evaluations of Vaxchora transmission and shedding.

Environmental Risk Assessment

In accordance with the National Environmental Policy Act, all BLAs must be accompanied by either an environmental analysis or a claim of categorical exclusion. PaxVax submitted an environmental assessment since the presence of a *mer* operon means the vaccine strain cannot be considered to “occur naturally in the environment” per 21 CFR 25.31(c).

The environmental risk assessment identified five hazards listed below. The likelihood of these hazards occurring and the estimated risk were determined to be low or negligible.

1. Pathogenicity of the modified strain:
 - Potential reversion of the attenuated vaccine strain to a pathogenic strain through transfer of a functional *ctxA* gene to *V. cholerae* 103-HgR.
 - Pathogenicity may not be completely eliminated by the genetic modifications, as there could be other products manufactured by the *V. cholerae* 569B parent strain which cause disease.
2. Recovery of of hemolysin gene: Because a (b) (4) fragment of the *hlyA* gene was deleted during construction of CVD 103-HgR, the hemolysin gene locus could only be restored by transfer of a wild type hemolysin gene from a wild-type El Tor strain, not solely from a recombination event that results in deletion of the *mer* operon.
3. Unintended exposure or release into the environment of the vaccine strain in a household setting or release into the environment via excretion into the municipal sewage system, during shipping due to accidental spills or improper handling, and improper administration and controls at the point of use. [Of note, data from controlled laboratory studies have demonstrated that the vaccine strain does not multiply in soil or estuarine water.]
4. Components of the final product, including lactose and casein, could induce allergic response in susceptible individuals.
5. Long term persistence of the vaccine strain in aquatic environment

Management strategies to mitigate the potential harmful effects of three hazards determined to be of non-negligible (i.e., low) risk were identified as below:

1. Exposure of excreted vaccine product in a household setting could lead to infection or seroconversion of exposed individuals:
 - a. Good hygiene for at least 2 weeks after vaccination will be emphasized.
 - i. Wash hands after using the toilet and before handling food or eating
 - ii. Keep unwashed hands and items used for toilet purposes, away from the mouth, eyes, ears, nose, and wounds
 - iii. Do not use shared or unclean eating utensils, drinking cups, towels and handkerchief
 - iv. Instructing caregivers of infant/toddler recipients to properly manage handling and disposal of diapers for 3-4 weeks post-vaccination.
2. Exposure to the vaccine product through improper administration and controls at the point of use could lead to infection or seroconversion of exposed individuals.
 - a. Recommended procedures for vaccine management that eliminate potential routes of exposure include:
 - i. Destroying spilled liquid vaccine by chemical methods such as 10% bleach or 70% isopropyl alcohol before disposal and following institutional procedures for the disposal of biohazardous material

- ii. Destroying unused vaccine at the administration site following institutional procedures for the disposal of biohazardous material;
 - iii. Discarding waste generated during the administration of the vaccine, such as disposable cups and empty active component packets, into appropriate biohazard containers and disposing of the waste following institutional procedures for the disposal of biohazardous material;
- 3. Components of the final product, including lactose and casein, could induce allergenic response in susceptible individuals.
 - a. The product label will include all components of the vaccine; lactose and casein digest will be listed as inactive ingredients. A warning about possible (even rare) allergic reactions will be incorporated. The product will be dispensed only by order of qualified physicians who would evaluate individual patient history prior to administration. Patients with a history of allergy would not receive the vaccine product.

8.6 Safety Conclusions

An integrated review of safety data following administration of a single dose of Vaxchora finds that the Vaxchora safety profile is acceptable. There was one death reported in study PXVX-VC-200-004 due to suicide 84 days after receipt of Vaxchora. No death or SAE reports were assessed as related to vaccination by study investigators, the Applicant or the CBER clinical reviewer.

9. ADDITIONAL CLINICAL ISSUES

9.1 Special Populations

9.1.1 Human Reproduction and Pregnancy Data

Maternal cholera disease is associated with adverse pregnancy outcomes including fetal death.

CVD 103-HgR is not absorbed systemically following oral administration, and maternal use is not expected to result in fetal exposure to the bacteria.

The vaccine strain may be shed in the stool of the vaccinated mother for at least 7 days, with a potential for transmission of the vaccine strain from mother to infant during vaginal delivery.

Clinical Reviewer Note: Transmission to the infant during vaginal delivery is a theoretical risk based on shedding studies submitted to this BLA (see Section 6.4) and published literature demonstrating that constituents of the maternal fecal microbiota are transmitted to the infant during vaginal delivery.

Ten pregnancies occurred during the submitted pre-licensure studies (all in study PXVX-VC-200-004). Eight occurred among PXVX0200 vaccine recipients. Each of the 8 vaccine recipients who became pregnant delivered at term; and with regard to the infant, no adverse events were reported. Of the 2 placebo recipients, one delivered at 33 weeks. The other placebo recipient was lost to follow up and the outcome of the pregnancy is unknown. Seven of the eight vaccine recipients were determined to not be pregnant at the time that they received the study vaccine (based on last menstrual period and estimated date of delivery). For one of the eight vaccine recipients, the

temporal relationship between vaccination and date of conception was not determined. Neither placebo recipient was pregnant at the time of test article administration. Three subjects reported mild to moderate solicited adverse reactions within one week after vaccination (including the one subject who may have been pregnant at the time of vaccination). There were no vaccine-related unsolicited AEs reported among the 8 vaccine recipients who became pregnant during the study. There was one serious adverse event, a case of kidney infection almost 6 months after vaccination at which time the subject was 16 weeks pregnant and had recently been treated for a UTI (narrative provided in section 6.2.12.4) that was not related to vaccination.

Clinical reviewer note: Although the vaccine is not anticipated to cause adverse outcomes in pregnancy because it is not systemically absorbed, the submitted data are not sufficient to establish the presence or absence of risk with regard to use during pregnancy.

9.1.2 Use During Lactation

VAXCHORA is not absorbed systemically by the mother following oral administration, and breastfeeding is not expected to result in exposure of the child to VAXCHORA.

9.1.3 Pediatric Use and PREA Considerations

The safety and effectiveness of VAXCHORA in the pediatric population less than 18 years of age have not been established.

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), the submission of this original BLA required a Pediatric Study Plan for the claimed indication. PaxVax requested a partial waiver of the pediatric study requirement for children younger than 2 years of age, because Vaxchora does not represent a meaningful therapeutic benefit over existing measures recommended by the CDC for the prevention of cholera for children in this age group, and Vaxchora is not likely to be used by a substantial number of children in this age group (section 505B(a)(4)(B)(iii) of PREA). Children younger than 2 years of age traveling to cholera affected areas whose parents follow CDC's recommendations for the prevention of cholera, would have little chance of exposure to contaminated food or water. Breastfeeding and/or feeding formula prepared with sterile water would further reduce the possibility of exposure to contaminated food or water.^{48,49} Furthermore, the low incidence of cholera among US children suggests that Vaxchora is not likely to be used by a substantial number of children less than 2 years of age.

Under the provisions of Section 505B(a)(3)(A)(i) of the Federal Food, Drug, and Cosmetic Act, PaxVax requested a deferral of pediatric studies for the pediatric population 2 years to < 18 years of age on the basis that Vaxchora is ready for approval for use in adults and the pediatric studies have not been completed. PaxVax's deferred study, PXVX-VC-200-006, to evaluate the safety and effectiveness (immunogenicity) of Vaxchora in children 2 years through 17 years of age is a required postmarketing study under PREA. The status of this postmarketing study must be reported annually according to 21 CFR 601.70 and section 505B(a)(3)(B) of the Federal Food, Drug, and Cosmetic Act. PaxVax estimates that the final protocol will be submitted by December 31, 2016, the study will be completed by December 31, 2018, and the final clinical study report will be submitted by June 30, 2019.

The Pediatric Study Plan was presented to FDA's Pediatric Review Committee (PeRC) on May 11, 2016. The Committee agreed with the Pediatric Study Plan.

9.1.4 Immunocompromised Patients

The safety and effectiveness of Vaxchora have not been established in immunocompromised individuals. The immunologic response to Vaxchora may be diminished in immunocompromised individuals. Because Vaxchora may be shed in the stool of recipients for at least 7 days, there is a potential for transmission of the vaccine strain to non-vaccinated close contacts (i.e., household contacts). Therefore, caution is advised when considering whether to administer Vaxchora to individuals with immunocompromised close contacts.

9.1.5 Geriatric Use

The safety and effectiveness of Vaxchora have not been established in adults 65 years of age or older.

9.1.6 Persons Previously Exposed to Cholera

The safety and efficacy of Vaxchora was not studied in those previously exposed to cholera (e.g. from previous travel or residency in cholera-affected areas). Previous experience with Orochol/Mutachol Berna suggests that a higher dose may be needed in this population.

10. CONCLUSIONS

The efficacy of a single dose of Vaxchora has been established in adults 18 through 45 years of age for the prevention of moderate to severe diarrhea following challenge with a heterologous, live wild type *V. cholerae* O1 El Tor Inaba strain at 10 days and 3 months post-vaccination. Moderate to severe diarrhea was defined as cumulative diarrheal purge ≥ 3 liters through 10 days post-challenge. This approach to demonstrating vaccine efficacy is considered acceptable for the intended target population, which consists of adult U.S. travelers to cholera affected areas.

Vaccine effectiveness in adults 46 through 64 years of age was established based on demonstration of immunologic non-inferiority to a historical comparator group consisting of Vaxchora recipients 18-45 years of age. This immunologic parameter selected for bridging effectiveness to adults > 45 years of age was seroconversion rate against the vaccine strain, defined as a ≥ 4 -fold rise in serum vibriocidal antibodies from baseline to 10 days post-vaccination.

Manufacturing consistency was established by demonstrating immunologic equivalence of three different production lots of Vaxchora.

The safety of Vaxchora was evaluated in adults 18 through 64 years of age across the 4 clinical studies submitted to this BLA. A total of 3235 Vaxchora recipients and 562 placebo recipients contributed to the safety database. The safety data reviewed raised no safety concerns that would preclude licensure.

The Applicant has committed to conduct a post-licensure U.S. based pregnancy registry for 5 years to prospectively collect safety data on spontaneously reported exposures to Vaxchora occurring within 28 days prior to the last menstrual period or at any time during

pregnancy. The deferred pediatric study in 2 through 17 year old children and adolescents is a postmarketing requirement under PREA.

The data submitted by the Applicant in this BLA support the approval of Vaxchora for the active immunization against disease caused by *V. cholerae* serogroup O1 in adults 18 through 64 years of age traveling to cholera-affected areas. The safety and effectiveness of Vaxchora have not been established in persons living in cholera-affected areas and in persons with pre-existing immunity to cholera (due to previous exposure to *V. cholerae* or receipt of a cholera vaccine) traveling to cholera affected areas.

11. RISK-BENEFIT CONSIDERATIONS AND RECOMMENDATIONS

11.1 Risk-Benefit Considerations

Table 50 summarizes risk-benefit consideration with Vaxchora in adults 18 through 64 years of age.

Table 50: Risk Benefit Considerations of Vaccination with Vaxchora in Adults 18 through 64 Years of Age

Decision Factor	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	<ul style="list-style-type: none"> Severe cholera is characterized by acute diarrhea which leads rapidly (within 4 to 18 hours) to moderate or profound dehydration. Complications from cholera arise from the loss of fluid volume and electrolytes, resulting in hypovolemia, metabolic acidosis, and potassium deficiency. Secondary complications may include renal failure. If left untreated, cholera may lead to severe dehydration, hypovolemic shock and death within hours. Case fatality rates observed globally range from < 1% to 13.6%. There is an estimated burden of 1.4 to 4.3 million cases of cholera and 28,000 to 142,000 deaths per year worldwide due to cholera. In 2013, 47 countries reported 129,064 cases of cholera. Although > 200 <i>V. cholerae</i> serogroups can cause a cholera-like illness, only toxigenic strains of serogroups O1 and O139 that produce cholera-toxin have caused widespread epidemics and are reportable to the WHO as cholera. <i>V. cholerae</i> O1 is the predominant cause of cholera globally, and most <i>V. Cholerae</i> O1 cases are caused by O1 El Tor organisms. 	<ul style="list-style-type: none"> Cholera is a rapidly progressive, serious, life-threatening illness. The risk of morbidity and mortality is high if cholera is not treated in a timely manner. Potential exposure to <i>V. cholerae</i> represents a risk to U.S. travelers visiting cholera-affected areas.
Unmet Medical Need	<ul style="list-style-type: none"> There is currently no cholera vaccine licensed for use in the United States. Primary prevention currently consists of five recommendations made by the CDC. These include: 1) drinking and using safe water, 2) washing hands often with soap and water, 3) using latrines or burying feces, 4) cooking food well (especially seafood), keeping it covered, eating it hot, and peeling fruits and vegetables, and 5) bathing and washing diapers and clothes 30 meters (98 feet) away from drinking water sources. Although rates of cholera are low among travelers from the United States, including U.S. health care workers caring for cholera patients in endemic or outbreak settings, travelers at highest risk are those who are visiting remote areas where access to safe food and water and access to medical care is likely to be limited. Risk is also higher among persons with underlying medical conditions that predispose them to increased morbidity due to mild or worse diarrhea. 	<ul style="list-style-type: none"> In U.S. travelers, particularly those at increased risk of exposure or at increased risk of morbidity if infected with <i>V. cholerae</i>, there is an unmet medical need for effective prevention of cholera.
Clinical Benefit	<ul style="list-style-type: none"> Two clinical trials evaluating the effectiveness of a single dose of Vaxchora were submitted. In one study (PXVX-VC-200-003) conducted in adults 18 through 45 years of age, vaccine efficacy was demonstrated for the prevention of moderate to severe diarrhea following heterologous challenge with 1×10^5 CFU of live wild type <i>V. cholerae</i> El Tor Inaba at 10 days and 3 months post-vaccination. Efficacy was 90.3% (95.1% CI 2.7%, 100.0) at 10 days post-vaccination and 79.5% (95.1% CI 49.9%, 100.0%) at 3 months post-vaccination. These data supported the efficacy of Vaxchora in the prevention of cholera at 10 days and 3 months post-vaccination. In a second study (PXVX-VC-200-005) conducted in adults 46 through 64 years of age, immunogenicity was evaluated. The primary endpoint was seroconversion, defined as ≥ 4-fold rise in serum vibriocidal antibody titer against classical Inaba <i>V. cholerae</i>, at 10 days post-vaccination). This study demonstrated that seroconversion rates in 46 through 64 year old adults were noninferior to corresponding seroconversion rates in a historical comparator consisting of 18 through 45 year old adults. The specific immune mechanism responsible for conferring protection against cholera following receipt of Vaxchora has not been determined. However, data from the challenge study (PXVX-VC-200-003) showed that vibriocidal antibody seroconversion (defined as a 4-fold rise in vibriocidal antibody titer against classical Inaba <i>V. cholerae</i> at 10 days post-vaccination) is associated with protection against moderate to severe diarrhea. This was based on analyses of odds ratios calculated using multivariate logistic regression. 	<ul style="list-style-type: none"> Clinical benefit of a single dose of Vaxchora was demonstrated for the prevention of disease caused by <i>V. cholerae</i> serogroup O1 El Tor Inaba in adults 18 through 45 years of age who have not previously been exposed to cholera. Effectiveness of a single dose of Vaxchora in adults 46 through 64 years of age was established by immunogenicity bridging to the population in whom efficacy was demonstrated (18 to 45 year old adults). Effectiveness of a single dose of Vaxchora against each of the three major subtypes of <i>V. cholerae</i> O1 not contained in Vaxchora (classical Inaba, classical Ogawa and El Tor Ogawa) was supported by immunogenicity data. The effectiveness of Vaxchora in persons who live in cholera-affected areas has not been established. The effectiveness of Vaxchora in persons who have

	<ul style="list-style-type: none"> Serum vibriocidal antibody against the three types of <i>V. cholerae</i> not contained in the vaccine, namely classical Ogawa, El Tor Inaba and El Tor Ogawa, was also measured in studies -003 and -005. The percentages of vaccine recipients who seroconverted against each of the 4 major biotype/serotypes of <i>V. cholerae</i> serogroup O1 at 10 days post-vaccination ranged from 71.4% to 91.0%. Duration of protection beyond 3 months post-vaccination and the safety and effectiveness of revaccination was not evaluated. Vibriocidal antibody levels wane significantly at 3 months post-vaccination, especially in the older age group. 	<p>pre-existing immunity due to cholera infection or receipt of a cholera vaccine has not been established.</p> <ul style="list-style-type: none"> Duration of protection beyond 3 months is unknown.
Risk	<ul style="list-style-type: none"> The safety of Vaxchora was evaluated in adults 18 through 64 years of age across 4 randomized well controlled studies. A total of 3235 Vaxchora recipients and 562 placebo recipients contributed to the safety database. The most frequent adverse reactions following vaccination with Vaxchora were tiredness, headache, abdominal pain, nausea/vomiting, lack of appetite, and diarrhea. However, most reactions were mild or moderate in severity, and resolved relatively quickly and without sequelae. No other safety signals were apparent in the studied populations. No hypersensitivity reactions were noted during the Vaxchora pre-licensure clinical studies, however hypersensitivity reactions are theoretically possible. Shedding of the vaccine strain was detected in stool samples of vaccine recipients. The proportion of subjects shedding was highest on day 7 post-vaccination. The duration of shedding is unknown, because 7 days post-vaccination was the latest time point evaluated. The vaccine strain was not detected in stool samples or rectal swabs collected on day 7 post-vaccination in a study of 24 household contacts of vaccinees. No household contact seroconverted by Day 28. Vaxchora was not studied in pregnant women. For seven of the eight pregnancies that occurred during the clinical studies submitted to the BLA, conception occurred after vaccination. Although the vaccine would not be anticipated to cause adverse outcomes in pregnancy because it is not systemically absorbed, the submitted data are not sufficient to establish the presence or absence of risk with regard to use during pregnancy. 	<ul style="list-style-type: none"> The safety profile of Vaxchora is acceptable.
Risk Management	<ul style="list-style-type: none"> See "Clinical Benefit" and "Risk" sections above. 	<ul style="list-style-type: none"> If Vaxchora is approved for adults aged 18 through 64 years, routine measures, such as the package insert and the current pharmacovigilance plan, would be adequate to manage the risks. Use in pregnancy has not been studied. The Applicant has committed to conduct a post-licensure U.S. based pregnancy registry for 5 years to monitor safety in women exposed to VAXCHORA during pregnancy.

11.2 Risk-Benefit Summary and Assessment

Data submitted to this BLA establish a likelihood of benefit of vaccination with Vaxchora with respect to the prevention of moderate to severe diarrhea caused by *V. cholerae* serogroup O1 in adults 18 through 64 years of age traveling to cholera affected areas. The risks of vaccination with Vaxchora in this population have been found to be minimal. Therefore, the overall risk-benefit profile of this product for 18 through 64 year old adults who are traveling to cholera-affected areas is determined to be favorable.

The greatest benefit is expected among a subpopulation of adult U.S. travelers who are at increased risk of exposure or at increased risk of morbidity if infected with serogroup O1 *V. cholerae*. The benefit is unknown in persons living in cholera-affected areas and in persons with pre-existing immunity (due to previous exposure to *V. cholerae* or receipt of a cholera vaccine) traveling to cholera-affected areas.

11.3 Discussion of Regulatory Options

Please see below section 11.4.

11.4 Recommendations on Regulatory Actions

Vaxchora is recommended for approval as a single dose for active immunization against disease caused by *V. cholerae* serogroup O1 in adults 18 through 64 years of age traveling to cholera-affected areas.

11.5 Labeling Review and Recommendations

The prescribing information was reviewed and specific comments on the labeling were provided by CBER to the Applicant who made the requested revisions. All issues were satisfactorily resolved.

The package insert will address areas in which information gaps exist regarding the use of Vaxchora for its approved indication. Specific information gaps are listed below according to the corresponding section within the package insert.

1. Limitations of Use subsection of Indications and Usage
 - a. The effectiveness of Vaxchora has not been established in persons living in cholera affected areas.
 - b. The effectiveness of Vaxchora has not been established in persons who have pre-existing immunity due to previous exposure to *V. cholerae* or receipt of a cholera vaccine.
 - c. Vaxchora has not been shown to protect against disease caused by *V. cholerae* serogroup O139 or other non-O1 serogroups.
2. Dosage and Administration
 - a. The safety and effectiveness of revaccination have not been established with Vaxchora.
3. Warnings and Precautions
 - a. The safety and effectiveness of Vaxchora have not been established in immunocompromised persons.
4. Drug Interactions
 - a. There are no data available on concomitant administration of Vaxchora with other vaccines.

The CMC review issues below were addressed in the package insert. Please refer to section 4.1 of this review for further details.

1. Based on the data submitted to the BLA and a review of FDA regulations pertaining to bottled water, CBER requested that the package insert only specify use of purified bottled water for reconstitution.
2. CBER recommended that the package insert instruct reconstitution within 15 minutes after removal of the two component packets from the freezer and ingestion of the vaccine within 15 minutes after reconstitution, because this reflects the corresponding timeframes used in the clinical efficacy study (PXVX-VC-200-003).
3. Because of the complexities in storage, handling, preparing and administering Vaxchora, CBER recommended that the package insert specify that the preparation, reconstitution and administration of Vaxchora occur in a healthcare setting equipped to dispose of medical waste.

Section 8 of the package insert was revised according to the content and formatting requirements for pregnancy and lactation labeling (referred to as the “Pregnancy Lactation and Labeling Rule” or “final rule”) described in 21 CFR 201.57(c)(9)(i) through (iii). The key considerations pertaining to labeling of the pregnancy and lactation subsections are summarized here. The review committee determined that Vaxchora is not systemically absorbed following oral administration based on available scientific evidence available for wild type cholera (see Section 2.1).

Based on data from the available published literature,⁵⁰⁻⁵⁶ the review committee inserted language pertaining to the risk of adverse pregnancy outcomes, including fetal death associated with maternal cholera disease. Since Vaxchora is shed in the stool post-vaccination, the risk of transmission of vaccine strain to the infant during delivery was communicated under “Clinical Considerations.”

Data on pregnancy outcomes from women participants who became pregnant were reviewed. Since only 8 women enrolled in clinical trials with Vaxchora became pregnant 9 to 12 months after vaccination with Vaxchora, the human data did not establish the presence or absence of a drug-associated risk. Because the committee determined that Vaxchora is not systemically absorbed and not expected to result in fetal exposure to the drug, language pertaining to the clinical data was not required to be included in the package insert according to the final rule.

11.6 Recommendations on Postmarketing Actions

Post-marketing Requirements

- In accordance with PREA requirements, Study PXVX-VC-200-006 will be conducted to assess the safety and effectiveness of Vaxchora in children 2 through 17 years of age as a required postmarketing study.

Post-marketing Commitments

- The Applicant commits to conducting a post-licensure U.S. based pregnancy registry for 5 years to prospectively collect safety data on spontaneously reported exposures to Vaxchora occurring within 28 days prior to the last menstrual period or at any time during pregnancy.

APPENDIX 1

Data From Study PXVX-VC-200-003

Analyses of Secondary Endpoints in Study PXVX-VC-200-003 (Extension of Data Summarized in Section 6.1.11.2)

Volume of Grade 3 or Higher Stools

A smaller number (and proportion) of vaccine recipients in the 10-day and 3-month challenge groups experienced grade 3 or higher stools on days 1 through 10 post-challenge compared to placebo recipients in the combined 10-day and 3-month challenge groups (Table 51). The peak number of subjects generally occurred on day 3 or 4, and grade 3 stools subsided within 10 days post-challenge in all study groups challenged.

In general, a smaller number (and proportion) of vaccine recipients in the 10 day challenge experienced grade 3 or higher stools compared to vaccine recipients in the 3 month challenge, suggesting some waning protection by 3 months post-vaccination. However, the volume of grade 3+ stools was sometimes higher among vaccine recipients challenged at 10 days compared to those challenged at 3 months post-vaccination. Among subjects who had grade 3 or higher stools, the geometric mean of the overall volume of grade 3 or higher stools was 624.2 mL (95% CI 147.2, 2645.9) for the 10 day challenge vaccine recipients and 487.9 mL (95% CI 237.3, 1003.5) for the 3-month challenge vaccine recipients; both geometric mean volumes were less than the geometric mean volume among combined 10-day and 3-month challenge group placebo recipients who had grade 3 or higher stools (3495.1 (95% CI 2651.3, 4607.4). In general, the daily mean volume of grade 3+ stools had large confidence intervals, precluding meaningful interpretation. Therefore, table 54 below does not include mean or median summaries of volume of grade 3+ stools.

Table 51. Post-Challenge Grade 3 or Higher Stools^a – Intent-to-Treat Population

	PXVX0200 10 Day Challenge N=35 n^b (%)	PXVX0200 3 Month Challenge N=33 n (%)	Combined Placebo Challenges N=66 n (%)
1 Day Post-Challenge			
Number of Subjects with Grade 3+ Stools	2 (5.7)	1 (3.0)	3 (4.5)
Number of Grade 3+ Stools, Min, Max	1, 2	1, 1	1, 3
Volume (mL) of Grade 3+ stools, Min, Max	97, 436	104, 104	34, 321
2 Days Post-Challenge			
Number of Subjects with Grade 3+ Stools	4 (11.4)	9 (27.3)	51 (77.3)
Number of Grade 3+ Stools, Min, Max	2, 6	1, 17	1, 29
Volume (mL) of Grade 3+ stools, Min, Max	104, 1562	22, 5303	67, 6445
3 Days Post-Challenge			
Number of Subjects with Grade 3+ Stools	4 (11.4)	7 (21.2)	60 (90.9)
Number of Grade 3+ Stools, Min, Max	1, 24	1, 16	1, 29
Volume (mL) of Grade 3+ stools, Min, Max	50, 12047	75, 3424	73, 13231

	PXVX0200 10 Day Challenge N=35 n^b (%)	PXVX0200 3 Month Challenge N=33 n (%)	Combined Placebo Challenges N=66 n (%)
4 Days Post-Challenge			
Number of Subjects with Grade 3+ Stools	2 (5.7)	14 (42.4)	58 (87.9)
Number of Grade 3+ Stools, Min, Max	1, 19	1, 15	1, 26
Volume (mL) of Grade 3+ stools, Min, Max	148, 4902	83, 3592	31, 7308
5 Days Post-Challenge			
Number of Subjects with Grade 3+ Stools	2 (5.7)	13 (39.4)	52 (78.8)
Number of Grade 3+ Stools, Min, Max	1, 12	1, 16	1, 14
Volume (mL) of Grade 3+ stools, Min, Max	85, 1216	36, 2281	22, 3387
6 Days Post-Challenge			
Number of Subjects with Grade 3+ Stools	2 (5.7)	7 (21.2)	37 (56.1)
Number of Grade 3+ Stools, Min, Max	2, 2	1, 6	1, 13
Volume (mL) of Grade 3+ stools, Min, Max	176, 350	34, 1188	47, 1599
7 Days Post-Challenge			
Number of Subjects with Grade 3+ Stools	2 (5.7)	6 (18.2)	24 (36.4)
Number of Grade 3+ Stools, Min, Max	1, 2	1, 2	1, 14
Volume (mL) of Grade 3+ stools, Min, Max	168, 390	51, 335	39, 1036
8 Days Post-Challenge			
Number of Subjects with Grade 3+ Stools	1 (2.9)	3 (9.1)	16 (24.2)
Number of Grade 3+ Stools, Min, Max	1, 1	1, 2	1, 8
Volume (mL) of Grade 3+ stools, Min, Max	383, 383	168, 266	20, 835
9 Days Post-Challenge			
Number of Subjects with Grade 3+ Stools	1 (2.9)	1 (3.0)	5 (7.6)
Number of Grade 3+ Stools, Min, Max	1, 1	1, 1	1, 6
Volume (mL) of Grade 3+ stools, Min, Max	124, 124	223, 223	35, 1036
10 Days Post-Challenge			
Number of Subjects with Grade 3+ Stools	0 (0.0)	0 (0.0)	1 (1.5)
Number of Grade 3+ Stools, Min, Max	0, 0	0, 0	2, 2
Volume (mL) of Grade 3+ stools, Min, Max	0, 0	0, 0	363, 363
Overall (i.e., within 10 days post-challenge)			
Number of Subjects with Grade 3+ Stools	8 (22.9)	23 (69.7)	63 (95.5)
Number of Grade 3+ Stools, Min, Max	1, 55	1, 49	2, 79
p-value for Number of Grade 3+ stools ^c	0.0042	< 0.0001	
Volume (mL) of Grade 3+ stools, Min, Max	154, 18164	22, 9950	140, 24374
p-value for Volume of Grade 3+ stools ^c	0.0073	<0.0001	

^a Stool did not need to meet the definition of mild diarrhea to be counted in this table. Min and max summary statistics were calculated from all subjects with any grade 3 (liquid) or higher stools on the indicated day(s).

^b n=number of subjects with grade 3+ stools.

^c p-value calculated using Wilcoxon rank sum test.

Source: STN 125597_17. Module 1.11.3 (Efficacy Information Amendment) Table 16 and Module 5.3.5.1. PXVX-VC-200-003 Clinical Study Report, Table 17.

Number of Days with Grade 3 or Higher Stools (Exploratory Variable)

The number of days that subjects had grade 3 or higher stools is described for the 10-Day and 3-Month Challenge groups (Table 52). In this table, the statistics were calculated from all subjects in the group including those who had no grade 3 or higher stools. The median number of days with grade 3 or higher stools was 0.0 (min 0.0, max 8.0) in vaccine recipients in the 10-Day Challenge group and 1.0 (min, 0.0, max 6.0) in vaccine recipients in the 3-Month Challenge group; both medians were less than the median of 5.0 days (min 0.0, max 9.0) for the combined placebo group ($p < 0.0001$ for 10-Day and 3-Month Challenges; Wilcoxon rank sum test). The data in Table 55 show that a higher proportion of vaccine recipients in the 3 month challenge experienced grade 3 or higher stools for more days compared to vaccine recipients in the 10 day challenge, suggesting some waning protection by 3 months post-vaccination.

Table 52. Number of Post-Challenge Days with Grade 3 or Higher Stools – Intent-to-Treat Population

Total Number of Days with Grade 3+ Stools	PXVX0200 10 Day Challenge N=35 n^a (%)	PXVX0200 3 Month Challenge N=33 n (%)	Combined Placebo Challenges N=66 n (%)
0	27 (77.1)	10 (30.3)	3 (4.5)
1	3 (8.6)	8 (24.2)	1 (1.5)
2	3 (8.6)	4 (12.1)	5 (7.6)
3	1 (2.9)	4 (12.1)	9 (13.6)
4	0 (0.0)	3 (9.1)	11 (16.7)
5	0 (0.0)	3 (9.1)	12 (18.2)
6	0 (0.0)	1 (3.0)	15 (22.7)
7	0 (0.0)	0 (0.0)	6 (9.1)
8	1 (2.9)	0 (0.0)	3 (4.5)
9	0 (0.0)	0 (0.0)	1 (1.5)
10	0 (0.0)	0 (0.0)	0 (0.0)
Median (Min, Max)	0.0 (0.0, 8.0)	1.0 (0.0, 6.0)	5.0 (0.0, 9.0)
p-value ^b	<0.0001	< 0.0001	

^a n=number subjects who had grade 3 or higher stools for the corresponding number of days given in the first column.

^b p-value calculated using Wilcoxon rank sum test.

Source: STN 125597_0. Module 5.3.5.1 PXVX-VC-200-005 Clinical Study Report, Table 18.

Number of Days and Peak Concentration of Fecal Shedding

The number of days and peak concentration of fecal shedding through 10 days post-challenge for the 10-Day and 3-Month Challenge groups are described in Table 53. In this table, the statistics were calculated from all subjects in the group including those who had no fecal shedding. The median number of days with a positive stool culture was 0 for 10-Day Challenge vaccine recipients and 2 for 3-Month Challenge vaccine recipients; both medians were less than the median of 3 days with a positive stool culture for the combined placebo group ($p < 0.0001$ for 10-Day and 3-Month Challenges; Wilcoxon rank sum test). Median peak *V. cholerae* excretion was 0 CFU for 10-Day Challenge vaccine recipients and 135,500 CFU for 3-Month Challenge vaccine recipients; both medians were less than the median peak of 31,500,000 CFU for the combined placebo group ($p < 0.000$ for 10-Day and 3-Month Challenges; Wilcoxon rank sum test). A larger proportion of vaccine recipients in the 3 month challenge experienced

fecal shedding for a greater number of days compared to vaccine recipients in the 10 day challenge, suggesting waning protection by 3 months post-vaccination.

Table 53. Number of Days and Peak Concentration of Fecal Shedding – Intent-to-Treat Population

Fecal Shedding: Total Number of Days with Positive Stool Culture	PXVX0200 10 Day Challenge N=35 n (%)^a	PXVX0200 3 Month Challenge N=33 n (%)	Combined Placebo Challenges N=66 n (%)
0	18 (51.4)	8 (24.2)	2 (3.0)
1	8 (22.9)	2 (6.1)	0 (0.0)
2	5 (14.3)	8 (24.2)	9 (13.6)
3	2 (5.7)	10 (30.3)	24 (36.4)
4	2 (5.7)	5 (15.2)	26 (39.4)
5	0 (0.0)	0 (0.0)	5 (7.6)
6	0 (0.0)	0 (0.0)	0 (0.0)
7	0 (0.0)	0 (0.0)	0 (0.0)
8	0 (0.0)	0 (0.0)	0 (0.0)
9	0 (0.0)	0 (0.0)	0 (0.0)
10	0 (0.0)	0 (0.0)	0 (0.0)
Median (Min, Max)	0.0 (0.0, 4.0)	2.0 (0.0, 4.0)	3.0 (0.0, 5.0)
p-value for Median # days ^b	<0.0001	< 0.0001	
Median peak <i>V. cholerae</i> O1 concentration (CFU/G) ^c	0	135500	31500000
p-value for Median peak concentration ^b	<0.0001	< 0.0001	
Geometric mean for peak <i>V. cholerae</i> O1 concentration	23 (95% CI 3, 133)	23385 (95% CI 1659, 329534)	10336355 (95% CI 3450948, 30959667)

^a Number and proportion of subjects who had positive qualitative stool cultures for the corresponding number of days in the first column.

^b p-value calculated using Wilcoxon rank sum test.

^c Peak excretion concentration (i.e., the maximum quantitative result over the 10-days post-challenge) was calculated for each subject. The median peak concentration is the median of those individual peak concentrations.

Source: STN 125597_17. Module 1.11.3 (Efficacy Information Amendment) Table 19.

Table 54. Post-Challenge Vibriocidal Antibody Response Against Homologous Classical Inaba *V. cholera* and against Three Heterologous *V. cholera* Serotypes and Biotypes (El Tor Inaba, Classical Ogawa, and El Tor Ogawa). Immunogenicity Evaluable Population.

Cholera Strain	Vibriocidal Antibody GMT (95% CI)			Cumulative % with 4-fold rise ^a in Vibriocidal Antibody		
	10-Day Challenge Group PXVX0200	3-Month Challenge Group PXVX0200	Combined Placebo	10-Day Challenge PXVX0200 n(%)	3-Month Challenge PXVX0200 n (%)	Combined Placebo Challenges n (%)
Classical Inaba	N=35	N=33	N=63-64 ^b	N=35	N=33	N=66
28 Days post-challenge	2461 (1523, 3975) ^c	1647 (1112, 2439) ^c	17409 (13828, 21918) ^c	2 (5.7) ^c	20 (60.6) ^c	64 (97.0)
Day 181	295.6 (183, 477) ^c	224 (148, 338) ^c	2032 (1465, 2819) ^c	2 (5.7) ^c	20 (60.6) ^c	66 (100.0)
El Tor Inaba						
Day 181	414 (243, 705) ^c	541 (321, 911) ^c	3190 (2314, 4398) ^c	5.7 ^c	18.2 ^c	89.4
Classical Ogawa						
Day 181	346 (187, 643) ^c	259 (149, 452) ^c	1429 (996, 2049) ^c	5.7 ^c	18.2 ^c	78.8
El Tor Ogawa						
Day 181	247 (132, 463) ^c	288 (172, 484) ^c	1294 (896, 1869) ^c	8.6 ^c	18.2 ^c	78.8

^a Statistics describe the percentage of subjects who had at least a 4-fold rise in the vibriocidal antibody titer by the given time point over the last titer measured prior to challenge (Day 1, Day 8 or Day 11).

^b N=63 for Classical Inaba data. N=63 for El Tor Inaba, Classical Ogawa and El Tor Ogawa data.

^c p value < 0.0001 compared to placebo.

Source: STN 125597, Module 5.3.5.1. PXVX-VC-200-003 Clinical Study Report Tables 24-27 and Tables 14.4.17.1 – 14.4.22.2.

Table 55. Post-Challenge Anti-Cholera Toxin (CT) Antibody Responses by Challenge Group (Immunogenicity Population).

Anti-CT GMT (95% CI) (Day 181)			Cumulative % with 4-fold rise ^a in Anti-CT Antibody (Day 181)		
PXVX0200 10 Day Challenge	PXVX0200 3 Month Challenge	Combined Placebo	10-Day Challenge PXVX0200 n(%)	3-Month Challenge PXVX0200 n (%)	Combined Placebo Challenges n (%)
N=35	N=33	N=63	N=35	N=33	N=66
1237 (874, 1750) ^b	6073 (3870, 9530) ^c	21001 (13590, 32452)	10 (28.6) ^b	19 (57.6) ^d	56 (84.8)

^a Percentage of subjects who exhibited ≥ 4 -fold increase in anti-CT titer at Day 181 over last value obtained prior to challenge.

^b p-value < 0.0001

^c p value = 0.0004

^d p-value=0.0054

Source: STN 125597, Module 5.3.5.1. PXVX-VC-200-003 Clinical Study Report Tables 14.4.23.1 – 14.4.24.2.

APPENDIX 2

Additional Data From Study PXVX-VC-200-004

Evaluation of Antibody Response Profile for Up to 6 Months Post-Vaccination

GMTs for serum vibriocidal antibody against homologous classical Inaba on Days 1, 11, 29, 91, and 181 in Immune Sub-study participants in all lots are shown in Table 56. Serum vibriocidal GMT in vaccine recipients peaked on Day 11 and declined at subsequent time points. Seroconversion in vaccine recipients was 91% (95% CI 71, 99) by Day 11 and peaked at 96% (95% CI 80, 100) by Day 29.

Analogous results for anti-Cholera Toxin GMT and seroconversion are shown in Table 57. Anti-CT GMT in vaccine recipients was 1780 on Day 11 and continued to increase to 2007 on Day 29. Anti-CT seroconversion in vaccine recipients was 42% by Day 11 and peaked at 46% by Day 29.

Of note, fold-increase in serum vibriocidal titer after vaccination was 140 (9688/69) (Table 56) in vaccine recipients compared with little to no change from baseline in placebo recipients, while post-vaccination fold-increase in anti-CT was 4 (1780/445) (Table 57) in the vaccine group compared with no increase in the placebo arm.

Table 56. Vibriocidal GMT and Seroconversion by Study Day, Immune Sub-Study Population

Study Day Statistic	PXVX0200 All Lots N=26	Placebo N=6	p-value
Day 1			
N (Analyzable) ^b	26	6	
GMT (95% CI)	91 (52, 162)	143 (12, 2728)	0.5481 ^a
Day 11			
N (Analyzable) ^b	22	4	
GMT (95% CI)	9922 (4306, 22865)	320 (6, 16216)	0.0037 ^a
Seroconversion n [% (95% CI)]	20 91% (71, 99)	0 0% (0, 60)	< 0.0001 ^c
Day 29			
N (Analyzable) ^b	26	6	
GMT (95% CI)	6170 (3798, 10025)	127 (10, 1623)	< 0.0001 ^a
Seroconversion n [% (95% CI)]	25 96% (80, 100)	0 0% (0, 46)	< 0.0001 ^c
Day 91			
N (Analyzable) ^b	26	6	
GMT (95% CI)	560 (322, 975)	127 (10, 1623)	0.0491 ^a
Seroconversion n [% (95% CI)]	18 69% (48, 86)	0 0% (0, 46)	0.0033 ^c

Day 181			
N (Analyzable) ^b	26	5	
GMT (95% CI)	386 (211, 703)	53 (8, 348)	0.0107 ^a
Seroconversion n [% (95% CI)]	13 50% (30, 70)	0 0% (0, 52)	0.0580 ^c

Note: Assay results assess vibriocidal activity against the classical Inaba biotype of *V. cholerae*.

Note: Values below the LLQ were assigned the value of the LLQ.

Note: A subject is considered to have seroconverted at a visit if they achieve a titer at that visit that is at least 4-fold higher than their Day 1 titer.

a The p-value is from a one-way analysis of variance (ANOVA) model with log titer as the outcome and treatment group as the sole predictor.

b N Analyzable is the number of subjects with an analyzable sample available at the indicated visit for GMT calculation and the number of subjects with analyzable samples available from both Day 1 and the indicated visit for seroconversion estimation.

c P-value is from a Fishers exact test of equality of seroconversion across groups.
Source: STN 125597_0. Module 5.3.5.1. PXVX-VC-200-004 Clinical Study Report
Tables 14.4.1.5 – 14.4.1.6.

Table 57. Anti-Cholera Toxin GMT and Seroconversion by Study Day, Immune Sub-Study Population

Study Day Statistic	PXVX0200 All Lots N=26	Placebo N=6	p-value ^a
Day 1			
N (Analyzable) ^b	26	6	
GMT (95% CI)	445 (405, 489)	951 (296, 3057)	0.0023 ^a
Day 11			
N (Analyzable) ^b	26	6	
GMT (95% CI)	1780 (901, 3517)	800 (319, 2008)	0.2726 ^a
Seroconversion n [% (95% CI)]	11 42% (23, 63)	0 0% (0, 46)	0.0711 ^c
Day 29			
N (Analyzable) ^b	26	6	
GMT (95% CI)	2007 (1075, 3748)	848 (316, 2275)	0.2033 ^a
Seroconversion n [% (95% CI)]	12 46% (27, 67)	0 0% (0, 46)	0.0613 ^c
Day 91			
N (Analyzable) ^b	26	6	
GMT (95% CI)	1276 (771, 2111)	599 (370, 972)	0.1588 ^a
Seroconversion n [% (95% CI)]	11 42% (23, 63)	0 0% (0, 46)	0.0711 ^c
Day 181			
N (Analyzable) ^b	26	6/5 ^d	

GMT (95% CI)	1116 (687, 1814)	528 (329, 846)	0.1833 ^a
Seroconversion n [% (95% CI)]	9 35% (17, 56)	0 0% (0, 52)	0.2862 ^c

Note: Values below the LLQ were assigned the value of the LLQ.

Note: A subject is considered to have seroconverted at a visit if they achieve a titer at that visit that is at least 4-fold higher than their Day 1 titer.

a The p-value is from a one-way analysis of variance (ANOVA) model with log titer as the outcome and treatment group as the sole predictor.

b N Analyzable is the number of subjects with an analyzable sample available at the indicated visit for GMT calculation and the number of subjects with analyzable samples available from both Day 1 and the indicated visit for seroconversion estimation.

c p-value is from a Fishers exact test of equality of seroconversion across groups.

d N=6 for GMT calculation. N=5 for seroconversion estimation.

Source: STN 125597_0. Module 5.3.5.1. PXVX-VC-200-004 Clinical Study Report
Tables 14.4.2.1 – 14.4.4.2.