30 January 2020
EMA/82271/2020
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Vaxchora

Common name: cholera vaccine, oral, live

Procedure No. EMEA/H/C/003876/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

ABFP	Aqueous Buffer Finished Product
AE	Adverse Event
AS	Active Substance
BFP	bulk finished product (vaccine)
BHC	Biohazard cabinet
BSE	Bovine Spongiform Encephalopathy
CFU	Colony-forming units
CI	Confidence Interval
CPP	Critical process parameters
CQA	Critical quality attributes
CRF	Case Report Form
CT	Cholera toxin
$CTAB_5$	Cholera Holotoxin
CTB	Non-toxic B subunit of cholera toxin
CTM	Clinical Trial Material
ctxA	Cholera toxin A subunit gene
ctxB	Cholera toxin B subunit gene
CVD 103-HgR	*V. cholerae* vaccine strain
DoE	Design of Experiments
EDC	Electronic data capture
Effer-Soda® 12	Sodium bicarbonate/ sodium carbonate
EU	European Union
FMEA	Failure Mode and Effects Analysis
FP	Finished Product
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
HCP	Host Cell Proteins
hlyA	Hemolysin A gene

Hy-Case® SF	Casein acid hydrolysate acids
LICH	International Committee on Harmonization
KPA	Key performance attributes
KPP	Key process parameters
mer	Mercury resistant operon
MSL	Master Seed Lot
NOR	Normal operating range
PAR	Proven acceptable range
PCR	Polymerase chain reaction
Ph.Eur.	European Pharmacopeia
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SSVI	Swiss Serum and Vaccine Institute
SVA	Serum Vibriocidal Antibody
TFF	Tangential flow filtration
TSE	Transmissible Spongiform Encephalopathy
USP	United States Pharmacopoeia
WHO	World Health Organisation
WSL	Working Seed Lot
ΔctxA	Cholera holotoxin negative gene
ΔhlyA	Hemolysin A negative gene

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Emergent Netherlands B.V. submitted on 11 January 2019 an application for marketing authorisation to the European Medicines Agency (EMA) for Vaxchora, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication 'Vaxchora is indicated for active immunisation against disease caused by Vibrio cholerae serogroup O1 in adults and children aged 6 years and older. This vaccine should be used in accordance with official recommendations.'

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0381/2018 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0381/2018 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

The applicant requested the active substance *V. cholerae* live attenuated strain CVD 103-HgR contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Scientific advice

The Applicant received Scientific Advice on the development of Vaxchora for the prevention of disease caused by *Vibrio cholerae* serogroup O1 by the CHMP on 21 February 2013 (EMA/CHMP/SAWP/768988/2012), 20 November 2014 (EMEA/CHMP/SAWP/699798/2014) and on 09 November 2017 (EMA/CHMP/SAWP/713319/2017). The Scientific Advices pertained to the following aspects:

- Genetic comparability of the Vaxchora to the Orochol/Mutacol Berna vaccine
- Use of Orochol/Mutacol Berna data as supportive information to provide additional assurance of the clinical profile of PXVX0200
- Manufacturing process and controls
- Technical transfer, scale-up and optimization of manufacturing processes and associated comparability exercises
- Environmental risk assessment
- Waiving non-clinical studies to support registration given the lack of appropriate preclinical model and the extensive clinical experience with Orochol/Mutacol Berna
- Design of the phase II challenge study
- Absence of field efficacy trials to support a travellers' vaccine indication
- Design of safety and immunogenicity studies in i) children, and ii) older adults/elderly and parameters, methodology to support immunobinding from the challenge study to these populations
- Design of a lot to lot consistency, safety and immunogenicity study
- Agreement on the use of placebo control in all studies
- SmPC claims that can be supported by literature data with Orochol/Mutacol Berna vaccine
- Size of the safety database

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Bjorg Bolstad Co-Rapporteur: Filip Josephson

The application was received by the EMA on	11 January 2019
The procedure started on	30 January 2019
The Rapporteur's first Assessment Report was circulated to all CHMP members on	23 April 2019
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	23 April 2019
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	02 May 2019
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	29 May 2019
The applicant submitted the responses to the CHMP consolidated List of Questions on	15 August 2019

The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	23 September 2019
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	03 October 2019
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	14 October 2019
The applicant submitted the responses to the CHMP List of Outstanding Issues on	17 October 2019
The name of the Applicant changed from PaxVax Ltd. to Emergent Netherlands B.V.	20 December 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	16 January 2019
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Vaxchora on	30 January 2020

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease

Cholera is an acute toxigenic diarrhoeal illness caused by toxigenic strains of Vibrio cholerae O1. Clinical infection with cholera is often mild but can be severe and life-threatening.

2.1.2. Epidemiology

Contaminated water supplies are the main source of infection, although raw shellfish, uncooked fruits and vegetables and other foods can harbour V. cholerae and therefore also present a risk of infection.

Although cholera can occur in Europe e.g. via contaminated shellfish, most cholera cases are imported from EU nationals travelling to cholera endemic areas. Popular cholera-endemic travel destinations for Europeans include countries in Asia (India, Pakistan, Vietnam, Malaysia, and the Philippines), Africa (Morocco, Tanzania, Kenya) and the Caribbean (Cuba, Haiti, the Dominican Republic). These include poverty affected regions with poor infrastructure and sanitation.

Cholera epidemics can also arise sporadically, for example due to flooding following natural disasters such as the recent cyclone Idai in Mozambique, Zimbabwe and Malawi. European travellers to these cholera-affected regions include aid workers and military personnel.

In general, European travellers should be informed about the availability of a cholera vaccine before travelling and should follow good personal hygiene practices and drink only bottled water to prevent or minimise the risk for Vibrio cholerae infection when residing in endemic areas.

Globally in 2017, 34 countries reported 227,391 cases and 5,654 deaths (source: WHO). However, due to limited diagnostic capability and other factors contributing to underreporting, these figures are considered to represent only a small fraction of the actual cholera cases and deaths. Correcting for this underreporting, WHO estimates that 1.3-4.0 million cases and 21,000 to 143,000 deaths occur each year around the world.

2.1.3. Aetiology and pathogenesis

The bacterium *Vibrio cholerae* is the etiological agent of cholera and humans are its only hosts. Vibrio cholerae can be divided into over 200 serogroups, but only serogroups O1 and O139 have been known to cause epidemic cholera. The vibrio predominantly associated with epidemic cholera is V. cholerae serogroup O1, which is divided into two biotypes, Classical and El Tor, based on genotypic and phenotypic differences. Both biotypes contain two major serotypes, Inaba and Ogawa. The Inaba serotype differs from Ogawa by the absence of a 2-O-methyl group in the non-reducing terminal sugar of the O-specific polysaccharide (OSP) component of the lipopolysaccharide (LPS). During cholera outbreaks the prevalent serotype may switch between Ogawa and Inaba. Worldwide, *V. cholerae* O1 El Tor is currently the predominant biotype. O1 vibrios contain an enterotoxin (cholera toxin) which is responsible for causing diarrhoea.

Cholera toxin (CT), secreted by *V. cholerae* O1 confers pathogenicity to the organisms. Injection of as little as 5 µg of purified cholera toxin can elicit severe cholera diarrhoea. The toxin is an 84 kDa polymeric protein consisting of two subunits. The A subunit (28 kDa) is responsible for the biological activity of the toxin (diarrhoea). It is linked by non-covalent interactions to five identical B subunits (11.5 kDa each). The B

subunit aids pathogenicity by binding the toxin to receptors on intestinal cell membranes. Since the A subunit is surrounded by B subunits, the predominant antitoxin immune response is elicited against the B subunit. The A subunit is the enzymatically active portion of the toxin while the B subunit is the immunologically dominant portion. After receptor binding by the B subunit, the A subunit is taken up by cells and leads to hypersecretion of chloride, bicarbonate and water from epithelial cells into the lumen of the small intestine. This results in the characteristic voluminous stool. *V. cholerae* is also shed in high concentrations in the stool of severely ill patients.

Infection with wild-type V. cholerae provides prolonged protective immunity against subsequent infection. Protection against cholera is serogroup specific; hence infection with V. cholerae O1 provides no cross-protection from cholera caused by V. cholerae O139, and vice versa. The immunity is mediated by local mucosal secretory IgA (sIgA) produced in the small intestine, which is the anatomical site of colonization. Specifically, sIgA targets both the lipopolysaccharide (LPS) coat of the bacterium and the cholera toxin (CT). Antibodies directed against LPS appear to confer more robust protection than those against CT. The anti-LPS response is mostly addressed against the O-specific polysaccharide (OSP) component of the LPS and may mediate protection against V. cholerae via multiple mechanisms including inhibition of motility.

The presence of naturally acquired serum vibriocidal antibodies (SVA) correlates with protection against subsequent cholera infection at both the individual and population level. Also, SVA responses are directed primarily against OSP. Recently, plasma antibody responses against OSP have been shown to correlate with protection against cholera in household contacts, strengthening the mechanistic link between the SVA correlate and the OSP-specific mucosal antibodies that directly mediate protection. In addition, naturally acquired peripheral memory B cell responses against OSP have also been shown to correlate with protection.

2.1.4. Clinical presentation, diagnosis and management

Clinical symptoms are usually sufficient to diagnose cholera and begin treatment. However, laboratory diagnosis of *V. cholerae* in stool specimens is required to confirm the presence of cholera, characterise the organism, and determine its antibiotic sensitivity pattern.

Laboratory diagnosis is important in the initial cases, for the purpose of official notification and for monitoring the progress of the disease or epidemic. Rapid diagnostic tests kits for detecting V cholerae O1 and O139 are available.

Cholera can be successfully treated with prompt and adequate replacement of lost fluid and electrolytes. Individuals with mild and moderate cholera can usually be treated with oral rehydration salts (ORS), pre-packaged mixtures of glucose and salts mixed with safe drinking water and orally administered.

Approximately 5% of infected persons will have severe disease (cholera gravis), characterized by profuse watery diarrhoea, vomiting and leg cramps. In these people, rapid loss of body fluids leads to dehydration and pre-renal azotaemia. These individuals typically require intravenous fluid replacement. Without treatment, death can occur within hours and mortality rates may exceed 70%. Prompt rehydration dramatically reduces cholera mortality; less than 1% of cholera patients may die with appropriate hydration. Antibiotics may shorten the course of the diarrhoea and/or diminish the severity of the illness (i.e. the total diarrhoeal stool volume), but do not substitute for rapid rehydration.

In Europe an inactivated cholera vaccine is available for travellers (Dukoral). This contains inactivated V. cholerae Classical and El Tor biotypes and the Inaba and Ogawa serotypes for each biotype. In addition, recombinant cholera toxin B subunit is also included from V. cholerae O1 Classical Biotype, serotype Inaba.

About the product

Vaxchora (CVD 103-HgR) consists of live attenuated V. cholerae O1 strain Classical biotype. It is administered orally and is therefore expected to induce a local mucosal immune response in the small intestine in a similar way to wild type *V. cholerae* infection. During its previous clinical development, CVD103-HgR was shown to induce SVA responses as well as intestinal anti-LPS antibodies.

Based on the above, the proposed mechanism of action of Vaxchora is to induce a broad protective mucosal immune response like that induced by natural *V. cholerae* infection. This response includes induction of antigen-specific mucosal antibodies and memory B cells, which can be reliably assessed by measurement of SVA.

2.2. Quality aspects

2.2.1. Introduction

The finished product (FP) is presented as an effervescent powder and powder for oral suspension containing not less than 4 x 108 colony forming units- CFU/ dose of *V. cholerae* live, attenuated strain CVD 103-HgR (serotype O1 Inaba) as active substance.

Other ingredients are: sucrose, ascorbic acid, hydrolysed casein and lactose. The effervescent buffer contains sodium bicarbonate, sodium carbonate, ascorbic acid and lactose.

The product is available in a carton box containing an active substance (AS) sachet (2 g powder) and an effervescent buffer sachet (4.5 g powder). The product is intended to be administered by reconstituting the buffer contents in 100 ml of cold/ room-temperature non-carbonated bottled drinking water in a cup (water and cup not supplied) followed by addition of the active component powder and stirring for 30 seconds, prior to oral administration within 15 minutes.

2.2.2. Active Substance

General information

Vaxchora (also referred to as PXVX0200 in the dossier) is a cholera vaccine (recombinant, live, oral), containing as active substance 4 x 108 to 2 x 109 viable cells of *V. cholerae* live, attenuated strain CVD 103-HgR (produced by recombinant technology). Attenuation is affected by the deletion of a substantial portion of the catalytic subunit A of the two copies of the cholera enterotoxin gene (*ctxA*). A mercury resistance marker is inserted within the hemolysin A (*hlyA*) locus to allow the vaccine strain to be distinguished from the wild-type V. cholerae O1 Inaba. Vaxchora contains a new active substance, *V. cholerae* live, attenuated strain CVD 103-HgR.

Manufacture, characterisation and process controls

Manufacturing of the active substance takes place at Emergent BioSolutions Berna GmbH, Oberriedstrasse 68, CH-3174 Thörishaus, Switzerland. The GMP status of all sites involved with manufacture/ testing of the AS has been confirmed.

Description of manufacturing process and process controls

The active substance manufacturing process consists of the following process steps: reconstitution of the WSL, fermentation, concentration, lyophilisation, milling and curing of the active substance.

PXVX0200 active substance manufacturing process starts with the thawing of working seed lot vials, followed by fed-batch fermentation, then the main culture is harvested and concentrated. The suspension is transferred to a mixing tank containing a specified stabilising solution (containing ascorbic acid, sucrose and casein hydrolysate). After mixing, the cell suspension is lyophilised. The lyophilisate is milled and cured (stabilised) to become the final active substance. The curing step makes it easier to predict the future titre and define the quantity of active substance required for further processing. A proportion of the bacteria remains alive and this comprises the active substance. Curing is the final step in the active substance manufacturing process. Once cured, the active substance is stored in specified storage bottles for long term storage under approved conditions. The batch size is defined. No reprocessing is claimed.

The ranges of critical process parameters and the routine in-process controls along with acceptance criteria, including controls for microbial purity and endotoxin, are described for each step. The description of the active substance manufacturing process is generally considered acceptable. Exclusion of identity as an in-process control has been appropriately justified and in process samples collected, ensure deviations from AS identity can be investigated.

Control of materials

Information on raw materials used in the manufacture of the PXVX0200 master seed lot (MSL), working seed lot (WSL) and AS are included and provide adequate assurance of their quality and consistency. For most of the raw materials used in manufacture, testing is performed in accordance with pharmacopoeial monographs. The non-pharmacopoeial materials are tested according to in-house specifications, which are provided and considered appropriate.

The animal sourced materials used during production are limited to casamino acids and casein acid hydrolysate, both derived from bovine milk sourced from New Zealand. The fermentation culture medium contains casamino acids and the stabilisation solution contains casein acid hydrolysate (Hy-Case® SF). Related BSE/TSE statements are included and deemed to be adequate and also cover manufacture of the MSL.

A general description of the origin and source of the strains used historically to establish the final CVD 103-HgR is provided in the dossier. The construction of the final CVD 103-HgR from the *V. cholerae* classical Inaba O1 strain 569B, based on allelic exchange strategies, is described and relevant information is included regarding the donor organisms for the inserts used in the final construct as well as all plasmids used in the process. CVD 103-HgR is defined by two major genetic modifications:

- Deletion of the majority (94 %) of the sequence of the cholera toxin A subunit gene (*ctxA*) from both replicons corresponding to the catalytic domain, to remove the toxinogenicity of the vaccine strain. CVD 103-HgR retains the *ctxB* gene and the ability to synthesise the non-toxic B subunit of cholera toxin (CTB), which is immunogenic and able to generate neutralising antibodies preventing the toxic activity of cholera enterotoxin.
- Insertion of a mercury resistance operon in the hemolysin A gene (*hlyA*) locus which enables differentiation of the vaccine strain from wild type *V. cholerae*.

The information provided on the approach used for the construction of CVD 103-HgR strain is considered sufficient.

The confirmation of the identity of CVD 103-HgR is based on the analysis of several genotypic and phenotypic markers of the strain. Most of these tests are performed as part of the release specifications of the MSL, WSL and AS and include appropriate tests for purity, identity, potency and safety.

The presence of the intended genetic modifications included in CVD 103-HgR is evaluated using PCR and gel point assays. These methods are sufficiently presented, and the provided data are considered sufficiently robust to confirm the identity of the current CVD 103-HgR MSL and WSL.

The presented information regarding the manufacture of the MSL and WSL is considered sufficient.

Relevant specifications have been established for the commercial MSL and WSL. The vials are stored at ≤-60° C for long term storage and shipping. Stability data on MSL and WSLs are provided and a protocol for WSL qualification is included. Related BSE/TSE statements are included and deemed to be adequate.

Similarity of the PXVX0200 and Orochol/Mutacol vaccines (earlier vaccines manufactured using the same construct and not authorised in the EU- see manufacturing development section for AS and FP) was also investigated. Additional physicochemical characterisation exercises (based on historical data and relevant tests used for the release of MSL/WSL) were performed on several lots of the MSL and WSL.

Genome sequencing studies confirmed the expected *ctxA* cholera toxin sequence as well as the presence of the *mer* operon in the interrupted *hlyA* gene all tested strains. It can be concluded that no genetic modifications occurred during the transfer of the parental strain nor during subsequent manufacturing of the vaccine active substance. Genetic stability of the construct has therefore been established.

Control of critical steps and intermediates

Process parameters for the manufacturing process are provided together with their respective operating set points or ranges. The criticality of the parameters was determined based on a risk analysis, which is provided. During development, a design of experiments (DoE) approach was used to anticipate potential parameter alterations, which might occur during manufacture. This exercise was designed to gain a better process understanding and was used to establish the acceptable range of key processes. Failure mode and effects analysis (FMEA) was used to identify the critical parameters of the upstream steps and the parameter ranges established through development and characterisation studies were validated.

The definition of critical process parameters is not fully supported since a parameter is considered non-critical by the applicant if run at set points or within claimed proven acceptable ranges (PARs), e.g. lyophilisation parameters. It is accepted that they do not impact the critical quality attributes (CQA) if within their limits but they still have to be controlled to be shown to be within the range that does not impact the CQA and put under sufficient regulatory oversight. However, as there are no claims of differences in regulatory action for critical vs non-critical attributes and parameters, it is understood that changes to all attributes and parameters mentioned in section S.2.2 and S.2.4 will be handled in the same way. With the above clarification, the definition of critical process parameters can therefore be accepted.

Process validation

The commercial active substance manufacturing process for the PXVX0200 AS has been validated at the commercial manufacturing site. The production steps of the AS manufacturing process include: fermentation (pre-culture, sub-culture, main culture), stabilisation, lyophilisation and milling (uncured AS), and curing (cured AS). There are no viral clearance steps or aseptic processing steps in the PXVX0200 AS production

processes that require specific validation although media hold times validation studies are presented. The data and evaluation of the validation study show that all results met the pre-defined acceptance and release criteria.

Three separate commercial-scale lots of PXVX0200 AS were produced using the commercial manufacturing process. Critical quality attributes and key performance attributes (KPA) were identified within the criticality analysis. Parameters were categorised into non-critical, potentially critical and critical depending on their impact on the respective CQAs and KPAs, based on the information available from development studies and the current commercial production process of Vaxchora. Ranges for critical process parameters (CPPs) and key process parameters (KPPs) were tested within the characterisation studies and multiple analyses were performed for the CQAs and KPAs in order to obtain further information on the criticality of the respective parameters.

Based on the results from the characterisation studies, the parameters were re-assessed for criticality and when a PAR was successfully shown, a narrower normal operating range (NOR) was defined for the individual parameter. In the validation, all parameters were set within their NOR. The goal of the validation was to show in three consecutive runs that the Vaxchora fermentation and lyophilisation process is consistent when run with parameters within the defined ranges and capable of yielding results fulfilling the acceptance criteria for critical quality attributes and key performance attributes.

Considering the history of the product, including the development studies performed at the transfer to the Swiss site and further described below, it is acceptable to build the process validation on development data from the US process. The data from the Swiss site show that the transfer/ adaptation of the process at the Swiss site has been successful. As mentioned above, the claim that certain steps do not include any critical parameters/attributes is not supported but since the parameters and attributes described in the S.2.2 and S.2.4 have been included in the validation, even if not considered critical, no further information is needed.

Manufacturing process development

The CVD 103-HgR vaccine strain of *V. cholerae* was generated at the Center for Vaccine Development (CVD) at the University of Maryland, Baltimore, and was used to produce the (non-EU) marketed vaccine known as Orochol/Mutacol Berna. This progenitor strain was later expanded to form the expanded progenitor strain which was subsequently used to generate the PXVX0200 MSL.

The PaxVax active substance process was first developed and manufactured in the USA, including the phase 3 clinical batches and commercial batches, this is referred to as the "US licensed process". Preliminary feasibility and optimisation studies were performed to evaluate the medium concentrations, cell culture steps, harvest, and lyophilisation. The optimised process was transferred to a new site where pilot runs were conducted at the fermentation scale and the process was subsequently scaled up to the commercial scale. In parallel, several DoE studies were performed to establish the acceptable range of key process operating parameters for the fermentation, TFF and lyophilisation unit operations to support a robust and reproducible process. FMEA was used to identify the critical parameters of the upstream steps and the acceptable ranges of these parameters were verified by two rounds of DoE at small scale.

The active substance formulation and process were optimised and then transferred to and scaled up at the commercial site Emergent BioSolutions Berna GmbH, Switzerland, this process is called the "global process". The optimisation was performed to achieve a finished product storage condition of 2°C to 8°C, the US licensed finished product is stored at -20°C. At the same time the manufacturing process was scaled-up. The technical transfer demonstrated that the global process is robust and has been successfully transferred to the recipient site. Active substance and finished product comparability studies were performed. Several

commercial scale active substance batches (including from the US-Licensed, which includes AS used for Phase 3 clinical trial material (CTM) manufacturing, and some for the global process) were evaluated for comparability. This comparability analysis includes verification of equipment qualification, performance of process validation, critical control parameter and critical quality attribute determination and impact analysis and, sampling and testing results analysis. The changes implemented in the AS manufacturing process were evaluated and found to be comparable and found not to impact product quality and safety. Changes to the stabiliser solutions used for Vaxchora compared to Orochol have also been justified.

Characterisation

For characterisation of Vaxchora, the applicant has investigated the CVD 103-HgR bacterial strain, deletion of a substantial portion of *ctxA* on both chromosomes and subsequent absence of cholera holotoxin expression, the mercury resistance marker inserted within the hemolysin A (*hlyA*) locus, antibiotic resistance, cell viability and expression of cholera toxin B subunit. The results from the characterisation studies confirm the expected genomic structure.

The agglutination assay is used to identify the CVD-103 HgR as *V. cholerae* O1. Serotype identification is based on agglutination in antisera to type-specific O1 antigens. One of the main characteristics of the CVD-103 HgR is the substantial deletion of the A subunit (*ctxA*) of the cholera toxin. The deletion in *ctxA* on both chromosomes of the strain is crucial and assessed by PCR. Gene modifications of the strain have to be confirmed to guarantee safety of the strain. Another characteristic of the CVD 103-HgR is the insertion of the mercury resistance (*mer)* gene. The presence of the *mer* operon at the hemolysin gene (*hylA)* locus is verified by PCR. *Mer* insertion serves as an indication to distinguish the vaccine strain CVD 103-HgR from the wild-type 569B strain and does not have any impact on the protective action of the strain.

The potency of the CVD 103-HgR is determined by viable cell count.

Wild type *V. cholerae* strain produces an enterotoxin made up of A and B subunits. CVD-103 HgR produces only the B toxin subunit (CTB). CTB is expressed during fermentation and is present at low levels in the active substance. Though these CTB levels are too low to be considered to be immunologically significant, CTB is considered clinically relevant as neutralising antibodies to it are claimed by the applicant, to be a part of the mechanism of action. Upon request, a AS release test has been included.

The Y1 adrenal cell assay is used as a safety test to demonstrate the absence of the cholera holotoxin ($CTAB_5$). The Y-1 adrenal cells are very sensitive to the presence of enterotoxins from *V. cholerae*, which can be detected by a change of the morphology of the cells (rounding of cells).

The issue of the number of dead cells in the active substance had not originally been discussed in the characterisation part as such. It could not be assured that the vaccine was not working in a dual mode both through the live bacteria and the dead. In characterising the seed lots, a test was performed to determine the proportion of dead cells in FP and WSL. This is considered to have no impact on the active substance as long as the number of live cells in the WSL is sufficient. A flow cytometry assay used for finished product characterisation has also been used to bridge the Global Process to the clinical trial material. The amount appears also to be consistent between batches. The levels of dead cells are considered much too low to present a risk to safety and efficacy with the current manufacturing process/fraction of dead cells in DP. However, the company is asked to monitor dead cells following substantial AS manufacturing changes in case they were to rise significantly (recommendation).

No product-related impurities have been identified. No antibiotics are used in the manufacturing process. The components in the medium for the fermentation culture include specified, compendial raw, as well as non-

compendial raw materials. The medium components are substantially utilised by the bacterial culture during fermentation and converted to biomass. The leftover components are present in the active substance since there is no purification step in the process. However, these are further decreased when the majority of the spent medium is removed by microfiltration prior to addition of the stabilization solution. Further dilution occurs at the finished product manufacturing stage (see finished product manufacture section) and are considered to be at an acceptable level. The applicant identified cyclohexane and ethanol as possible residual solvents. Their limits comply with the ICH requirements. Stated impurities have been studied in nonclinical and clinical studies as relevant. Although the clearance of specified elemental impurities from fermentation has been well-documented, the applicant should perform a risk assessment for the presence of elemental impurities (as per Ph.Eur. monograph on pharmaceutical preparations (2619)) and control the levels of elemental impurities using the principles of risk management according to ICH Q3D (recommendation).

Specification

The proposed Vaxchora AS specification includes general tests (appearance, moisture), identity tests (PCR test for *ctxA* deletion and presence of *hlyA*/*mer*), test for biological activity (viable cell count) and tests for purity (microbiological examination and absence of specific organisms).

The specification tests are considered appropriate and their acceptance criteria are appropriate.

The applicant has provided justification for the proposed acceptance criteria. The AS potency criteria are appropriate and ensures the AS maintains acceptable potency throughout the shelf-life. For the moisture content, a specification limit has been proposed using US produced clinical batches. Using these batches is considered justified.

Analytical methods

Vaxchora AS is tested using non-compendial methods. Overall, appropriate descriptions are provided for all methods. All analytical procedures used for control of the Vaxchora AS have been validated and validation reports are included in the dossier. The inclusion of a second potency assay to harmonise AS and FP potency testing is acceptable. For the potency (viable cell count method), a sample of active substance is reconstituted in 100 mL cooled aqueous buffer finished product (ABFP). The ABFP is prepared by diluting a packet of buffer finished product in 100 mL chilled water for injection. The determination of the number of CFUs is carried out by serial dilution of the reconstituted sample and inoculated onto specified agar plates. After incubation of the plates, the CFU are counted. Appropriate positive and negative controls are in place.

Batch analysis

Batch information details for AS (including their use) and analytical results of the three validation batches are provided which were manufactured according to the commercial process and scale. The AS batches were manufactured according to the validated manufacturing process at the commercial site and were released according to the commercial specification using validated analytical methods.

Reference materials

There are no reference standards for Vaxchora. Different lots of Vaxchora reference material are used. The description of the use of reference standards is considered acceptable. The qualification of future lots described above is referenced to a master specification and annual re-qualification is conducted. For the standard used in the viable cell count potency assay, a suitable qualification protocol has been submitted.

Container closure

The active substance primary container closure system consists of specified square bottles with screw caps. The containers are then placed in stainless steel barrels for protection during storage. The container/closure system comply with European requirements and an identity test of the material is performed by the applicant. The assessment of risks arising from leachables and extractables as a result of the use of polymer materials that get in contact with the product during production, packaging, and storage has been provided.

Stability

A shelf-life and storage conditions were proposed for the AS.

Stability studies are ongoing at long-term and accelerated conditions, in line with ICH guidelines, for several batches of AS stored in a reduced volume container closure system (the type mentioned in section 3.2.S.6, the commercial bottles). Some of these batches are characterisation batches and three are process validation batches. All of these AS batches were manufactured at full commercial scale with the commercial process at the commercial site and were subsequently processed into FP.

Stability data for process validation lots are available. Stability data for the characterisation batches are used to set the active substance expiry, which is acceptable since they are representative of the same manufacturing process, testing and packaging as the process validation batches. The AS release and stability requirements are the same. All AS batches met stability acceptance criteria when stored at the long-term condition. For accelerated conditions, AS met the stability criteria for up to eight weeks. All of the stability studies are still ongoing.

The overall strategy and design of the stability studies are considered acceptable. Based on available data, the company's shelf-life proposal and storage conditions for the active substance is endorsed.

2.2.3. Finished Medicinal Product- Vaccine

Description of the product and pharmaceutical development

Vaxchora is supplied as a 4.5 g effervescent powder (buffer) and a 2 g powder (vaccine) for oral suspension in two separate sachets. Each dose of vaccine contains not less than 4 x 10^8 CFU/dose of recombinant, live attenuated *V. cholerae* vaccine strain CVD 103-HgR.

The composition of the vaccine sachet is given in **Table 1**.

Table 1 Composition of the vaccine sachet

Ingredient	Function	Reference to Standard	Quantity (per dose)
Vaccine			
Viable CVD 103-HgR	Active Ingredient	None	Not less than 4×10^8 CFU*
Sucrose	Cryoprotectant	Ph. Eur./NF	
Hydrolyzed casein (Hy-Case SF®)	Stabilizer (Cryoprotectant)	Manufacturer	
Ascorbic acid	Stabilizer (Antioxidant)	Ph. Eur./NF	
Lactose	Bulking Agent	Ph. Eur./NF	

*CFU = colony forming units

The vaccine finished product consists of a co-package of a vaccine and buffer sachet. The vaccine powder for reconstitution is contained in a single-dose, multilayer (four-ply multilayer foil containing an outer layer of paper, a layer of low-density polyethylene, a layer of aluminium foil and an inner layer of low-density polyethylene) sachet. The separate buffer powder is contained in a single-dose, multilayer (three-ply multilayer foil containing an outer layer of paper, middle layer of aluminium foil and an inner layer of low-density polyethylene) sachet. For the primary container closure system, the packet foil contains no animal derived components except for the lubricant component linear low-density polyethylene film (finished product contact). The lubricant is based on substances that are derived from animal fats (tallow). These substances are approved for food-contact use. The production of these additives is subject to very severe processing conditions that meet or exceed the recommendations for complete inactivation of TSE agents. The reconstitution procedure requires dissolution of the buffer in 100 mL of bottled water, followed by addition of the vaccine powder and mixing. The reconstituted vaccine is administered orally after reconstitution.

The composition of Vaxchora reconstituted FP is presented in **Table 2** below.

Table 2. Composition of PXVX0200 reconstituted FP

Ingredient	Function	Reference to Standard	Quantity (per dose)
Viable CVD 103-HgR	Active Ingredient	None	Not less than 4×10^8 CFU*
Sucrose	Cryoprotectant	Ph. Eur./NF	
Hydrolyzed casein (Hy-Case SF®)	Stabilizer (Cryoprotectant)	Manufacturer	
Ascorbic acid	Stabilizer (Antioxidant)	Ph. Eur./NF	
Lactose	Bulking Agent	Ph. Eur./NF	
Buffer	Buffer	None	100 mL

*CFU = colony forming units

Two excipients of animal origin are present in the finished product. Both the casein acid hydrolysate (Hy-Case SF) and lactose are derived from bovine milk. Specifications for all excipients used (except for Hy-Case SF) conform to the respective Ph. Eur. monograph. Casein is derived from milk fit for human consumption and complies with the relevant EU TSE guidance. The final product does not contain novel excipients. All excipients with the exception of the lactose are actually incorporated during the manufacture of the AS. Lactose is added during the blending step during the preparation of the bulk finished product.

The applicant has acceptably described the composition of the finished product. The pharmaceutical development has been thoroughly described. The composition of the finished product intended for the EU market (global process) is based on the US-licensed version produced by PaxVax which in turn is based on the composition of Orochol/Mutachol licensed outside of the EU in the late 90ies. No initial formulation development studies were conducted for this reason, for the US licensed product. The presentation and route of administration are the same as Orochol: a vaccine packet and a buffer packet which are reconstituted in water prior to oral administration. As part of the technical transfer and commercial scale-up for the global process, one of the key objectives was to produce a vaccine that was also stable at 2°C to 8ºC long term storage condition while the US product was stored at -20 ºC. The formulation components listed for the

global vaccine are the same as the US-licensed product with the exception of the removal of the sodium chloride component and the replacement of the dried lactose excipient with anhydrous lactose.

There is no formulation overage used for the PXVX0200 finished product. Buffer pH is another critical parameter affecting the biological activity of the PXVX0200 vaccine after reconstitution. *V. cholerae* is a very acid-labile organism and it has been shown (in literature) that administration of buffer to neutralise stomach acid reduces the dose of *V. cholerae* required to establish infection in volunteers. It has also been shown in literature, that persons with impaired gastric acid production may be predisposed to infection with *V. cholerae*. The buffer formulation was adopted from the buffer used in the studies of Orochol. In order to maintain viability of the live PXVX0200 vaccine bacteria, the buffer has a pH range of 6.9 ± 0.2. At higher pH, the viability of the bacteria will start to decline therefore the FP is reconstituted in the buffer solution to maintain stability at the time of preparation. The buffer also neutralises the stomach acidity of the recipient, facilitating the passage of viable bacteria through the stomach and into the intestinal tract, where they replicate and induce the desired immune response. Studies have been provided to justify the feasibility of the reconstitution procedure as described in the SmPC.

The effect of chlorine in bottled water was investigated on the vaccine potency. Chlorination is one of the most widely used methods to control microbial growth in water sources. Chlorine is very effective at inactivating *V. cholerae*, with the capacity to inactivate 100 % of the bacteria in less than 30 seconds at dilute concentration (0.5 ppm). The buffer that is used to reconstitute the vaccine is formulated with ascorbic acid (1.5 g to 1.8 g per dose), which effectively neutralises chlorine. The vaccine is stable for 30 minutes at chlorine concentrations greater than recommended by the WHO for drinking water.

Manufacturing changes

For the US-licensed process, the finished product manufacturing process starts with the curing step of the active substance. For the global process the curing step is considered part of the active substance and the finished product manufacturing step starts with the premix. There are some further changes, particular use of anhydrous lactose instead of dried lactose, different blending equipment and pre-mix with lactose. The anhydrous lactose used for the global Vaxchora process is far less hygroscopic than dried lactose. Thus, anhydrous lactose is better able to protect the product from excessive water uptake during open manufacturing steps. New larger blending equipment is employed for the global process. This change is considered to be a like for like replacement, given that both mixers function along the same principal. Both are diffusion mixers. It was believed that splitting the blending of AS with lactose in two steps at such a large scale, by the inclusion of a pre-mix step, would better ensure a homogeneous final bulk finished product (BFP).

The clinical studies have been performed with the US material. A number of commercial scale finished product lots (several for the US-Licensed, which includes Phase 3 CTM and commercial lots, and some for the global process) were evaluated for comparability. This comparability analysis included verification of equipment qualification, performance of process validation, critical control parameter and critical quality attribute determination and impact analysis, and sampling and testing results analysis. Overall the quality improvements implemented in the FP manufacturing process were evaluated in this comparability report and the material produced pre- and post-modification was found to be comparable. These modifications were deemed not to negatively impact product quality and safety. The impact of the changes continues to be monitored under the stability program to ensure maintenance of the expected improvement in quality. No further action is required beyond continuance of the ongoing stability studies.

Microbial attributes

PXVX0200 is not considered to be a sterile product. An orally-administered live bacterial vaccine such as PXVX0200 should not be required to meet a sterility standard, since it is not possible to have any inactivation steps in the process. Furthermore, the vaccine is not intended to be delivered to a severely immunosuppressed population and the human digestive tract is designed to inactivate most types of organisms which are routinely ingested with food. Similarly, the human digestive tract is designed to accommodate the presence of endotoxins. However, it is important that particular organisms that do represent a potential risk for infection via the alimentary tract are specifically excluded from the vaccine finished product, and that the bioburden level of the vaccine is controlled below specific limits. The absence of these specified organisms is tested for each batch.

Manufacture of the product and process controls

PXVX0200 vaccine finished product manufacturing, packaging, testing and batch control sites are specified in the dossier. Importation of the finished product and batch release into the EEA is the responsibility of IL-CSM GmBH, Marie-Curie-Str. 8, 79539 Loerrach, Germany. Suitable GMP documentation has been provided for all sites.

The manufacturing process for PXVX0200 finished product involves three primary unit operations: 1) premixing of the AS with a portion of lactose to form the premix, 2) followed by blending of the premix with the additional lactose to form the BFP, 3) and then filling the BFP into multi-layer foil packets. The batch size is specified.

Hold times for the premix and the BFP have been submitted, supporting the proposed storage times, respectively.

The description of the manufacturing process is acceptable and includes appropriate in-process controls, critical and non-critical process parameters. A similar risk assessment as performed for the AS has been performed for the FP as well. The criticality assessment has been properly described and the outcome is agreed. Process validation for the PXVX0200 FP manufacturing process was completed at the commercial manufacturing facility. A suitable number of batches of PXVX0200 AS were successfully processed through the premix, blending and filling manufacturing steps.

The production operations were performed per approved and effective procedures and batch records. Critical and non-critical control and monitoring parameters for the manufacturing process steps were confirmed to operate according to defined set points and within the defined parameter limits for the lots. As there are no claims of differences in regulatory action for critical vs non-critical attributes and parameters, it is understood that changes to all attributes and parameters mentioned in section S.3.3 and S.3.4 will be handled in the same way.

Test results for the lots produced confirmed that the material produced met defined specifications for product quality. Deviations generated during production of the validation lots were resolved successfully with no impact to the lot produced.

Product specification

The specification for PXVX0200 FP contains appropriate tests for identity, potency, purity and physicochemical attributes. The FP specification is based on characterisation, development, stability data and lots produced for clinical and commercial, as well as consideration of the applicable guidelines (e.g., ICH,

USP, Ph. Eur.). Most of the data used to develop the specifications were derived from the FP batches manufactured with the US-licensed process. Some specifications have been tightened upon request, during the procedure. The potency limit is clinically justified. There are no additional process-related impurities in the manufacturing and filling of the PXVX0200 FP. There are no product-related impurities introduced during finished product manufacture. Although the clearance of specified elemental impurities from fermentation has been well-documented, the applicant should perform a risk assessment for the presence of elemental impurities (as per Ph.Eur. monograph on pharmaceutical preparations (2619)) and control the levels of elemental impurities using the principles of risk management according to ICH Q3D (recommendation).

Analytical methods

All analytical procedures used for control of the PXVX0200 finished product (FP) have been validated for their intended purpose. The compendial methods for residual moisture and uniformity of mass was verified for suitability for use.

Batch analysis

Three FP lots were manufactured according to the validated commercial manufacturing process at commercial scale at PaxVax Berna and were released according to the current specification at the time using validated analytical methods. The acceptance criteria were fulfilled for all batches. There are no additional process-related impurities in the manufacturing and filling of the FP. All identified impurities from AS have been present in product studied in trials.

Container closure system

Finished product is filled into packets (60 mm x 90 mm) made from a four-ply multilayer foil. Each vaccine packet contains a single-dose (two grams) of vaccine finished product. The materials of construction for each layer of the four-ply multilayer foil packet and their corresponding functions, and manufacturer's specification have been listed. This foil was selected because it is designed to be impervious to light and to prevent the ingress of moisture. Vaccine packets are packed into secondary cartons. The documentation submitted is considered sufficient and the four-ply multilayer foil complies with European food contact regulations. The compatibility of vaccine drug product and its container closure has been evaluated and will continue to be assessed in drug product long-term stability studies.

Stability of the product

Based on the stability study a shelf life of 18 months at 2–8 °C is proposed.

Stability studies, in accordance with ICH guidelines, are ongoing for three lots of finished product (FP) manufactured for process characterisation which are representative of the final process and scale, and three lots of finished product manufactured for process validation. In accordance with EU GMP guidelines[1], any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

Stability studies for the characterisation and validation lots are being evaluated in a 36 months stability study and 18 months data have been reported. The results have met the proposed commercial specification and stability requirements in general. With this, the proposed shelf life of 18 months when stored under refrigerated conditions, can be accepted. A photostability study was not performed because the finished

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product is heat-sensitive and photostability conditions (i.e. heat from the light source) would negatively bias the results of any such study.

To cover potential quality aspects related to self-medication of the vaccine, further stability studies have been submitted. A stability study was performed to evaluate the impact on potency when the vaccine is removed from the refrigerator prior to reconstitution at 25°C, 30°C, and 32°C, and the long term stability of Vaxchora sachets placed at 25°C and put on long-term stability at 2-8°C.

From the data submitted, it appears that the levels seen following 3x 8 h or 1x12 hours of exposure to 25 °C respectively of the un-reconstituted vaccine, compares to the levels of the reference stored at 2-8°C. Therefore, a storage period of up to 12 hours at 25 °C could be acceptable. It is also shown that reconstituted vaccine is stable for 30 minutes at room temperature (no decrease seen in titre). These data would support the stability at the recommended excursions at 25°C even for samples having reached the plateau close to the expiry date. As a safety precaution the time allowed from reconstitution to drinking the vaccine is limited to not more than 15 minutes as this minimises the risk linked to self-medication in that the administration is not interrupted. The SmPC has been updated accordingly.

The storage temperature recommended is 25 °C (after removing from the refrigerator and after reconstitution). The data demonstrate that if the exposure to 25 °C is limited to what is proposed, an acceptable dose can be guaranteed throughout the shelf life. It is noted that stability at 30 and 32 °C is poorer. It is expected that temperatures during summertime in certain parts of Europe may exceed 25 °C and the recommendation based on the 25 °C data may not be fully applicable. The current description of reconstitution indicates that the buffer should be mixed with cold or room tempered bottled water no higher than 25 °C. The following instructions have been added to the SmPC to ensure product potency is assured at point of use:

To prepare the vaccine for administration the Vaxchora active and buffer component sachets are removed from the refrigerator for no more than 12 hours at 25°C prior to reconstitution.

It is important to mix the sachets in the order described. First, the contents of the buffer sachet 1 (a white-to-off-white powder) are mixed with 100 mL of cold or room temperature (≤25°C) bottled non-carbonated drinking water in a cup. Second, the contents of the active component sachet 2 (a white-to-beige powder) are then added and the mixture is stirred for at least 30 seconds. The reconstituted vaccine forms a slightly cloudy suspension that may contain some white particulates. The dose should be administered within 15 minutes of reconstitution.

Note: if the sachets are reconstituted in the incorrect order, the vaccine must be discarded.

An 18 month shelf-life at 2–8 °C for the (un-reconstituted) vaccine finished product is acceptable. This is also the agreed overall shelf-life.

It is noted that vaccine sachet and buffer sachet have different shelf lives (18 months and 36 months respectively-see finished product-buffer section). However, in line with the QRD template on the SmPC (section 6.3) only one overall shelf life for the finished product is to be given in the product information (which is 18 months in this case) even if different components of the product have a different shelf life. The SmPC, package leaflet and outer carton for Vaxchora will state the overall shelf-life. However, the buffer sachet could reflect its individual expiry date (36 months).

Potential Reconstitution Errors

A study was performed to re-verify the impact of the vaccine reconstitution preparation on potency of the active component. A study was conducted to evaluate if varying water volumes for reconstituting the buffer and vaccine would impact potency, where the vaccine was reconstituted as instructed except for the amounts of bottled water 50, 100 (control), and 150 mL used for reconstitution of the buffer. The potency was evaluated over time and remained within the potency acceptance criterion when the starting potency was above 1 x 10^9 CFU/dose.

With reference to the issue of potential reconstitution errors, the presented data indicate that Vaxchora potency remains within the acceptance criteria following variations in reconstitution volume, reconstitution order (adding active substance sachet at the same time as the buffer sachet) and incomplete emptying of active substance sachet. It is agreed that these data support self-administration, with respect to potential reconstitution error.

2.2.4. Finished Medicinal Product- Buffer, Effervescent powder

Description of the product and pharmaceutical development

The buffer for Vaxchora consists of ascorbic acid, sodium bicarbonate, sodium carbonate and anhydrous lactose. See Table 3 for the composition of the buffer component.

Table 3 Quantitative composition of the buffer component.

Ingredient	Function	Reference to Standard
Buffer		
Sodium Bicarbonate	Buffer	Manufacturer
Sodium Carbonate		
Ascorbic Acid	Buffer; Water Chlorine Neutralizer	NF, Ph. Eur.
Lactose	Mfg Flowability	Ph. Eur./NF

Documentation was originally provided for the US manufactured buffer which is actually supportive for the information provided during the MAA procedure, for the buffer manufactured at the commercial site (commercial FP). The production of the buffer had been transferred from the US to the global manufacturing site. The applicant has clarified that the US buffer will not be used in commercial batches of Vaxchora.

The applicant has provided the following information on the difference between the buffers manufactured at the two sites. For the composition of the buffer, the non-compendial dried lactose has been replaced with anhydrous lactose (Ph.Eur quality). Furthermore, the buffer finished product long term storage condition has been changed from -20°C to 5°C. The controls for the buffer are the same as for the US buffer. Sufficient justification has been provided for certain specifications that become in-process tests for the global process. A buffer comparability plan has been submitted describing the process changes made between the Vaxchora US-licensed and global process in order to identify and evaluate the potential impact of the changes to product quality and safety. The buffer formulation for PXVX0200 finished product was adapted from buffer used for the Orochol CVD 103-HgR vaccine; therefore, limited formulation development studies were conducted. The US manufactured buffer was used for all three Phase 3 clinical trials which evaluated PXVX0200.

The vaccine is acid labile and needs to be protected from stomach acid during administration. Based on historical information from Orochol, a pH of <5 during and/or after administration of the vaccine results in inactivation of the bacteria; therefore, the amount of 1M HCl required to change from pH from 6.9 ± 2 to pH 5 is tested, for acid neutralisation, based on USP<301>. Adequate pH during administration and passage into the stomach is assured.

Several BFP lots and (including PaxVax US Phase 3 CTM lots, Vaxchora US-licensed lots and PaxVax Berna validation lots) have been evaluated for comparability. An acceptable comparability report has been provided.

A suitable compatibility study has been performed between the commercial buffer and active (vaccine) components showing that the vaccine is stable for at least 30 minutes in non-carbonated purified bottled and bottled spring waters, however the recommended time allowed from reconstitution to drinking the vaccine is 15 minutes to minimise the risk for interruption of administration. Buffer is filled into packets made from a three-ply multilayer foil. Each packet contains a single-dose of buffer. The materials of construction for each layer of the three-ply multilayer foil packet, their corresponding functions and manufacturer's specification have been provided. The four-ply multilayer foil complies with relevant EU/ Ph.Eur requirements. The foil contains no animal derived components except for a component utilized in the production of the polyethylene film which uses an additive which is derived from animal fats (tallow). See the adventitious agents section.

Manufacture of the product and process controls

Sufficient documentation has been provided in the updated dossier on the manufacture of the buffer and process control. The manufacturing process for the buffer, including the controlled process parameters, is therefore suitably described.

Product specification

The applicant has provided satisfactory full documentation on the buffer specifications in the updated dossier. The buffer specification is based on the US Licensed product and has been adapted for the Global Vaxchora Process and includes appropriate specifications for identity, content, safety and physicochemical attributes. The specification is based on manufacturing and stability data collected during development, as well as consideration of applicable guidelines (e.g. ICH, Ph. Eur., USP).

Batch analysis

The analytical results for a suitable number of buffer FP process validation lots were provided. These were manufactured according to the commercial manufacturing process and were released according to the specification at the time using validated analytical procedures. The data are acceptable. Analytical methods are described and validation data provided, as appropriate. There are no reference standards or materials for Buffer finished product. This is acceptable.

Stability of the product

A shelf-life of 36 months for the buffer FP when stored at 5°C is proposed.

The applicant has provided stability data on a suitable number of process validation lots of buffer finished product (BFP) manufactured at the commercial scale and packaged into multi-layer foil packets. These lots have been monitored at the long-term storage conditions at 5°C ± 3°C (5°C) and satisfactory results are

provided up to 12 months. In accordance with EU GMP guidelines[2], any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

Based on supportive stability data from lots manufactured at another site and lots manufactured with the US licensed process, a 36 month shelf-life at 5°C is considered acceptable. It is noted that vaccine sachet and buffer sachet have different shelf lives (18 months and 36 months respectively). However in line with the QRD template on the SmPC (section 6.3) only one overall shelf life for the finished product is to be given in the product information (which is 18 months in this case) even if different components of the product have a different shelf life. The SmPC, package leaflet and outer carton for Vaxchora will state the overall shelf-life. However, the buffer sachet could reflect its individual expiry date (36 months).

Adventitious agents

PXVX0200 is not considered to be a sterile product. It is not possible to have any inactivation steps in the process for a live bacterial vaccine. Furthermore, the vaccine is not intended to be delivered to a severely immunosuppressed population, and the human digestive tract is designed to inactivate most types of organisms which are routinely ingested with food. Similarly, the human digestive tract is designed to accommodate the presence of endotoxins. However, it is important that particular organisms that do represent a potential risk for infection via the alimentary tract are specifically excluded from the vaccine finished product, and that the bioburden level of the vaccine is controlled below specific limits. The PXVX0200 manufacturing process has controls in-place to limit the bioburden present in the product. These controls are:

- raw material selection, control and testing
- manufacturing procedures and facility controls
- testing performed on cell banks, product intermediates, AS and FP.

The materials of animal origin used in the manufacturing process are casamino acids, casein acid hydrolysate (Hy-Case SF), lactose and packet foil. For the primary container closure system, the packet foil contains a lubricant component (finished product contact) which is derived from animal fats (tallow). These substances are approved for food-contact use. The production of these additives is subject to very severe processing conditions that meet or exceed the recommendations for complete inactivation of TSE agents. The manufacturer of this material has declared it safe according to European regulations laid out in Table 1, Section 3.2.P.7. The BSE/TSE statement is provided.

Raw materials are assessed for their ability to introduce non-viral adventitious agents, such as bovine spongiform encephalopathy / transmissible spongiform encephalopathy (BSE/TSE). BSE/TSE statements for the raw materials have been provided. Lactose is tested to ensure acceptable microbiological limits. Steam sterilisation of culture media and sterile filtration of culture additives to remove adventitious bacteria and fungi provide additional assurance regarding the purity of the final product. The provided documentation is acceptable. Justifications why no TSE certification is needed for the animal derived components have been submitted.

Sufficient information has been presented to give reassurance on adventitious agent safety.

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GMO

The genetically modified CVD 103-HgR strain was generated from the *V. cholerae* classical Inaba O1 strain 569B, based on allelic exchange strategies. CVD 103-HgR is defined by two major genetic modifications:

- Deletion of the majority (94 %) of the sequence of the cholera toxin A subunit gene (*ctxA*) from both replicons corresponding to the catalytic domain, to remove the toxinogenicity of the vaccine strain. CVD 103-HgR retains the *ctxB* gene and the ability to synthesise the non-toxic B subunit of cholera toxin (CTB), which is immunogenic and able to generate neutralising antibodies preventing the toxic activity of cholera enterotoxin.
- Insertion of a mercury resistance operon in the hemolysin A gene (*hlyA*) locus which enables differentiation of the vaccine strain from wild type *V. cholerae*.

Please refer to the non-clinical environmental risk assessment (ERA) for genetically modified organism (GMO) evaluation.

2.2.5. Discussion on chemical, pharmaceutical and biological aspects

The product is provided as a dual pack of two sachets, one with vaccine and stabilisers and one with effervescent buffer to be dissolved in water. The submitted dossier describes in great detail the manufacture, control and stability of the active substance and vaccine finished product. Thorough reports from validation studies and manufacturing development have been provided supporting the proposed manufacturing process and control strategy. Following a request in the initial assessment, acceptable updates to most attributes tested in-process or at active substance or finished product have been introduced. As regards the effervescent buffer the manufacture has recently been transferred to the commercial site and validation data and results from routine control and stability studies were initially missing. Full documentation was subsequently provided for the buffer in the updated dossier. This issue was considered a major objection since important information was missing; however, the issue is now considered solved. All of the questions have now been adequately addressed by the applicant.

Two recommendations are made:

- The issue of the number of dead cells in the active substance has not been discussed in the characterisation part as such. It could not be assured that the vaccine was not working in a dual mode both through the live bacteria and the dead. Further to investigations, the results show that the fraction of dead cells present in the FP is much lower (approximately 5 %) than in the WSL. The amount appears also to be consistent between batches. The levels of dead cells are then considered much too low to present a risk to safety and efficacy with the current manufacturing process/fraction of dead cells in DP. However, the company is asked to monitor dead cells following substantial AS manufacturing changes in case they were to rise significantly (recommendation).
- Although the clearance of specified elemental impurities from fermentation has been well-documented, the applicant should perform a risk assessment for the presence of elemental impurities (as per Ph.Eur. monograph on pharmaceutical preparations (2619)) and control the levels of elemental impurities using the principles of risk management according to ICH Q3D. (recommendation).

2.2.6. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data have been presented to give reassurance on viral/TSE safety.

2.2.7. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

Area	Number	Description	Classification
Quality	1	The applicant should commit to studying and submitting the data on, the fraction of dead cells if substantial changes to the manufacture of the active substance are introduced via post-authorisation changes to the MA.	Recommendation
Quality	2	The applicant should perform a risk assessment for the presence of elemental impurities (as per Ph.Eur. monograph on pharmaceutical preparations (2619)) and control the levels of elemental impurities using the principles of risk management according to ICH Q3D.	Recommendation

2.3. Non-clinical aspects

2.3.1. Introduction

Vaxchora is a live attenuated bacterial vaccine containing the CVD 103-HgR vaccine strain of Vibrio cholera (*V. cholerae*) serogroup O1, biotype classical, serotype Inaba. V. cholera is a strictly human pathogen. Cholera bacilli do not colonize or replicate in healthy adult animals. Relevant animal models are not available to assess safety, efficacy via live cholera challenge, or predict the immune response to a live attenuated cholera vaccine such as Vaxchora. The applicant has therefore not performed any non-clinical animal pharmacology, pharmacokinetics or toxicology studies with Vaxchora.

Extensive clinical safety data are available from the historical studies conducted with the previously marketed CVD 103-HgR Orochol, a total of 3,563 individuals to date who received Vaxchora during clinical development, and safety experience from 55,808 doses distributed up to June 30, 2018 in the US during the post-marketing phase of Vaxchora. Based on the large amount of data available in humans and lack of specific safety or efficacy concerns, the CHMP deemed that non-clinical studies are not needed for this vaccine.

Relevant non-clinical data from the scientific literature were submitted by the Applicant and are summarised in this section.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Mice were given an intraperitoneal (IP) dose of 108 CFU of CVD103-HgR, followed by challenge with cholera toxin (CT). IP immunization was chosen as the route of administration since only mice less than 7 days old are susceptible to oral infection with V. cholera. Immunized mice in groups of 10 were given 1, 2.25 or 3 LD50 doses of CT administered IP, and were then observed for 7 days. All of the mice survived CT doses of 1 and 2.25 LD50, and 70% of mice survived a CT dose of 3 LD50. Control mice in groups of 8, given only phosphate buffered saline, were less protected following CT challenge of 1, 2.25, and 3 LD50, i.e., 50%, 12%, and 0% survival, respectively (Dragunsky 1992).

CVD103-HgR has been used as a vaccine vector to deliver other antigens by oral administration in rabbits. One study evaluated administration of CVD103-HgR (pInt248), which expressed intimin, an outer membrane antigen of E. coli. Immunized rabbits produced anti-intimin antibodies and showed moderate protection from enteropathogenic E. coli challenge (Keller 2010). A similar study explored the protective immunity afforded by CVD103-HgR (pDA60) expressing a Shiga-like toxin I B subunit antigen. All rabbits developed neutralizing antibodies against Shiga-like toxin I (Acheson 1996).

2.3.3. Pharmacokinetics

Pharmacokinetics studies are generally not required for vaccines, in line with available guidelines.

2.3.4. Toxicology

The only animal models of cholera disease are infant rabbits (Ritchie 2010), infant mice (Taylor 1987, Klose 2000), rabbits that have been surgically modified using ligated ileal loops (Formal 1961), adult mice with ligated ileal loops (Sawasvirojwong 2013) or the Removable Intestinal Tie-Adult Rabbit (RITARD) model (Pierce 1988, Dziejman 2005, Russell 1992, Morris 1990). None of these models are suitable for toxicology studies.

Genotoxicity and carcinogenicity

Genotoxicity and carcinogenicity studies are not required based on the type of product and in line with current guidelines on non-clinical evaluation of vaccines.

2.3.5. Ecotoxicity/environmental risk assessment

Vaxchora contains genetically modified organisms (GMOs). These can be introduced into the environment via faeces (due to shedding), accidental spilling and unconsumed residues. An environmental risk assessment was performed according to the relevant guidelines, and no risk to the environment was identified.

V. cholerae does not survive well in dry conditions such as on fabric, paper, plastic and metal surfaces and dies off within a few days (Felsenfeld 1965). Vaxchora is required to be transported in refrigerated conditions and stored in a refrigerator because may loses its potency if left at room temperature for extended periods. A study performed to evaluate the persistence of high concentrations of Vaxchora on stainless steel surfaces at room temperature in the manufacturing facility (CH-SYR-55-01) showed the bacterial concentration was substantially reduced after 3 days. The Vaxchora sachet contains a maximum of 2×10^9 CFU. Any release of

the bacteria into the environment would therefore be negligible, with a short survival time. No risk to the environment was identified during the preparation of the environmental risk assessment. Notwithstanding, the package leaflet provides instructions regarding clean up and disposal of the unused medicine, and also contains information regarding faecal shedding of vaccine bacteria and the need to exercise good hygiene in the period after taking Vaxchora.

2.3.6. Discussion on non-clinical aspects

The applicant has not performed any non-clinical pharmacology, pharmacokinetic or toxicological studies with Vaxchora. The main reason is that Cholera bacilli do not colonize or replicate in healthy adult animals, making it difficult to perform relevant non-clinical studies. Relevant non-clinical data from the literature has been assessed.

Further, clinical experience with CVD103-HgR is sufficient to characterize the safety profile of Vaxchora. Vaxchora has been on the market in the US since June 2016, and safety data is available from over 50,000 distributed doses. Further, more than 3,000 individuals have received Vaxchora during clinical development. In addition, clinical data has also been gathered for CVD103-HgR when the vaccine was marketed under the name Orochol.

2.3.7. Conclusion on the non-clinical aspects

In view of the substantial amount of clinical safety data with CVD103-HgR, and lack of appropriate animal models for pharmacology and safety assessment, the omission of non-clinical studies is considered adequately justified. No risk to the environment was identified. Nevertheless, since Vaxchora is a GMO, the waste should be disposed of according to the recommendations provided in order to minimize any potential risks to the environment.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 4 Overview of Vaxchora Clinical Trials

Type of Trial	Trial No.	Male/ Female n/n (age)	Objectives of the Trial	Trial Design and Type of Control	Test Product(s); Route of Administration	Number of Subjects Randomized (*Number of Subjects who Received Study Vaccine)*	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Trial Status; Type of Report
Phase 1	PXVX-VC-200-002	33/33 (18-50)	Safety immunogenicity	Randomized, double-blind, placebo-controlled	4.43×10^8 CFU/dose; oral	66 55 vaccine, 11 placebo *(55 vaccine, 11 placebo)*	Healthy Subjects	Single dose	Complete; full report
Challenge Phase 3	PXVX-VC-200-003	124/73 (18-45)	Demonstrate protection from challenge	Randomized, double-blind, placebo-controlled	5×10^8 CFU/dose; oral	197 95 vaccine, 102 placebo *(95 vaccine, 102 placebo)*	Healthy Subjects	Single dose	Complete; full report
Lot Consistency Phase 3	PXVX-VC-200-004	1423/1723 (18-45)	Demonstrate clinical lot consistency	Randomized, double-blind, placebo-controlled	1×10^9 CFU/dose; oral	3146 2795 vaccine, 351 placebo *(2789 vaccine, 350 placebo)*	Healthy Subjects	Single dose	Complete; full report
Older Adults Phase 3	PXVX-VC-200-005	182/216 (46-64)	Safety immunogenicity	Randomized, double-blind, placebo-controlled	1×10^9 CFU/dose; oral	398 299 vaccine, 99 placebo *(296 vaccine, 99 placebo)*	Healthy Subjects	Single dose	Complete; full report
Paediatric Phase 4	PXVX-VC-200-006	198/183 (2-18§)	Safety immunogenicity	Randomized, double-blind, placebo-controlled	1×10^9 CFU/dose; oral	381 327 vaccine, 54 placebo *(328 vaccine, 50 placebo)*	Healthy Subjects	Single dose	Ongoing; interim report

§ Only ages 6-18 are evaluated in this report. Children 2-6 years are outside the proposed indication for Vaxchora

2.4.2. Pharmacokinetics

Pharmacokinetic studies are usually not required for vaccines (EMEA/CHMP/VWP/164653/2005 – Guideline on clinical evaluation of new vaccines). Vaxchora (PXVX0200) is a live attenuated bacterial vaccine. No pharmacokinetic studies were conducted.

2.4.3. Pharmacodynamics

Mechanism of action

Vaxchora contains live attenuated cholera bacteria (V. cholerae O1 classical Inaba strain CVD 103-HgR) that replicate in the gastrointestinal tract of the recipient and induce serum vibriocidal antibody and memory B cell responses. Immune mechanisms conferring protection against cholera following receipt of Vaxchora have not been determined, however, rises in serum vibriocidal antibody 10 days after vaccination with Vaxchora were associated with protection in a human challenge study.

According to the Guideline on clinical evaluation of new vaccines (EMEA/CHMP/VWP/164653/2005), pharmacodynamic studies for vaccines are essentially comprised of the immunogenicity studies that characterise immune responses to the vaccine. This section will therefore focus on the bioanalytical methods used for evaluating the immunogenicity endpoints in the Vaxchora clinical trials. The trials are discussed in the Efficacy section of this report.

The serum vibriocidal antibody assay (SVA)

The serum vibriocidal antibody (SVA) assays for all Classical Inaba, El Tor Inaba, Classical Ogawa and El Tor Ogawa are validated functional assays that involved complement-mediated bacteriolysis and were used to measure vibriocidal antibody levels in serum. The SVA assay detected mainly IgM antibodies, which correspond mainly to de novo immune responses. Briefly, serial dilutions of serum were mixed and incubated at 37°C with equal volumes of standardized *V. cholerae* (including guinea pig complement). Titres were expressed as the reciprocal of the dilution of the most diluted sample associated with bacterial growth of 75% or less compared with the negative control. To achieve validation of the assay, intra- and inter-assay precision, accuracy/dilutability, specificity, sample stability, and robustness were evaluated.

The cholera toxin (CT) ELISA assay

The CT ELISA assay measured serum antibodies against cholera toxin. It is a validated ELISA assay specific for detecting IgG antibodies, which are usually induced approximately 2 weeks after exposure to antigen. Briefly, samples were serially diluted from 1:200 to 1:12,800 in duplicate. Following incubation on CT coated wells, antibodies to CT are detected with a peroxidase labelled goat anti-human IgG and a tetramethylbenzidine (TMB) substrate. The titre is equal to the reciprocal of the dilution of the least diluted sample with an OD450 greater than or equal to 0.2. To achieve validation of the assay, intra and inter-assay precision, accuracy/dilutability, specificity, sample stability, and robustness were evaluated.

Memory B-cells specific for CT and LPS, Inaba strain ELISPOT assay

Percentages of memory B-cells that are specific for V. cholerae toxin B (CT) and lipopolysaccharide (LPS, Inaba strain) in the peripheral blood mononuclear cells (PBMC) of clinical trial subjects were measured using an enzyme-linked immunospot (ELISPOT) assay.

Spots appearing in assay wells corresponding to B-cells within a PBMC sample that secreted IgG or IgA antibody specific for CT or LPS were enumerated. PBMC isolated from blood samples collected from each subject are cryopreserved in liquid nitrogen and then batch tested in the same experiment.

The ELISPOT assay was conducted at the site of the MAH. Since the memory B cell evaluation was only an exploratory endpoint in clinical studies, this assay was qualified rather than validated.

O-specific polysaccharide antibody ELISA assay

A research study was conducted post-hoc on samples from the PXVX-VC-200-003 trial (challenge study) using an ELISA assay to detect different anti-O-specific polysaccharide (OSP) antibody subclasses directed against the lipopolysaccharide of V. cholerae serogroup O1. The seroconversion threshold was defined as >1.5 fold increase from baseline. The findings from this study were included in the dossier based on a publication by Islam et al. (2018).

Concomitant administration with other vaccines

No clinical studies are included that assess the concomitant use of PXVX0200 with particularly oral travel vaccines, however the Applicant provided supportive data in the form of medical literature on Orochol (vaccine previously registered and marketed by SSVI/Berna, Switzerland, which contains the strain CVD103-HgR). Vaccines such as Oral Ty21a, oral polio vaccine and yellow fever vaccine were studied in coadministration with Orochol.

It was found that Orochol and Ty21a could be administered concomitantly with no adverse effect on immunogenicity of either vaccine, although this study used a non-enteric coated formulation of the Ty21a vaccine (Cryz 1995b, Kollaritsch 1996). The current marketed formulation of Vivotif (typhoid vaccine, live, oral, Ty21a) consists of enteric coated capsules, and it is possible that the buffer used with Vaxchora vaccine could adversely affect the protective effect of the enteric coating of Vivotif. Historically, the Orochol package insert advised that the administration of Orochol and Vivotif should be separated by 8 hours for this reason. However, the applicant assessed to what extent a separation of concomitantly administered Vaxchora and Vivotif is necessary based on the nature of the buffer used in Vaxchora on published literature to this topic. The current marketed formulation of Vivotif (typhoid vaccine, live, oral, Ty21a) consists of enteric coated capsules, and it is possible that the buffer used with Vaxchora vaccine could adversely affect the protective effect of the enteric coating of Vivotif. It is estimated that the buffer of Vaxchora rapidly interacts with the acid in the stomach allowing a return to normal pH values within a time frame of two hours, which is therefore the suggested separation time of two hours between the administration of Vivotif and Vaxchora.

Studies of concomitant vaccination with oral polio vaccine and yellow fever vaccine (YF 17D) demonstrated that these vaccines did not suppress the immune response to Orochol and that the immune response to YF 17D was also not suppressed by Orochol (Kollaritsch 1997, Tsai 1999).

There are no plans for any further interference studies to be conducted with other travel vaccines, which is acceptable.

2.4.4. Discussion on clinical pharmacology

Vaxchora is a live attenuated oral vaccine. The mode of administration therefore corresponds to the natural mode of infection with the aim of providing protection at the site of infection.

No clinical studies are included that assess the concomitant use of PXVX0200 with particularly oral travel vaccines. The applicant relied on information from an earlier version of the vaccine (Orochol) as supportive information to provide additional assurance of the clinical profile of the product.

Humoral immunity, induced by V. cholerae infection, specifically sIgA, targets both the lipopolysaccharide (LPS) coat of the bacterium and the cholera toxin (CT). Antibodies directed against LPS appear to confer more robust protection than those against CT (Apter 1993). The anti-LPS response is focused on the O-specific polysaccharide (OSP) (Wang 1998, Villeneuve 2000, Johnson 2012). The serum vibriocidal antibody (SVA) assay measures lysis of standardised V. cholera (according to serotype). SVA seroconversion was pre-specified in the protocol as a >4-fold increase over baseline.

All analyses of immunogenicity are based on samples taken from peripheral blood (serum and B-cells).

Immunogenicity analyses used assays that were validated and carried out at a central laboratory for determining serum vibriocidal antibodies (SVA) and anti-cholera toxin (CT) antibodies. The only assay that was not validated was the ELISPOT assay to measure memory B-cells, which was carried out In House by the Sponsor.

The serum vibriocidal antibodies correspond to a simple means of assessing the induction of functional vibriocidal antibodies. SVA measurements in serum may not adequately reflect immune responses produced locally in the mucosa. Assessment of the duration of immunity was limited to detecting B-cell memory responses up to 6 months post-vaccination. Since the assay used was not validated, findings from this assay are considered exploratory.

Clinical pharmacology documentation of this orally administered live bacterial vaccine has shortcomings regarding interactions with antimicrobials and effects of food intake. The Applicant circumvents these shortcomings by including relevant prescribing information to avoid antimicrobials and eating.

2.4.5. Conclusions on clinical pharmacology

No clinical studies have been carried out to determine the potential for immune interference should Vaxchora be taken with other, particularly oral, vaccines prior to travel, but supportive information applicable to Vaxchora was included in the SmPC to inform prescribers.

Serum vibriocidal antibodies (SVA) were detected using a validated functional assay that involved complement-mediated bacteriolysis. The SVA assay detected mainly IgM antibodies, which correspond mainly to de novo immune responses. Detection of anti-CT antibodies used a validated ELISA assay specific for detecting IgG antibodies, which are usually induced approximately 2 weeks after exposure to antigen. Detection of memory B-cells involved a qualified but not validated ELISPOT assay using frozen peripheral blood lymphocytes. No criteria for cell recovery and viability were used; however the viability of the cells was acceptable.

The omission of non-clinical studies can be considered acceptable based on the accrued clinical experience with similar vaccines.

No major concerns were identified and from a pharmacology perspective the application is considered acceptable.

2.5. Clinical efficacy

2.5.1. Dose response study(ies)

Defined dose-finding studies have not been carried out.

The final to-be-marketed formulation selected for Vaxchora is between $4x10^8$ to $2x10^9$ CFU/dose, and the recommended regimen is a single dose to be administered at least 10 days prior to potential exposure to cholera.

The challenge study used a dose of $4x10^8$ CFU/dose. This is in line with historical data from a challenge trial using Orochol at a concentration of $3\text{-}5x10^8$ CFU/dose (Losonsky 1993), which supports the proposed lower end of the specification.

Vaccine lots with a concentration of $1x10^9$ CFU/dose were used in the lot consistency, older adults and paediatric studies in order to provide evidence of safety of a "higher" concentration for use in setting release specifications. The concentration of $1x10^9$ CFU/dose was shown to be well tolerated. The highest concentration of Vaxchora evaluated in the clinical trials was $1.39x10^9$ CFU/dose (rounds to $1x10^9$ CFU/dose). A higher concentration of Vaxchora is being used in an investigator-initiated study in Mali ($1x10^{10}$ CFU/dose). These data support the recommendation for the upper potency specification limit for Vaxchora of $2x10^9$ CFU/dose.

2.5.2. Main studies

The Applicant submitted five clinical studies on Vaxchora: one Phase 1 study, three Phase 3 studies and one Phase 4 study. One of the Phase 3 studies (PXVX-VC-200-003) is a challenge study, which was important for determining efficacy.

The clinical efficacy study is described first, followed by the remaining immunogenicity studies as well as the bridging analysis. Information related to Orochol is considered relevant for Vaxchora since Orochol was based on the same V. cholerae strain as Vaxchora, and is described under Supportive Studies.

PXVX-VC-200-003

A Phase 3 randomized, double-blind, placebo-controlled multi-centre clinical study was carried out to evaluate efficacy, immunogenicity and safety of a single dose of the live oral cholera vaccine candidate, PXVX0200, CVD103-HgR Strain compared with placebo following challenge with virulent *Vibrio Cholera* 01 El Tor Inaba at 10 days or 3 months post-vaccination in healthy volunteers aged 18 to 45 years. Healthy adults were randomised to receive Vaxchora (PXVX200), or placebo (physiological saline) in a 1:1 ratio.

Methods

Study Participants

Main inclusion criteria:

Healthy male and female adults, age 18 to 45 years (inclusive). Women of childbearing potential had to have a negative pregnancy test at screening, prior to vaccination and challenge. All participants had to be of non-childbearing potential or use contraception/birth control within 2 months of vaccination, and had to agree to continue such precautions during the study and for 30 days post-challenge. Male subjects had to agree not to

father a child for 30 days post-vaccination. Participants had to be able to pass a written examination with at least a score of 70% correct in order to demonstrate their comprehension of the study procedures and possible side effects before inoculation with the challenge strain, V. cholerae. If the subject scored at least 50% correct, he/she could take the test a second time after undergoing re-education, but could not participate if the second score was less than 70%. Participants had to agree not to participate in another investigational vaccine or drug trial during the duration of the study.

Main exclusion criteria:

- 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, current drug or alcohol abuse.
- History of hospitalization for psychiatric illness, suicide attempt, or confinement for danger to self or others, within the past 10 years.
- Elevated blood pressure, ≥150 systolic or ≥90 diastolic mm Hg, before vaccination.
- Abnormal stool pattern defined as fewer than 3 stools per week or more than 2 stools per day in past 6 months, and loose stools during the 1–2 day acclimation period before challenge.
- Known allergy to, or known medical condition that precludes the use of both tetracycline and/or ciprofloxacin. Previously received a licensed or investigational cholera vaccine. Travel to a cholera-endemic area in the previous 5 years.
- Malignancy (excluding non-melanotic skin cancers) or lymphoproliferative disorders diagnosed or treated during the past 5 years.
- Positive serology for HIV, hepatitis B antigen, or hepatitis C.
- 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. Received or planned to receive antibiotics (other than protocol-specified) or chloroquine within 14 days prior to the study vaccination through to 28 days post-vaccination or challenge, whichever is longer.

Participants that were due to be challenged had an additional eligibility requirement in that they should continue their consent to participate in the study and pass a written examination with a minimum score of 70% to demonstrate their comprehension of the study procedures and possible side effects. If subjects scored at least 50% correct but less than 70% correct, they could retake the exam a second time after undergoing re-education, but were ineligible if the second score was less than 70%.

Treatments

Each subject was randomised to receive a single vaccination of either:

- Vaxchora (PXVX0200), administered orally as a suspension with buffer, or
- Placebo (physiological saline), administered orally.

On the day of challenge (Day 11 or Day 91), each subject selected for challenge was administered $1x10^5$ CFU of wild type *V. cholerae* O1 El Tor Inaba strain N16961.

All challenged subjects were carefully monitored in the in-patient unit, had intravenous or oral rehydration when they had stool output that met the definition of diarrhoea (grade 3). All subjects who did not receive antimicrobials due to diarrhoea received antimicrobials starting 96 hours after challenge or earlier if they had stool output that met the definition of severe diarrhoea (cumulative stool output >5L).

Objectives and associated endpoints

Primary

Co-primary objective: Demonstrate that the lower 95% confidence bound on the protective efficacy (PE) of a single dose of PXVX0200 is ≥30% following a challenge with virulent *V. cholerae* O1 El Tor Inaba 10 days or 3-months post-vaccination.

Associated endpoint: The occurrence of moderate or severe diarrhoea (≥3.0 L purge)

Comparator Group: PXVX0200 recipients challenged at 10 days compared with a pooled group of placebo recipients challenged at either 10 days or 3 months.

Success criteria: The lower, two-sided 95% confidence bound on protective efficacy must be ≥30%.

Secondary

Objective 1: Evaluate the impact of vaccination on disease severity.

Associated post-challenge endpoints include total weight (converted to volume) of diarrhoeal stools, incidence of diarrhoea of any severity, incidence of faecal shedding of wild type V. cholerae, peak concentration of wild type V. cholerae detected in stool.

Objective 2: Evaluate the tolerability of vaccine.

Associated pre-challenge endpoints include incidence and severity of signs and symptoms of reactogenicity such as diarrhoea and fever, incidence and severity of unsolicited AEs.

Tertiary

Objective 1: Evaluate the pre-challenge immunologic response to PXVX0200.

Associated pre-challenge endpoints include serum vibriocidal antibody and serum IgG anti-CT geometric mean titre (GMT) at all available pre-challenge time points; the percentage of subjects who demonstrated a 4-fold rise in vibriocidal antibody prior to challenge, the percentage of subjects who demonstrated a 4-fold rise in anti-CT prior to challenge; the percentage of subjects who demonstrated a vibriocidal antibody titre ≥2560.

Objective 2: Evaluate the post-challenge immunologic response to PXVX0200.

Associated post-challenge endpoints include the percentage of subjects who exhibited a 4-fold rise, when comparing last pre-challenge vibriocidal antibody or anti-CT antibody to levels following challenge.

Exploratory

- Explore the relationship between post-vaccination, pre-challenge vibriocidal and/or anti-CT concentration and the incidence of moderate/severe diarrhoea, mild diarrhoea, any diarrhoea, or measures of diarrhoea severity such as total number or total volume of diarrhoeal stools.

- Explore the association between age and immunologic response, and evaluate whether the relationship between immunologic response and outcome varies with age.
- Explore the impact of blood type – group 0 vs. non-0 – on the incidence and severity of diarrhoea.
- Explore the relationship between pre-challenge memory B cell concentration and the incidence or severity of diarrhoea.
- Explore whether the memory B cell response to PXVX0200 vaccination is similar to the response induced by cholera infection. Associated memory B cell endpoint: Anti-O1 LPS IgA memory B cell concentration measured at 180 days in non-challenged vaccine recipients and in placebo recipients challenged at 10 days.

Sample size

The sample size calculations depended on three key assumptions: the expected protective efficacy of the vaccine; the attack rate of moderate or severe diarrhoea among placebo recipients; and the expected proportion of subjects of blood group O (a priori knowledge that the attack rate varies by blood group). Protective efficacy has been estimated at 90% based on the results of a challenge trial that was similar to the current study (Tackett 1999). The expected attack rate of moderate or severe diarrhoea was estimated from two previous trials involving a total of 63 placebo recipients challenged with the same dose and strain of virulent V. cholerae used in the current trial (Sack 1998; Tacket 1999). Summing across trials, 13 of 24 (54%) subjects of blood group O and 14 of 39 (36%) non-O subjects experienced moderate or severe diarrhoea following challenge. The proportion of subjects in the trial with blood group O was controlled during enrolment with the aim of having 60% of all enrolled subjects with blood group O. The expected attack rate, however, has been estimated under the more conservative assumption that 50% of challenged subjects will be blood group O. Under that assumption, the attack rate among placebo recipients was expected to be 45%.

Since the 10-day and 3-month challenges target different aspects of the vaccine's performance, they were analysed separately. However, vaccinees in each challenge were compared to a common control group comprised of all placebo recipients challenged at any time during the trial. That implied that the treatment arms contained an unequal number of subjects in each challenge analysis with roughly twice as many placebo recipients as vaccines. As a precaution against introducing bias, there was a roughly equal balance between vaccinees and placebo recipients within each challenge. The sample size for the 10-day challenge study (31 subjects per treatment arm combined with the 27 placebo recipients in the 3-month challenge) was chosen to have 95% power of meeting the primary objective to show that the lower 95% confidence bound on protective efficacy exceeds 30%. Like the 10-day challenge, the analysis for the 3-month challenge compared vaccinees challenged at 3 months to the group of placebo recipients pooled across both challenges. The resulting sample of 27 vaccinees and 58 placebo recipients yielded approximately 93% power to establish that the lower 95% confidence bound is at least 30% at the 3-month challenge. An allowance was built to accommodate a shortfall in the number challenged. Allowing for a 10% shortfall inflated the ultimate sample size to 34 subjects per treatment arm in the 10-day challenge and 30 subjects per arm in the 3-month challenge.

This minimal sample size calculated was estimated to achieve both co-primary endpoints at a power level of at least 95%*93%=88%. Ultimately, the total sample size for the trial was 95 subjects in the treatment arm and 102 subjects in the placebo arm.

Randomisation and blinding

A randomization schedule was created using randomized permuted blocks, and randomized subjects within each site were assigned the treatment (PXVX0200 or placebo) associated with the next available row on the schedule within the appropriate Blood Group stratum. The subject's identification (ID), as well as the date and time of randomization, were populated. Subjects were automatically assigned by the interactive web randomization system (IWRS) upon randomization. Prior to the day of vaccination, a report was generated for each site based on subjects that met entry criteria, providing blood group and Subject IDs. The unblinded statistician reviewed the list and selected a subset of subjects that met target enrolment within site that satisfied at least 60% blood group 0. This information was reported in the EDC, in order for the sites to obtain a list of subjects and alternates to be invited back for randomization and vaccination. At the time of the vaccination visit, the user accessed the randomization system to enrol each subject and randomize the subject. In the event that a subject no longer qualified for vaccination, the user accessed the system to identify which alternate subject should be randomized.

After enrolment, sites screened subjects to eligibility into the Challenge Phase of the trial. It was expected that approximately 30% of subjects would not be challenged in the 10-day challenge group, and approximately 40% of subjects would not be challenged in the 3-month challenge group.

Blinded study personnel received a randomization notification once a subject had been randomized into the study, excluding the assigned treatment. An unblinded site pharmacist who was qualified to handle and dispense medication received an unblinded notification from the system generated at the time the subject was randomized and used that information to dispense the PXVX0200 vaccine or placebo as appropriate.

Statistical methods

Vaccine protective efficacy (PE) was estimated by comparing the attack rates of moderate/severe diarrhoea in the two vaccinated challenge groups to those in the combined placebo group as per the protocol and Statistical Analysis Plan (SAP).

Protective Efficacy = [(Attack Rate in Placebo Group – Attack Rate in Vaccine Group)/Attack Rate in Placebo Group] x 100

Confidence intervals (CIs) for the PE estimates were calculated using the method of Farrington and Manning (Farrington 1990). PE was estimated using similar methods for subgroups of subjects defined by blood type, sex, and race. Fisher's exact tests were used to compare vaccine and placebo groups on the number and volume of loose stools experienced post-challenge, while Wilcoxon rank test were used to compare groups on the number of days with loose stools and the number of days shedding V. cholerae. Fisher's exact tests were also used to compare the percentage of subjects in the vaccine and placebo groups that reported signs and symptom of reactogenicity.

Vibriocidal and anti-CT antibody were summarized by GMT and seroconversion defined as the percentage of subjects with a 4-fold or higher increase in titre over baseline. For discrete/categorical variables, the number and percentage of non-missing subjects was generated.

Bridging across studies and immune correlates

In order to develop criteria to bridge the protective efficacy established in the younger adults ages 18-45 who participated in the challenge trial to other age groups and populations, analyses were conducted on the data from the challenge study to assess potential immune correlates of protection. SVA GMT, fold rise,

seroconversion and seroprotection levels were evaluated using the results from the 10-Day and 3-Month challenge cohorts. Seroprotection was defined as a range of vibriocidal titre cut-offs evaluated to investigate correlation with the development of moderate/severe cholera. SVA seroconversion was defined as a ≥4-fold rise in SVA titre from pre-vaccination to Day 11. Administration of a single $5x10^8$ CFU oral dose of PXVX0200 led to serum vibriocidal seroconversion in 89.4% of vaccine recipients by Day 11 (10 days after vaccination).

There was a significant correlation between SVA seroconversion and protection from cholera diarrhoea (refer to the section on Exploratory outcomes). Fold-rises in SVA titre at Day 11 were observed frequently in Vaxchora recipients and extremely rarely in placebo recipients. Furthermore, only 2 of 62 seroconverting Vaxchora recipients developed moderate/severe cholera after challenge: 1 of 33 at Day 11 and 1 of 29 at Day 91. Based on this near 1:1 relationship between seroconversion and protection, along with the fact that approximately 90% of Vaxchora recipients seroconverted, SVA seroconversion at Day 11 was used as the best immunologic measure for establishing a bridge between populations.

Seroconversion performed better as an immune marker than titre or a protective level of vibriocidal antibody, perhaps because it was better measure of "vaccine take" and better reflected unmeasured variables such as local (gastrointestinal) immune responses.

Results

Participant flow

A total of 197 subjects were randomized, 95 to Vaxchora (PXVX0200) and 102 to saline placebo, approximately at a 1:1 ratio. Each randomized subject received a single dose (Day 1) of vaccine or placebo, administered orally. The study disposition is shown in the figure below.

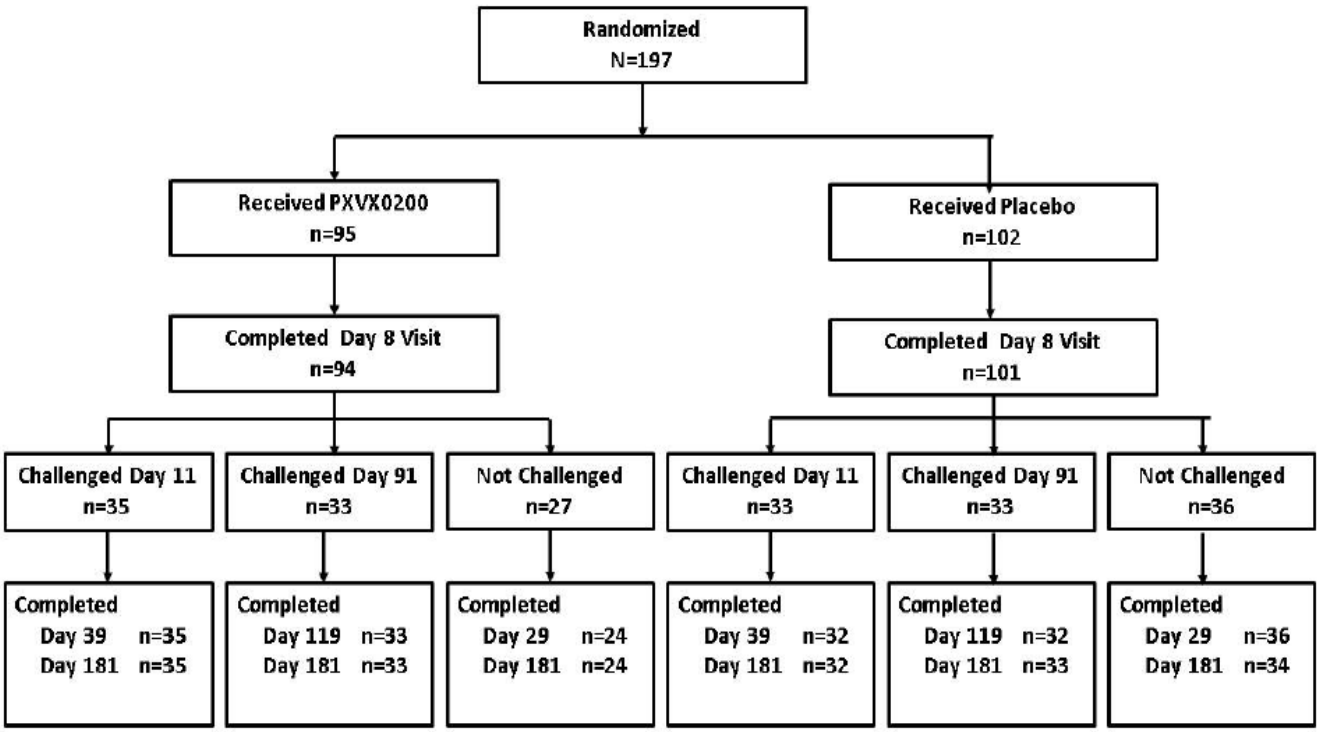


Figure 1 Study Disposition Flow Chart – Randomised Population

Recruitment

Study Period: Duration: 8 months. Date of first enrolment: 07 May 2012. Date of last completed: 07 Jan 2013.

Conduct of the study

One subject was dosed from a previously utilised vial. The randomisation was appropriate, but dosing was inappropriately low and resulted in the subject being removed from the pre-specified Immunogenicity Evaluable Cohort.

After the trial began, but before unblinding, it was discovered that the two clinical sites were using different techniques to prepare the vaccine and placebo for administration. Specifically, the University of Kentucky site reconstituted lyophilised vaccine or the placebo powder using tap water while the CVD at the University of Maryland used sterile, non-bacteriostatic water.

Given that concern was raised over whether chlorine or other additives in tap water could affect dose of PXVX0200 administered, and consequently could affect immunogenicity, several new analyses were added to the statistical analysis plan to investigate potential differences in antibody response between the two sites.

In particular, the two clinical sites were compared based on the seroconversion rate and GMT from both vibriocidal and anti-CT antibody assays using data from vaccine recipients only. The results from each site were also compared to the group of placebo recipients pooled over both sites. Hochberg's procedure (Hochberg 1988) was used to adjust for the multiple comparisons involved in the analyses. The additional site-specific analyses were specified after data unblinding.

Baseline data

The baseline demographic and clinical characteristics of vaccinated/placebo groups that were challenged is shown in the table below.

Table 5 Demographics – Randomised Population

	Challenged				Unchallenged	
Baseline Characteristics	**PXVX0200 10 Day N=35**	**Placebo 10 Day N=33**	**PXVX0200 3 Month N=33**	**Placebo 3 Month N=33**	**PXVX0200 N=27**	**Placebo N=36**
Age in years						
Mean±SD	30.5±6.68	31.6±8.43	33.1±8.23	30.3±7.73	30.8±8.28	29.8±7.54
Median (Min – Max)	31.0 (18 - 45)	31.0 (20 - 45)	32.0 (18 - 45)	30.0 (18 - 45)	29.0 (18 - 45)	28.5 (18 - 44)
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
Native Hawaiian or other Pacific Islander	0	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 (21.2%)	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%)

Numbers analysed

Participants were divided into analysis groups as shown in the table below.

Table 6 Study Groups in Clinical Study PXVX-VC-200-003

Population	PXVX0200 N=95	Placebo N=102	Total N=197
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[a]	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[b]	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[c]	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[d]	55 (57.9%)	26 (25.5%)	81 (41.1%)
10-Day Challenge Group	0	26 (25.5%)	26 (13.2%)
3-Month Challenge Group	33 (34.7%)	0[e]	33 (16.8%)
Unchallenged Subjects	22 (23.2%)	0	22 (11.2%)

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

a.ITT: All randomized subjects who received vaccination.

b.Safety: All subjects who received study treatment.

c. Immunogenicity Evaluable: subjects who received treatment and had evaluable, classical Inaba vibriocidal antibody results from Day 1 and at least one post-vaccination time point prior to challenge.

d. Memory B Cell Analysis: Subjects in the ITT population who received vaccine and were not challenged or who received placebo and were challenged 10-days post-vaccination and had evaluable anti-O1 LPS IgA memory B cell results at Day 1 and Day 181, as well as subjects who received vaccine and were challenged 3 months post-vaccination and had evaluable anti-O1 LPS IgA memory B cell results at Day 1 and Day 91.

e. Analyses of laboratory specimens obtained pre-challenge Day 91 for the 33 placebo recipients challenged at 3 months were added after initial data analysis was performed.

Outcomes and estimation

Primary efficacy endpoint

The primary efficacy endpoint was the occurrence of moderate or severe diarrhoea (≥3.0 L cumulative purge) post-challenge with virulent V. cholerae O1 El Tor Inaba 10 days (Day 11) and 3 months post-vaccination (Day 91) in two separate challenges. The co-primary objectives of the study were to demonstrate that the

lower 95% confidence bound on the protective efficacy was ≥30% at both of these time points. Success at both the 10-Day and 3-Month Challenges was required to achieve success for the trial as a whole.

Protective efficacy for the 10-Day and 3-Month Challenge groups is shown in Table 7. The co-primary objectives of the study were met in the ITT group with 10-Day protective efficacy of 90.3% (lower 95% CI bound of 62.7%) and 3-Month protective efficacy of 79.5% (lower 95% CI bound of 49.9%).

Table 7 Primary efficacy endpoint: Protective efficacy for 10-Day and 3-Month challenge (ITT)

Parameter	PXVX0200 10-Day N=35	PXVX0200 3-Month N=33	Combined Placebo N=66
Overall Severity			
No qualifying diarrhea	30 (85.7%)	18 (54.5%)	5 (7.6%)
Mild: <3 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%)
Protective Efficacy (PE)[b]	90.3%	79.5%	
Lower 95.1% CI[c]	[62.7%]	[49.9%]	

Note: Percentages were based on the number of subjects with a non-missing value within each treatment group. Mild diarrhoea was defined as the passage of 2 or more unformed stools (grades 3 to 5) over a 48-h period that equalled or exceeded 200 mL or a single unformed stool of 300 mL or greater and less than 3 L total diarrhoea.

a Attack Rate: Moderate or severe diarrhoea severity, as noted by ≥3 L of overall diarrhoeal purge.

b Protective Efficacy = [(Attack Rate in Placebo Group - Attack Rate in Vaccine Group)/Attack Rate in Placebo Group] * 100.

c Confidence interval was calculated using the Farrington and Manning method for a ratio of binomial variables where protective efficacy under the null hypothesis is 0.3.

Secondary efficacy endpoints

Secondary efficacy endpoints included incidence of diarrhoea of any severity, total weight of diarrhoeal stools converted to volume (1 g=1 mL) and incidence and peak concentration of shedding post-challenge with virulent V. cholerae O1 El Tor Inaba measured 10 days and 3 months post-vaccination.

The definition of stool grade is shown below. Diarrhoea was defined as grade 3 and above.

- Grade 1: formed (normal, does not take shape of the container)
- Grade 2: soft (normal, does not take shape of the container)
- Grade 3: thick (liquid diarrhoeal, takes the shape of the container)
- Grade 4: opaque watery
- Grade 5: rice water (clear watery).

The severity of diarrhoea was based on cumulative (total) diarrhoeal volume over a 10 day period following challenge when participants were in-patients.

Severity of diarrhoea:

- Mild: < 3 L of diarrhoea
- Moderate: ≥3 L - 5 L of diarrhoea
- Severe: > 5 L of diarrhoea

The **incidence of diarrhoea of any severity** (mild, moderate, or severe) for the 10-Day and 3-Month Challenge groups is shown in Table 8 below. Protective efficacy against diarrhoea of any severity (mild or worse) was 84.5% (95% CI, 67.0%-100.0%) 10 days post-vaccination and 50.8% (95% CI, 33.6%-66.8%) 3 months post-vaccination. The lack of overlap between the CIs for protective efficacy against diarrhoea of any severity at 10 days and at 3 months post-challenge suggests that efficacy for this secondary endpoint was higher at 10 days than at 3 months. The bulk of this difference was due to the higher incidence of mild diarrhoea among the 3-Month Challenge vaccine recipients (33.3%) than among the 10-Day Challenge vaccine recipients (8.6%) (Table 7 above). Protective efficacy against severe diarrhoea was 93.3% (95% CI, 56.2%-100.0%) 10 days post-vaccination and 85.7% (95% CI, 46.2%-100.0%) 3 months post-vaccination (Table 8).

Table 8 Secondary endpoint Protective efficacy for secondary endpoints for 10-Day and 3-Month challenge groups (ITT)

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

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

Note: Mild diarrhoea was defined as the passage of 2 or more unformed stools (grades 3 to 5) over a 48-h period that equalled or exceeded 200 mL or a single unformed stool of >300 mL or <3 L total diarrhoea. Worse diarrhoea includes all cumulative diarrhoea <5L.

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

a Protective Efficacy = [(Attack Rate in Placebo Group - Attack Rate in Vaccine Group)/Attack Rate in Placebo Group] * 100.

b Confidence interval was calculated using the Farrington and Manning method for a ratio of binomial variables where the protective efficacy under the null hypothesis is 0.

Daily and overall volume of grade 3 or higher stools through 10 days post-challenge for the 10-Day and 3-Month Challenge groups were measured. Among subjects who had grade 3 or higher stools, the median (min-max) overall volume of grade 3 or higher stools was 309 (154-18,164) mL for 10-Day Challenge vaccine recipients; 603 (22 – 9,950) mL for 3-Month Challenge vaccine recipients. Both medians were

significantly less than the median of 4,524 (140 – 24,374) mL for the combined placebo group (p=0.0073 for 10-Day Challenge and p<0.0001 for 3-Month Challenge; Wilcoxon rank sum test).

The number of days that subjects had grade 3 or higher stools through 10 days post-challenge is described for the 10-Day and 3-Month Challenge groups (Table 9). 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 in vaccine recipients in the 10-Day Challenge group and 1.0 in vaccine recipients in the 3-Month Challenge group; both medians were significantly less than the median of 5.0 days with grade 3 or higher stools for the combined placebo group (p<0.0001 for 10-Day and 3-Month Challenges; Wilcoxon rank sum test).

Table 9 Number of Days with Grade 3 or Higher Stools for 10-Day and 3-Month Challenge Groups (ITT)

Grade 3+ Stools	PXVX0200 10-Day[a] N=35	PXVX0200 3-Month[a] N=33	Combined Placebo N=66
Total number of days with Grade 3+ Stools			
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	3 (9.1%)	11 (16.7%)
5	0	3 (9.1%)	12 (18.2%)
6	0	1 (3.0%)	15 (22.7%)
7	0	0	6 (9.1%)
8	1 (2.9%)	0	3 (4.5%)
9	0	0	1 (1.5%)
10	0	0	0
Mean±SD	0.6±1.50	1.8±1.82	4.7±1.99
Median (Min – Max)	0.0 (0.0 - 8.0)	1.0 (0.0 - 6.0)	5.0 (0.0 - 9.0)
Interquartile Range	0.0 - 0.0	0.0 - 3.0	3.0 - 6.0
P-value[b]	<0.0001	<0.0001	

a Number and percentage of subjects who had grade 3 or higher stools for the corresponding number of days given in the first column.

b P-value was calculated using Wilcoxon rank sum test comparing total number of days with grade 3 or higher stools between treatment groups.

The number of days with faecal shedding through 10 days post-challenge for the 10-Day and 3-Month Challenge groups including peak concentration is described Table 10. The median number of days with a positive stool culture was 0.0 for 10-Day Challenge vaccine recipients and 2.0 for 3-Month Challenge vaccine recipients; both medians were significantly less than the median of 3.0 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 significantly 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).

Table 10 Number of days and peak concentrations of faecal shedding for 10-Day and 3-Month challenge groups (ITT)

Fecal Shedding	PXVX0200 10-Day N=35[a]	PXVX0200 3-Month N=33[a]	Combined Placebo N=66[a]
Total number of days with positive stool culture			
0	18 (51.4%)	8 (24.2%)	2 (3.0%)
1	8 (22.9%)	2 (6.1%)	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	5 (7.6%)
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0
Mean±SD	0.9±1.20	2.1±1.41	3.3±1.01
Median (Min – Max)	0.0 (0.0 - 4.0)	2.0 (0.0 - 4.0)	3.0 (0.0 - 5.0)
Interquartile Range	0.0 - 2.0	1.0 - 3.0	3.0 - 4.0
P-value[b]	<0.0001	<0.0001	
Median Peak *V. cholerae* O1 concentration (CFU/g)[c]	0	135500	31500000
P-value[d]	<0.0001	<0.0001	

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

b P-value was calculated using Wilcoxon rank sum test comparing total number of days with a positive qualitative stool culture between treatment groups.

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

d P-value was calculated using Wilcoxon rank sum test comparing peak V. cholerae O1 excretion between treatment groups.

Tertiary efficacy endpoints

Pre-Challenge Immunogenicity – serum vibriocidal antibodies (SVA)

The post-vaccination, pre-challenge immune response was a tertiary endpoint in this efficacy study. It was assessed by serum vibriocidal antibody (SVA) geometric mean titre (GMT) over time (Table 11), the percentage of subjects with a 4-fold rise in serum vibriocidal antibody from Baseline (Table 12) against homologous classical Inaba. Immune response was also assessed against three heterologous V. cholerae serotypes and biotypes: El Tor Inaba, classical Ogawa, and El Tor Ogawa (Table 13, Table 14).

Table 11 Serum Vibriocidal geometric mean titre prior to challenge, Classical Inaba V. cholerae, (Immunogenicity evaluable population)

Study Day	PXVX0200 N=94	Placebo N=102	P-value[a]
Day 1			
N	94	102	
Geometric Mean	46.0	63.1	0.0924
Median (Min – Max)	20.0 (20 - 2560)	40.0 (20 - 5120)	
95% CI	[36.5, 58.1]	[47.5, 83.7]	
Day 8			
N	93	100	
Geometric Mean	830.8	65.4	<0.0001
Median (Min – Max)	1280.0 (20 - 40960)	20.0 (20 - 5120)	
95% CI	[554.5, 1244.6]	[48.3, 88.6]	
Day 11			
N	93	99	
Geometric Mean	4313.4	64.8	<0.0001
Median (Min – Max)	5120.0 (20 - 81920)	20.0 (20 - 5120)	
95% CI	[2873.2, 6475.6]	[47.8, 87.9]	
Day 29[b]			
N	57	68	
Geometric Mean	1393.7	50.6	<0.0001
Median (Min – Max)	2560.0 (20 - 20480)	20.0 (20 - 2560)	
95% CI	[866.4, 2242.0]	[35.9, 71.2]	
Day 91[b]			
N	33	33	
Geometric Mean	270.5	48.3	<0.0001
Median (Min – Max)	320.0 (20-2560)	20.0 (20-2560)	
95% CI	[158.3, 462.2]	[29.8, 78.5]	
Day 181[c]			
N	24	33	
Geometric Mean	155.4	62.2	0.0299
Median (Min – Max)	160.0 (20 - 2560)	20.0 (20 - 2560)	
95% CI	[82.2, 293.9]	[35.9, 107.7]	

a P-value was calculated using a t-test comparing log results from vaccine recipients to placebo recipients.
b Day 29 and Day 91 results from subjects challenged at 10 days were not reported in this table.
c Day 181 results from challenged subjects were not reported in this table.

Seroconversion, pre-defined in the protocol as a 4-fold rise over time in SVA against homologous classical Inaba, is shown in Table 12. The cumulative percentage of vaccine recipients with seroconversion against homologous classical Inaba was 79.8% by Day 8, 89.4% by Day 11, 90.4% by Days 29, 91, and 181. Seroconversion among placebo recipients remained at 2.0% ($p<0.0001$) at all post-vaccination time points.

Table 12 Seroconversion at all post-vaccination time points

Study Day	PXVX0200 N=94	Placebo N=102	P-value[a]
Day 8	75(79.8%)	2 (2.0%)	<0.0001
Day 11	84 (89.4%)	2 (2.0%)	<0.0001
Day 29[b]	85 (90.4%)	2 (2.0%)	<0.0001
Day 91[b]	85 (90.4%)	2 (2.0%)	<0.0001
Day 181[c]	85 (90.4%)	2 (2.0%)	<0.0001

Note: Statistics describe the cumulative number and percentage of subjects who had at least a 4-fold rise in titer over the titer measured by Day 1.
a P-value was calculated using Fisher's exact test comparing number of vaccine recipients with a 4-fold rise with placebo recipients.
b Day 29 and Day 91 results from subjects challenged at 10 days were not reported in this table.
c Day 181 results from challenged subjects were not reported in this table.

The serum vibriocidal antibody GMT responses were found to peak at Day 11 (Table 11). Cross reactivity to other *V. cholerae* serotypes and biotypes was therefore compared at this time point, regarding GM SVA titre (Table 13) and 4-fold change over baseline (Table 14).

Table 13 Peak (Day 11) Serum Vibriocidal Antibody Geometric Mean Titre, All V. cholerae Biotypes and Serotypes. (Immunogenicity Evaluable Population)

Cholera Strain	PXVX0200 N=94	Placebo N=102	P-value[a]
Classical Inaba (95% CI)	4313.4 (2873.2-6475.6)	64.8 (47.8-87.9)	<0.0001
El Tor Inaba (95% CI)	6898.4 (4370.1-10889.3)	63.1 (44.6-89.1)	<0.0001
Classical Ogawa (95% CI)	2323.6 (1519.2-3553.9)	94.0 (67.4-131.1)	<0.0001
El Tor Ogawa (95% CI)	2238.6 (1492.3-3358.2)	71.5 (50.6-101.1)	<0.0001

a P-value was calculated using a t-test comparing log results from vaccine recipients to placebo recipients

Table 14 Day 11 Serum vibriocidal antibody 4-Fold Rise, All V. cholerae Biotypes and Serotypes (Immunogenicity Evaluable Population)

Cholera Strain	PXVX0200 N=94	Placebo N=102	P-value[a]
Classical Inaba	89.4%	2.0%	<0.0001
El Tor Inaba	90.4%	3.9%	<0.0001
Classical Ogawa	86.2%	2.9%	<0.0001
El Tor Ogawa	88.3%	4.9%	<0.0001

Note: Statistics describe the cumulative number and percentage of subjects who had at least a 4-fold rise in titer over the titer measured by Day 1.
a P-value was calculated using Fisher's exact test comparing number of vaccine recipients with a 4-fold rise with placebo recipients.

Post-Challenge Immunogenicity- serum vibriocidal antibodies (SVA)

The post-challenge immune response is presented for the 10-Day Challenge group and the 3-Month Challenge group separately. It was assessed by serum vibriocidal antibody GMT and the percentage of subjects with a 4-fold rise in serum vibriocidal antibody against homologous classical Inaba 28 days after

challenge as well as by Day 181. Immune response was also assessed against heterologous *V. cholerae* serotypes and biotypes including El Tor Inaba, classical Ogawa, and El Tor Ogawa on Day 181.Overall, the serum vibriocidal antibody responses post-challenge were higher in placebo recipients compared to in vaccine recipients. This is in contrast to the immune response after vaccination, which were higher in vaccine recipients than placebo recipients at all time points.

Post-challenge vibriocidal antibody GMT against classical Inaba was 2460.6 at 28 days after challenge and 295.6 at Day 181 in the 10-Day Challenge vaccine recipients. In the 3- Month Challenge recipients, vibriocidal antibody GMT against classical Inaba was 1646.9 at 28 days after challenge and 223.9 at Day 181. In the combined placebo group vibriocidal antibody GMT against classical Inaba was 17409.1 at 28 days after challenge and 2031.9 at Day 181 ($p<0.0001$ for both time points in both the 10-Day and the 3-Month Challenge groups).

Post-challenge the percentage of subjects with a 4-fold rise in serum vibriocidal antibody against classical Inaba by 28 days was 5.7% in the 10-Day Challenge vaccine recipients, 60.6% in the 3-Month Challenge vaccine recipients, and 97% in the combined placebo group ($p<0.0001$ for both time points in both the 10-Day and the 3-Month Challenge groups; t-test comparing log results from vaccine recipients with placebo recipients).

Post-Challenge serum vibriocidal antibody responses against other serotypes and biotypes (Day 181)

The main findings on post-challenge on the geometric mean titres against other biotypes and serotypes are summarised in the Table 15 and Table 16 below.

Table 15 Serum Vibriocidal Antibody geometric mean titre at day 181 following challenge against other biotypes and serotypes, Day 10 challenge group

Biotype	PXVX0200 (n=35)	Combined placebo (n=66)	p-value[a]
El Tor, Inaba	414 (n=35)	3190.1 (n=63)	<0.0001
Classical, Ogawa	346.4 (n=35)	1428.9 (n=63)	<0.0001
El Tor, Ogawa	247.4 (n=35)	1294.2 (n=63)	<0.0001

a P-value is from a t-test comparing log results from PXVX0200 vaccinees to placebo recipients.

Table 16 Serum Vibriocidal Antibody geometric mean titre at day 181 following challenge against other biotypes and serotypes, 3-Month challenge group

Biotype	PXVX0200 (n=33)	Combined placebo (n=66)	p-value[a]
El Tor, Inaba	541 (n=33)	3190.1 (n=63)	<0.0001
Classical, Ogawa	259.4 (n=33)	1428.9 (n=63)	<0.0001
El Tor, Ogawa	288.1 (n=33)	1294.2 (n=63)	<0.0001

a P-value is from a t-test comparing log results from PXVX0200 vaccinees to placebo recipients.

The main findings on post-challenge on the 4-fold increases in SVA against other biotypes and serotypes are summarised in Table 17 for the Day 10 challenge group and Table 18 for the 3-month challenge group.

Table 17 Post-challenge (Day 181) percentage of subjects with 4-fold rise in serum vibriocidal antibodies, Day 10 challenge group

Biotype	PXVX0200	Combined placebo	p-value[a]

	(n=35)	(n=66)	
El Tor Inaba	2 (5.7%)	59 (89.4%)	<0.0001
Classical, Ogawa	2 (5.7%)	52 (78.8%)	<0.0001
El Tor, Ogawa	3 (8.6%)	52 (78.8%)	<0.0001

a: P-value is from Fisher's exact test comparing number of PXVX0200 vaccinees with a 4-fold increase to placebo recipients.

Table 18 Post-challenge (Day 181) percentage of subjects with 4-fold rise in serum vibriocidal antibodies, 3-month challenge group

Biotype	PXVX0200 (n=33)	Combined placebo (n=66)	p-value[a]
El Tor Inaba	6 (18.2%)	59 (89.4%)	<0.0001
Classical, Ogawa	6 (18.2%)	52 (78.8%)	<0.0001
El Tor, Ogawa	6 (18.2%)	52 (78.8%)	<0.0001

a: P-value is from Fisher's exact test comparing number of PXVX0200 vaccinees with a 4-fold increase to placebo recipients.

Pre-challenge Anti-Cholera toxin CT antibody responses

The post-vaccination, pre-challenge anti-cholera toxin (CT) immune responses were assessed. For these analyses the vaccine and placebo groups respectively were combined. Seroconversion was defined as a 4-fold increase in antibody titre from baseline.

On Days 8, 11, 29, 91, and 181, vaccine recipients had a higher immune response than placebo recipients with respect to anti-CT GMT and in the percentage of subjects who seroconverted.

Geometric mean values peaked at 1609.8 at Day 29, rather than Day 11 as seen with vibriocidal immune responses. The cumulative percentage of subjects who seroconverted reached a maximum of 38.3% by Day 181 for anti-CT antibody.

Post-challenge Anti-Cholera toxin CT antibody responses

Post-challenge anti-CT responses were assessed on Day 181. Overall, the immune response post-challenge was higher in placebo recipients than in vaccine recipients. This is in contrast to the immune response after vaccination, which was higher in vaccine recipients than placebo recipients at all time points as described.

Post-challenge anti-CT antibody GMT was 1236.8 at Day 181 in the 10-Day Challenge vaccine recipients 6072.7 at Day 181 in the 3-Month Challenge vaccine recipients, and 21000.7 at Day 181 in combined placebo group ($p<0.0001$ for the 10-Day Challenge group and $p=0.0004$ for the 3-Month Challenge group. Post-challenge the percentage of subjects with a 4-fold rise in serum anti-CT antibody by Day 181 was 28.6% after challenge in the 10-Day Challenge vaccine recipients, 57.6% in the 3-Month Challenge vaccine recipients and 84.8% in combined placebo group ($p<0.0001$ for the 10-Day Challenge group and $p=0.0054$ for the 3-Month Challenge group.

Exploratory Outcomes

Serum vibriocidal antibody as an immune correlate of protection

The exploratory outcomes were to a) correlate serum vibriocidal antibodies (SVA) and anti-cholera toxin (CT) antibodies with protective effect, b) Explore the association between age and immunologic response, and evaluate whether the relationship between immunologic response and outcome varies with age, c) Explore

the impact of blood type (group O vs. non-O) on the incidence and severity of diarrhoea and d) explore the relationship between pre-challenge memory B cell concentration and the incidence or severity of diarrhoea.

A comparison of efficacy and immunogenicity results from the 10-day and 3-month challenge groups is presented in Table 19.

Table 19 Comparison of efficacy endpoints and immunogenicity across the 10-Day and 3-Month challenges

Endpoint	PXVX0200 10-Day N=35	PXVX0200 3-Month N=33	Combined Placebo Group N=66
Number of Subjects (%) with Moderate/Severe Diarrhoea	2 (5.7%)	4 (12.1%)	39 (59. 1%)
Protective Efficacy [95% CI] against Moderate/Severe Diarrhoea	90.3% [62.7%, 100.0%]	79.5% [49.9%, 100.0%]	—
Number of Subjects (%) with Mild or Worse Diarrhoea	5 (14.3%)	15 (45.5%)	61 (92.4%)
Protective Efficacy [95% CI] against Mild or Worse Diarrhoea	84.5% [67.0%, 100.0%]	50.8% [33.6%, 66.8%]	—
Number of Subjects with Grade 3+ Stools (diarrhoea)	8 (22.8%)	23 (69.7%)	63 (95.5%)
Median (Min, Max) Volume (mL) of Grade 3+ Stools per Subject[a]	309 (154, 18164)	603 (22, 9950)	4524 (140, 24374)
Median (Min, Max) Number of Grade 3+ Stools per Subject[a]	4 (1, 55)	4 (1, 49)	24 (2, 79)
Mean (SD) Number of Days with Positive Stool Culture per Subject[b]	0.9 (1.20)	2.1 (1.41)	3.3 (1.01)
Median Peak *V. cholerae* O1 concentration (CFU/g)[c]	0	$1.36x10^5$	$3.15x10^7$
Day 11 Immunogenicity Pre-challenge			
N Analysable	35	33	66
GMT [95% CI]	5999 [3169, 11355]	3294 [1695, 6399]	66 [46, 96]

Seroconverted Through Day 11 [95% CI]	94% [81%, 99%]	88% [72%, 97%]	2% [0%, 8%]

— indicates not applicable.
a Median value for those subjects in the corresponding treatment group who had Grade 3+ stools
b Mean number of days for all subjects in the corresponding treatment group.
c Peak excretion concentration (ie, the maximum quantitative result over the 10 days post-challenge) was calculated for each subject. Statistic shown is the median of those individual peak concentrations.

Serum vibriocidal immune response is considered as an immune marker that correlates with protection in orally administered cholera vaccines (Holmgren 2010, Levine 1981). Since the serum vibriocidal immune response is an IgM response that peaks 7-14 days after vaccination, attempting to use serum vibriocidal titer measured at Day 11 alone as an immune marker introduces variability and is not likely to be reliable after peak response, owing to the decline in IgM levels as isotype switching occurs and the antibodies induced become IgG and IgA. A more reliable measurement of the transient IgM response is therefore vibriocidal seroconversion as defined by a 4-fold increase from baseline, since it accounts for individual variability in antibody titer levels.

The rate of seroconversion is shown in **Table 20**.

Table 20 Rates of seroconversion at Day 11 by treatment group

	Randomized	Seroconverted at Day 11	Proportion of Seroconverters	95% CI on Proportion
Vaccine - Day 11 Challenge	35	33	94%	[81% , 99%]
Vaccine - Day 91 Challenge	33	29	88%	[72% , 97%]
Placebo - Both Day 11 and Day 91 Challenges	66	1	2%	[0%, 8%]

From **Table 20** it is evident that 2 two subjects in the Day 11 challenge group did not seroconvert, and four subjects in the 3-Month challenge group did not seroconvert. In total 6/68 vaccinees did not seroconvert (8.8%). One placebo subject seroconverted at Day 11.

Table 21 shows the rates of seroconversion at Day 11 with respect to incidence of moderate/severe diarrhoea (>5L cumulative stool output). One of the subjects that did not seroconvert in the Day 11 challenge group experienced moderate/severe diarrhoea, whereas three of the four non-seroconverters in the Day 91 challenge group experienced moderate/severe diarrhoea.

Table 21 Rates of seroconversion at Day 11 vs. incidence of diarrhoea

	N	Mod/Sev Diarrhoea in Seroconverters	Mod/Sev Diarrhoea in Non-Seroconverters	Rate of Protection among Seroconverters	95% CI on Rate of Protection
Vaccine - Day 11 Challenge	35	1/33 (3%)	1/2 (50%)	97%	[84% , 100%]

Vaccine - Day 91 Challenge	33	1/29 (3%)	3/4 (75%)	97%	[82% , 100%]

For the 68 vaccine recipients in the 10-Day and 3-Month challenge studies, total post-challenge diarrhoeal 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) (Figure 4).

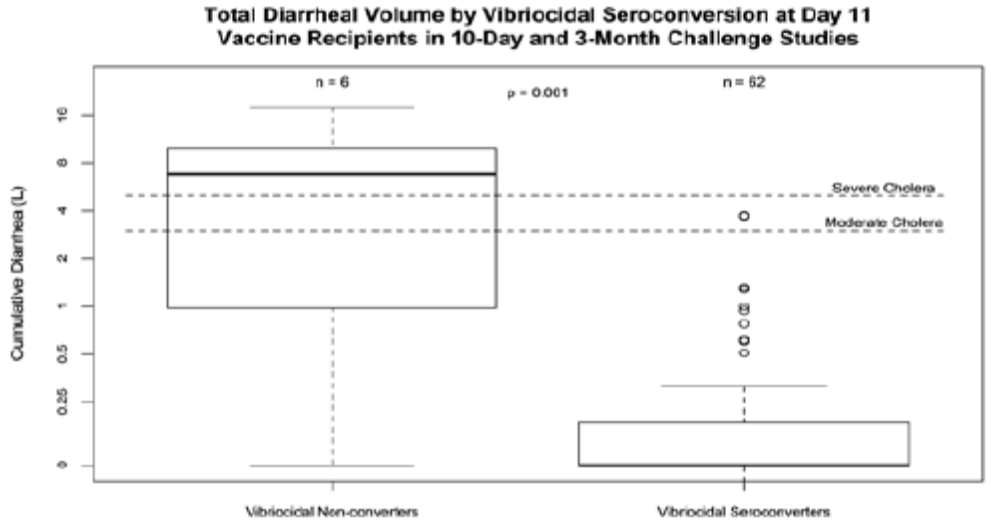


Figure 2 Total Diarrhoeal Volume by Vibriocidal Seroconversion on Day 11 – Vaccine Recipients in the 10-Day and 3-Month Challenge Studies

Diarrhoea severity was assessed in all subjects (both vaccinated and placebo) that were challenged at Day 11 with respect to vibriocidal titre and fold increase in titre from baseline. The fold increase in SVA for all participants is shown in Figure 5, where those with mild diarrhoea correspond mainly to the 3-month challenge group. One of the two vaccinees that did not seroconvert and did not experience diarrhoea. One placebo subject seroconverted but did not experience diarrhoea.

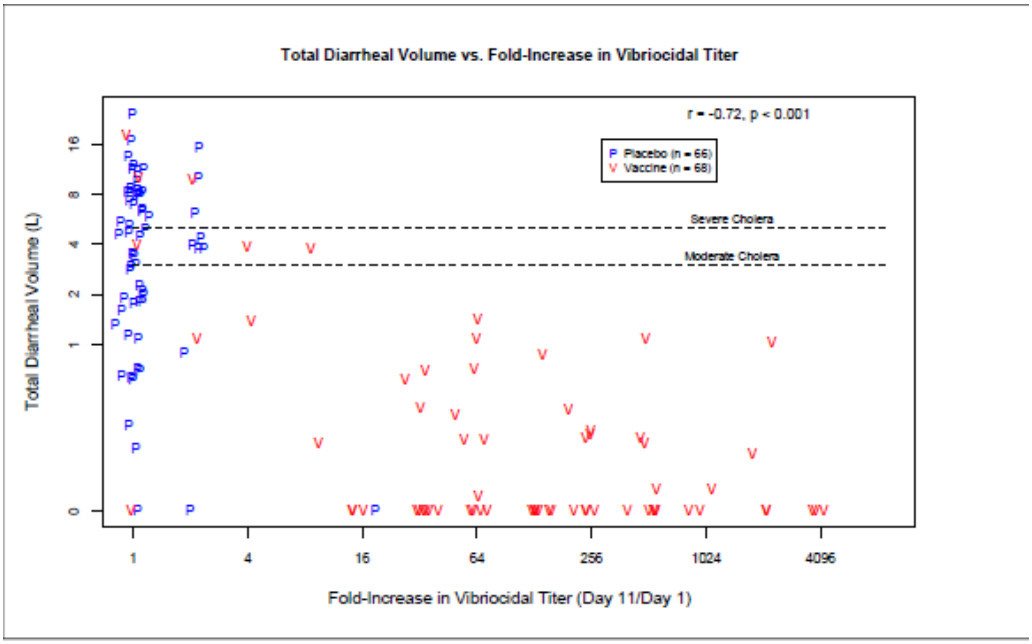


Figure 3 Vibriocidal Antibody Seroconversion correlates with Protection

Consistent with the case for post-vaccination vibriocidal titre at Day 11, there was a statistically significant association between fold-rise in serum vibriocidal antibody titre from Day 1 to Day 11 and total post-challenge diarrhoeal volume (Spearman's r=-0.72; p<0.001). Seroconversion, defined as a ≥4-fold rise in vibriocidal titre 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 near 1:1 relationship between seroconversion and protection, along with the fact that approximately 90% of vaccine recipients seroconverted, serum vibriocidal seroconversion at Day 11 was used as the best immunologic measure for establishing a bridge between populations.

Anti-CT antibody as an immune correlate of protection

The relationships between anti-CT antibody titres and diarrhoea-derived outcomes were also assessed. Since anti-CT antibody rises mores slowly and persists longer than vibriocidal antibody, serum anti-CT antibody titres 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 titres 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 enable measurements of association with diarrhoeal outcomes post-challenge. As with vibriocidal titre, the primary response variable for analyses of anti-CT antibody titre was moderate/severe cholera.

Anti-CT antibody titre was not strongly associated with outcome. Overall, anti-CT antibody titre increased post-vaccination in only a subset. Only slightly less than half, 16 of 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 diarrhoeal 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) (Figure 6).

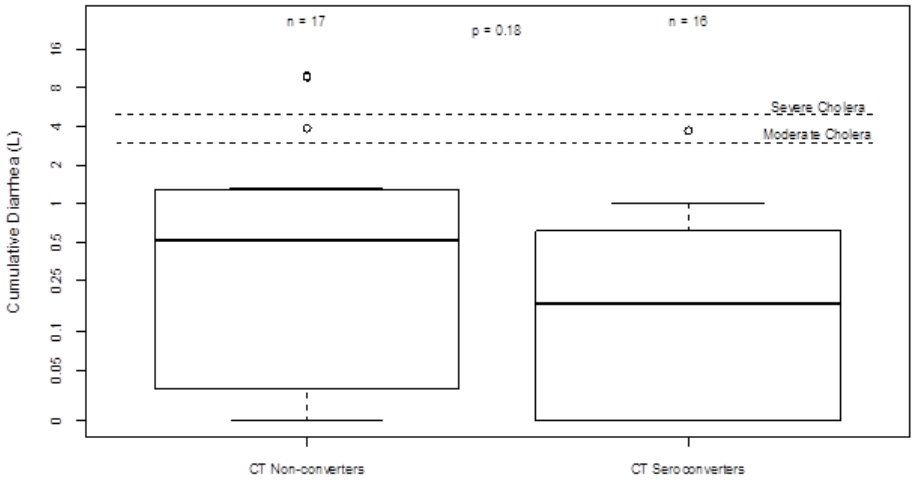


Figure 4 Total Diarrhoeal Volume by CT Seroconversion on Day 91 – Vaccine Recipients in the 3-Month Challenge Study.

Antigen-specific Memory B Cell Response as an Immune Correlate of Duration of Protection
The results of the challenge study demonstrated that following immunization with PXVX0200, serum vibriocidal response (particularly seroconversion at Day 11) was the best immune marker for protection both for volunteers challenged at 10 and at 90 days after immunization. Serum vibriocidal levels for the 33 vaccine recipients in the 90-day component of the study, however, declined over 90% from the peak geometric mean titre (GMT) of 3294 on Day 11 to a GMT of 271 on Day 91 and presumably continue to decline further with time. Memory B cell levels reflect a more durable immune response to vaccination and are considered to be potential predictors of duration of protection.

There was no statistically significant correlation between the percentages of anti-O1 LPS IgG, anti-CT IgG, and anti-CT IgA memory B cells at Day 91 and total stool volume post-challenge. However, the primary finding from the evaluation of memory B cells as an immune correlate of duration of protection was that there was a statistically significant association between the post-vaccination/pre-challenge percentage of anti-LPS IgA memory B cells at Day 91 and total post-challenge diarrhoeal volume in vaccine recipients (Spearman correlation=-0.39,p=0.02, n=33). The fold-increase from Day 1 to Day 91 in the percentage of anti-LPS IgA memory B cells was even more strongly associated with total diarrhoeal volume (Spearman correlation=-0.56, p<0.001, n=33).

Ancillary analyses

Further analyses on immunology evaluable patients from the challenge study (n=46) were included as a publication by Islam et al. 2018. This study evaluated the immunoglobulin subclass of antibodies to O-linked polysaccharides (OSP) using an ELISA assay. Seroconversion for anti-OSP antibody responses was defined as $\geq$1.5-fold rise in ELISA units over baseline.

Both titre and fold increase in anti-OSP antibodies were determined. Serum IgM OSP responses were found to be correlated with vibriocidal responses (Spearman r = 0.67; p < 0.0001). Serum IgA OSP responses correlated less well with vibriocidal responses (Spearman r = 0.32; p= 0.03), and there was no correlation of IgG OSP responses and vibriocidal responses. Serum anti-OSP antibody responses fell back toward baseline within 90 days of vaccination, although possible persistence of antibody responses in mucosal tissue was not assayed in this study. Whether CVD 103-HgR induces long-lived plasma cell responses or memory B cell

responses targeting V. cholerae OSP is currently unknown, as is the duration of protection afforded by vaccination.

Immunogenicity and bridging studies

Studies PXVX-VC-200-004, PXVX-VC-200-005, PXVX-VC-200-006 were considered pivotal and assessed immunogenicity only. A phase I clinical study PXVX-VC-200-200 also determined immunogenicity. Each clinical trial used a single oral dose of Vaxchora, and all studies were randomized, double-blind, and placebo-controlled. Immunogenicity data from the Lot consistency study PXVX-VC-200-004 used in adults 18-45 years was used to bridge to older adults up until the age of 64 years (PXVX-VC-200-005). Data from the Lot consistency study was also used to bridge immunogenicity data in children ≧6 to ≦18 years of age (PXVX-VC-200-006).

PXVX-VC-002-002 Phase I clinical study – summary

The Phase 1 trial was designed to evaluate the safety and immunogenicity of a single oral dose of PXVX0200 in healthy volunteers aged 18 to 50 years. The study enrolled 66 participants (vaccine n=55; placebo n=11). The dose of vaccine used in this trial was 4.43×10^8 colony forming units (CFU) formulated as a lyophilized powder in vials. Lactose powder was used as the placebo rather than physiological saline which was used in all subsequent studies.

The primary objectives of the Phase 1 trial were to evaluate the safety and clinical acceptability of PXVX0200, estimate the rate of seroconversion (≥4-fold rise) of serum Inaba vibriocidal antibody after a single oral dose, and estimate the between-subject variability of vibriocidal antibody response. Secondary objectives extended the safety evaluation to household contacts of vaccine and placebo recipients, evaluated the kinetics of serum Inaba vibriocidal antibody titres, estimated serum anti-cholera toxin (CT) immunoglobulin gamma (IgG) antibody seroconversion, and assessed faecal shedding of vaccine.

Administration of a single oral dose of $4.43x10^8$ CFU generated cumulative serum vibriocidal antibody seroconversion (cumulative seroconversion by a particular day indicates seroconversion on that day or on any previous day) in 88.9% [77.4, 95.8] of vaccine recipients by Day 15 (reported as Day 14 in the Phase 1 CSR, as the CDISC standard for designating the day of vaccination as Day 1 was not implemented for that report) and cumulative anti-CT seroconversion in 59.3% [45.0, 72.4] of vaccine recipients by Day 29 (reported as Day 28 in the Phase 1 CSR). The post-baseline time point with the greatest serum vibriocidal GMT was Day 11 (reported as Day 10 in the CSR), i.e., at 10 days post-vaccination. This Day 11 GMT was equal to 3025 [1720, 5320], titre levels were maintained through 14 days after vaccination, and these levels subsequently declined as expected for an IgM response.

PXVX-VC-200-004 – Phase 3 Clinical Lot Consistency Trial - Summary

The primary immunologic objective of this Phase 3 trial was to demonstrate the immunologic equivalence of three different production lots of PXVX0200 at Day 11 post-vaccination in healthy volunteers 18-45 years of age. The study enrolled 3146 participants (vaccine n=2795; placebo n=351). The three lots of vaccine were designated as Lot A (P700.550-1CA03), Lot B (P700.550-3CA03), and Lot C (P700.550-6BA03). Secondary immunogenicity objectives were to estimate the seroconversion rate of serum vibriocidal antibody by Day 11 as well as the antibody response profile for 6 months post-vaccination. The concentration of vaccine used in this trial was $1x10^9$ CFU/dose. Placebo was physiological saline. Both vaccine and placebo were administered orally.

The protocol's primary objective was met with the 95% confidence internal (CI) around each pairwise geometric mean ratio (GMR) falling within the pre-defined limits to demonstrate equivalence [0.67, 1.5].

GMRs were [0.78, 1.08] comparing lots A:B, [0.87, 1.20] comparing lots B:C, and [0.80, 1.10] comparing lots A:C. Day 11 GMTs of serum vibriocidal antibodies against homologous classical Inaba were 9220 [8219, 10343], 10034 [8942, 11260], and 9827 [8770, 11012], respectively. Serum vibriocidal antibody GMT against homologous classical Inaba on Day 1 prior to vaccination was 69 in vaccine recipients and 72 in placebo recipients. On Day 11, 10 days after vaccination, serum vibriocidal GMT increased to 9688 [9067, 10351] in vaccine recipients from all three lots combined and was relatively unchanged at 85 [72, 101] in placebo recipients. Administration of a single oral dose of PXVX0200 generated serum vibriocidal antibody seroconversion at Day 11 in 94% [93%, 94%] of vaccine recipients across the three lots of vaccine (n=2687, Immunogenicity Evaluable Population).

The results of the Phase 3 lot consistency trial met pre-specified immunologic equivalence criteria and demonstrated that the vaccine is well tolerated.

PXVX-VC-200-005 – Phase 3 Safety and Immunogenicity in Older Adults - Summary

This trial was designed to evaluate the safety of a single oral dose of the PXVX0200 vaccine in older adults (46–64) and to collect immunogenicity data to enable comparisons with the antibody responses of younger adults (18-45) from the Phase 3 Lot consistency trial (CSR PXVX-VC-200-004). Vaccinated younger adults were shown to be protected from moderate or severe cholera in the challenge trial; protection is presumed for the vaccinated subjects in the lot consistency trial since subjects in that trial are in the same age range. The lot consistency trial was chosen as the comparator group for the older adults simply because the large sample size of the lot consistency trial afforded high power to establish an immunologic bridge between older and younger adults. The concentration of vaccine used in this trial in older adults was $1x10^9$ CFU/dose, and the vaccine was derived from Lot No. P700.550-6BA03 in the Lot consistency trial. The placebo was physiological saline.

The primary bridging objectives of this trial were to demonstrate that seroconversion by classical Inaba vibriocidal antibody titres at Day 11 in older adults ages 46–64 was non-inferior to the analogous rate at Day 11 in younger adults ages 18–45 following vaccination with PXVX0200 and that the lower bound of the two-sided 95% CI on seroconversion was greater than 70% in older adults. This primary bridging endpoint was based on the analyses on immune markers correlating with protection that were derived using the data from the live cholera challenge trial. Secondary bridging objectives included comparing older and younger adults on classical Inaba vibriocidal GMT following vaccination with PXVX0200.

The primary bridging objectives were met. Following vaccination, 90.4% [86.4%, 93.5%] of older subjects and 93.5% [92.5%, 94.4%] of younger subjects seroconverted by classical Inaba vibriocidal antibody titers. Thus, it was established with 95% confidence that the proportion of seroconverters in the older adult population was greater than or equal to 86.4%, well above the pre-specified threshold of 70%. Furthermore, the lower bound of the two-sided 95% CI on the difference in the rate of seroconversion between older and younger adults was −6.7%, which satisfied the pre-specified non-inferiority requirement that the lower bound of the 95% CI should be greater than or equal to −10%.

For the secondary bridging endpoint of classical Inaba vibriocidal GMT, the peak GMT value of 4282 [3344, 5484] in older adults was significantly lower than the peak GMT of 9688 [9067, 10351] in younger adults ($p<0.0001$). The mean log2 fold-increase in classical Inaba vibriocidal titre between Days 1 and 11 was 6.6 [6.2, 7.0] in older adults, which was significantly lower than the mean log2 fold-increase of 7.1 [7.0, 7.3] attained by younger adults. Since the titres produced by the vibriocidal antibody assay lie on a scale for which each pair of levels differs by a factor of 2, a log2 fold-increase of 7 from Day 1 to Day 11 corresponds to an increase of 27 = 128, e.g., an increase from 40 to 5120 (5120/40 = 128). The results of this Phase 3

older adult trial met the pre-specified immunologic non-inferiority criteria. Based on the immune markers analyses and the non-inferiority in the rate of seroconversion, it is expected that efficacy in older adults would be similar to efficacy in younger adults.

PXVX-VC-200-006 – Phase 4 Study of Safety and Immunogenicity in children ≥6 and ≤18 years of age

This was part of a larger randomised, phase 4, multicentre, placebo-controlled, double-blind clinical study assessing safety and immunogenicity of PXVX0200 in children ≥2 years to ≤18 years. An interim report is provided with data from children ≥6 to ≤18 years of age.

The study had two primary immunogenicity objectives. The first objective was to demonstrate that the seroconversion rate at Day 11 in pediatric subjects was non-inferior to the seroconversion rate at Day 11 in previously studied adult subjects between the ages of 18 and 45 years (Lot study PXVX-VC-200-004). The second primary immunogenicity objective was to demonstrate that the seroconversion rate in pediatric subjects was greater than or equal to 70% with 98.3% confidence. Both objectives used the proportion of participants achieving seroconversion of serum vibriocidal antibody (SVA) against the classical Inaba biotype of *V. cholerae* at Day 11 following one dose of PXVX0200, defined as a 4-fold or greater rise over baseline Day 1 SVA titre. The secondary immunogenicity objective was to evaluate Seroconversion of SVA against the classical Inaba biotype of V. cholerae at Days 29, 91, and 181 (Days 91 and 181 in Cohort 1 only) following one dose of PXVX0200. This involved a single administration with a dose within the range $4 \times 10^8 - 1 \times 10^9$ based on the SmPC. The batch of PXVX0200 used in this study has not been part of the other clinical studies. The results are shown separately under 'Bridging analysis between studies in adults and children >6 -<18 years of age' described after the bridging analysis between studies in adults and older adults below.

Bridging analysis between studies in adults and older adults

Two bridging analyses were performed, one compared subjects from the older adult trial to the younger adults in the lot consistency trial (PXVX-VC-200-004), and the second compared subjects from the older adult trial to the younger adults in the challenge trial (PXVX-VC-200-003). These findings are derived from a report that does not include analyses in children (PXVX-VC-200-006).

The participant populations are shown in Table 22**.**

Table 22 Subject populations and groups

Population	PXVX200					Placebo				
	Phase 1	Challenge	Lot	Older	Total	Phase 1	Challenge	Lot	Older	Total
Challenge[a]	—	68	—	—	—	—	66	—	—	—
Immunogenicity Evaluable[b]	54	94	2688	291	3127	11	102	334	99	546
Immune Sub-study[c]	—	58	26	36	120	—	69	6	9	84

— indicates not applicable.
a The Challenge Population was defined as all subjects who were randomized and received either vaccine or placebo in the challenge trial, and were in either the 10-Day Challenge group or the 3-Month Challenge group.
b Eligibility for inclusion in the Immunogenicity Evaluable Population was determined by criteria established in individual trials that required at least valid one post-vaccination assessment of immunogenicity.
c The Immune Sub-study Population comprised a subset of subjects who had immunogenicity assessments at several time points between Days 1 and 181. The population is mostly useful for evaluating the development of the immune response over time.

The Immunogenicity Evaluable Population was the most inclusive set of subjects who have data available for immunogenicity analyses. In general, subjects needed to have only one valid post-baseline immunogenicity assessment to be included in this population. Subjects were included in the Immunogenicity Evaluable Population based on whether they satisfied the eligibility criteria for inclusion in the Immunogenicity Evaluable Population for the individual trial in which they participated. The eligibility criteria for the four individual trials were as follows:

- Phase 1 Trial: Subjects were required to have classical Inaba vibriocidal antibody assay results at both baseline and at least one post-baseline visit.
- Challenge Trial: Subjects were required to have evaluable classical Inaba vibriocidal antibody results from Day 1 and at least one post-vaccination time point prior to challenge.
- Lot Consistency Trial: Subjects were required to have evaluable classical Inaba vibriocidal antibody results from Day 11 and have no major protocol violations that affected immunogenicity.
- Older Adult Trial: Subjects were required to have evaluable classical Inaba vibriocidal antibody results from both baseline and Day 11 and have no major protocol violations that affected immunogenicity.

Immune Sub-study Populations, however, were only defined in two of the four trials included in this ISE. For those trials – the lot consistency trial and the older adult trial – vaccine recipients from the trial-specific Immune Sub-study Populations were also included in the ISE Immune Sub-study Population. For the other two trials in the ISE, eligibility for the Immune Sub-study Population was determined by the schedule of immunogenicity assessments within each trial. That is, since the Immune Sub-study Population was used to characterize long-term immune response – up to Day 181 – only those vaccine recipients who had immunogenicity assessments at either Day 91 or 181 were included in the ISE Immune Sub-study Population. Therefore, in the challenge trial 58 vaccine recipients who had immunogenicity assessments at Day 91 were included in the ISE Immune Sub-study Population. Note that the Day 91 data from challenged subjects was obtained prior to challenge administration. Since no immunogenicity assessments were made after Day 29 in the Phase 1 trial, no subjects from that trial were included in the ISE Immune Sub-study Population. This population was mostly useful for tracking the time course of the immune response to vaccination.

The demographics of participants in the immunogenicity evaluable population is shown in **Error! Reference source not found.** and for the immunogenicity sub-population is shown in Table 24**:**

Table 23 Demographics – Immunogenicity evaluable population

Baseline Characteristics	Phase 1 PXVX0200 N=54	Challenge PXVX0200 N=94	Lot PXVX0200 N=2688	Older PXVX0200 N=291	Total PXVX0200 N=3127	Total Placebo N=546
Age (years)						
Mean (SD)	30.8 (6.50)	31.4 (7.65)	30.0 (7.81)	53.8 (5.01)	32.3 (10.23)	34.1 (11.86)
Median ( )	29.0 (21, 48)	31.0 (18, 45)	29.0 (18, 46)	54.0 (46, 64)	30.0 (18, 64)	32.0 (18, 64)
Age Group						
18-31	34 (63.0%)	52 (55.3%)	1603 (59.6%)	0	1689 (54.0%)	268 (49.1%)
32-45	17 (31.5%)	42 (44.7%)	1083 (40.3%)	0	1142 (36.5%)	178 (32.6%)
46-55[a]	3 (5.6%)	0	2 (0.1%)	189 (64.9%)	194 (6.2%)	63 (11.5%)
56-64	0	0	0	102 (35.1%)	102 (3.3%)	37 (6.8%)

Sex						
Male	27 (50.0%)	67 (71.3%)	1206 (44.9%)	133 (45.7%)	1433 (45.8%)	258 (47.3%)
Female	27 (50.0%)	27 (28.7%)	1482 (55.1%)	158 (54.3%)	1694 (54.2%)	288 (52.7%)
Race						
American Indian or Alaskan Native	0	1 (1.1%)	11 (0.4%)	6 (2.1%)	18 (0.6%)	2 (0.4%)
Asian	2(3.7%)	1(1.1%)	56(2.1%)	0	59(1.9%)	7(1.3%)
Native Hawaiian or Other Pacific Islander	0	0	8(0.3%)	1(0.3%)	9(0.3%)	1(0.2%)
Black or African American	12 (22.2%)	63 (67.0%)	671 (25.0%)	65 (22.3%)	811 (25.9%)	201 (36.8%)
White	40 (74.1%)	26 (27.7%)	1855 (69.0%)	216 (74.2%)	2137 (68.3%)	324 (59.3%)
Multiracial	0	0	50 (1.9%)	2 (0.7%)	52 (1.7%)	7 (1.3%)
Other	0	3 (3.2%)	37 (1.4%)	1 (0.3%)	41 (1.3%)	4 (0.7%)
Ethnicity						
Hispanic or Latino	0	5 (5.3%)	268 (10.0%)	24 (8.2%)	297 (9.5%)	39 (7.1%)
Not Hispanic or Latino	54 (100.0%)	88 (93.6%)	2420 (90.0%)	266 (91.4%)	2828 (90.4%)	506 (92.7%)
ABO Blood Type						
Type O	20 (37.0%)	47 (50.0%)	1299 (48.3%)	109 (37.5%)	1475 (47.2%)	254 (46.5%)
Not Type O	34 (63.0%)	47 (50.0%)	1387 (51.6%)	182 (62.5%)	1650 (52.8%)	291 (53.3%)
Body Mass Index (kg/m^2)						
Mean (SD)	28.46 (6.978)	27.46 (6.855)	28.22 (6.994)	29.83 (6.738)	28.35 (6.980)	28.43 (7.201)
Median (Min, Max)	27.25 (17.0, 48.4)	26.46 (16.5, 48.6)	26.88 (15.2, 69.9)	28.82 (17.7, 54.1)	27.09 (15.2, 69.9)	26.71 (16.3, 60.2)

Note: Percentages were based on the number of subjects who had non-missing values in each trial.
a Birthdays during screening placed a total of 5 subjects in the 46 to 55 age group.

Table 24 Demographics – Immune sub-study population

Baseline Characteristics	Challenge PXVX0200 N=58	Lot PXVX0200 N=26	Older PXVX0200 N=36	Total PXVX0200 N=120	Total Placebo N=84
Age (years)					
Mean (SD)	32.0 (8.21)	34.8 (6.35)	53.1 (4.64)	38.9 (11.62)	32.9 (10.89)
Median (Min, Max)	30.5 (18, 45)	33.5 (24, 45)	52.0 (46, 63)	38.5 (18, 63)	31.0 (18, 64)
Age Group					
18-31	30 (51.7%)	8 (30.8%)	0	38 (31.7%)	43 (51.2%)
32-45	28 (48.3%)	18 (69.2%)	0	46 (38.3%)	32 (38.1%)
46-55	0	0	26 (72.2%)	26 (21.7%)	4 (4.8%)
56-64	0	0	10 (27.8%)	10 (8.3%)	5 (6.0%)
Sex					

Male	41 (70.7%)	12 (46.2%)	20 (55.6%)	73 (60.8%)	40 (47.6%)
Female	17 (29.3%)	14 (53.8%)	16 (44.4%)	47 (39.2%)	44 (52.4%)
Race					
American Indian or Alaskan Native	0	0	1 (2.8%)	1 (0.8%)	0
Asian	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	42 (72.4%)	7 (26.9%)	8 (22.2%)	57 (47.5%)	53 (63.1%)
White	15 (25.9%)	17 (65.4%)	26 (72.2%)	58 (48.3%)	31 (36.9%)
Multiracial	0	2 (7.7%)	1 (2.8%)	3 (2.5%)	0
Other	1 (1.7%)	0	0	1 (0.8%)	0
Ethnicity					
Hispanic or Latino	3 (5.2%)	2 (7.7%)	1 (2.8%)	6 (5.0%)	5 (6.0%)
Not Hispanic or Latino	55 (94.8%)	24 (92.3%)	35 (97.2%)	114 (95.0%)	78 (92.9%)
ABO Blood Type					
Type O	28 (48.3%)	12 (46.2%)	17 (47.2%)	57 (47.5%)	36 (42.9%)
Not Type O	30 (51.7%)	14 (53.8%)	19 (52.8%)	63 (52.5%)	48 (57.1%)
Body Mass Index (kg/m^2)					
Mean (SD)	27.83 (7.630)	26.61 (5.019)	30.10 (5.889)	28.25 (6.715)	28.39 (7.405)
Median (Min, Max)	26.41 (16.5, 48.6)	27.22 (17.7, 34.8)	28.90 (18.6, 47.5)	27.90 (16.5, 48.6)	26.30 (18.5, 56.6)

Note: Percentages were based on the number of subjects who had non-missing values in each trial.
a Ethnicity was missing for 1 subject in the combined placebo group.

Cumulative seroconversion of vibriocidal antibodies against classical Inaba V. cholerae through Day 11 is presented for the Immunogenicity Evaluable Populations for the combined group of placebo subjects from all four trials and for the vaccine recipients in each trial in Table 25. While serum vibriocidal antibody seroconversion occurred in 2.9% [1.7%, 4.7%] of the combined placebo group across all trials, the percentage of serum vibriocidal antibody seroconverters for the vaccine groups in the four trials ranged from 83.3% [70.7%, 92.1%] in the Phase 1 trial to 93.5% [92.5%, 94.4%] in the lot consistency trial. The variation in the widths of the CIs is mainly due to differences in sample size among trials.

Table 25 Cumulative vibriocidal antibody seroconversion against classical Inaba V. cholera through day 11 – immunogenicity evaluable population

Study Day Statistic	Phase 1 PXVX0200 N=54	Challenge PXVX0200 N=94	Lot PXVX0200 N=2688	Older PXVX0200 N=291	Total Placebo N=546

All Subjects					
Day 11					
N Analysable [a]	54	93	2687	291	544
N (%) Seroconverted Through Visit	45 (83.3%)	84 (90.3%)	2513 (93.5%)	263 (90.4%)	16 (2.9%)
95% CI on % Seroconverted [b]	[70.7%, 92.1%]	[82.4%, 95.5%]	[92.5%, 94.4%]	[86.4%, 93.5%]	[1.7%, 4.7%]

Note: A subject was considered to have seroconverted through a visit if they achieved a titre at or prior to that visit that was at least 4-fold higher than their Day 1 titre.
a N Analysable was the number of subjects with any analysable samples available between Day 1 and the indicated visit.
b 95% CIs of seroconversion rate were based on the Clopper-Pearson method.

The table above illustrates that the rate of seroconversion by classical Inaba vibriocidal antibody – the immune measure shown in the challenge trial to be a strong immune marker of protection against infection with *V. cholerae* – did not vary greatly across the three Phase 3 trials. In particular, the relatively small difference between the lot consistency and older adult trials affords evidence to support an immunologic bridge between younger and older adults.

Using the Immunogenicity Evaluable Populations from each of the four trials, GMTs of vibriocidal antibody against classical Inaba on Day 11 are depicted separately for each trial specific vaccine group and for all placebo subjects combined in Table 26. While serum vibriocidal GMT was only 71 [63, 81] on Day 11 in placebo recipients, the minimum Day 11 GMT across the vaccine groups in the four trials was 3064 [1759, 5338] in the Phase 1 trial.

Table 26 Vibriocidal geometric mean titre against Classical Inaba V. cholera at Day 1 and Day 11 – Immunogenicity Evaluable Population

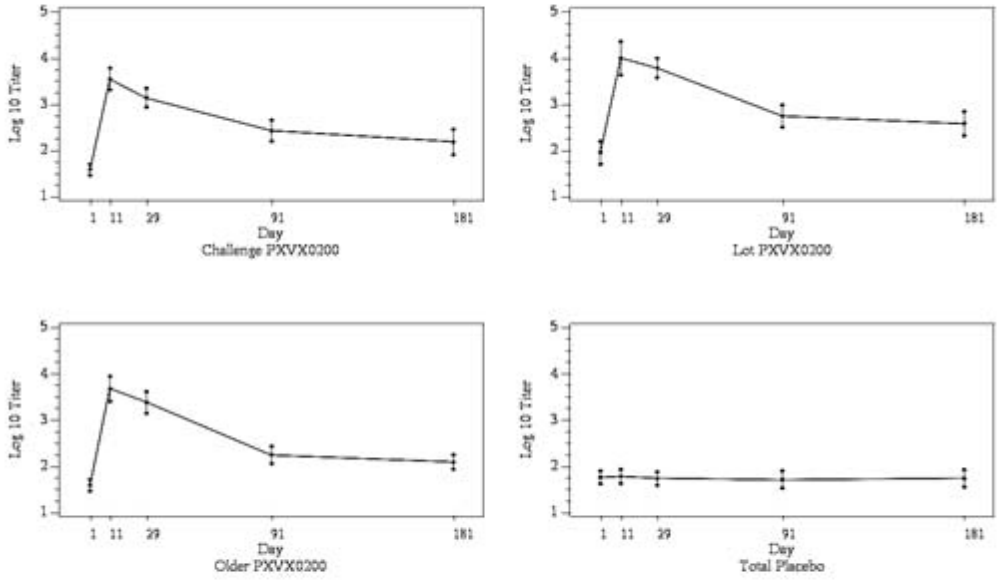
Study Day Statistic	Phase 1 PXVX0200 N=54	Challenge PXVX0200 N=94	Lot PXVX0200 N=2688	Older PXVX0200 N=291	Total Placebo N=546
Day 1					
N Analysable [a]	54	94	2687	291	546
Geometric Mean	68	46	69	44	64
95% CI on GMT	[47, 98]	[36, 58]	[65, 72]	[39, 50]	[57, 72]
Median [b]	40	20	40	20	40
95% CI on Median [b]	[20, 80]	[20, 40]	[40, 40]	[20, 40]	[40, 40]
Min, Max	20, 2560	20, 2560	20, 10240	20, 10240	20, 20480
Day 11					
N Analysable [a]	54	93	2688	291	543
Geometric Mean	3064	4313	9688	4282	71
95% CI on GMT	[1759, 5338]	[2873, 6476]	[9067, 10351]	[3344, 5484]	[63, 81]
Median [b]	5120	5120	10240	5120	40

95% CI on Median [b]	[2560, 10240]	[5120, 10240]	[10240, 10240]	[5120, 10240]	[40, 40]
Min, Max	20, 81920	20, 81920	20, 327680	20, 163840	20, 40960

Note: Values < the LLOQ were assigned the value of the LLOQ.
Note: Geometric mean and mean ratio's point estimates, together with their 95% CIs, and the p-value were based on t-statistics assuming normal distribution of the log titre.
a N Analysable was the number of subjects with an analysable sample available at the indicated visit.
b Median point estimates and their 95% CIs were distribution-free estimates.

The table above revealed that the subjects in the lot consistency trial had a higher classical Inaba GMT at Day 11 than subjects in either the challenge or older adult trial. Since the lot consistency and older adult trials were executed simultaneously, and subjects in the older adult trial were vaccinated with one of the vaccine lots used in the lot consistency trial, the most likely explanation for the difference in GMT between the trials is the age of the subjects in each trial. Age does not explain the difference between the lot consistency and challenge trials since subjects in both trials were roughly the same age. The most likely explanation for the difference between those trials is the concentration of the vaccine formulation used in each trial. Specifically, the challenge trial used a single vaccine lot with a concentration of $5x10^8$ CFU/dose while the lot consistency trial used three vaccine lots each with a concentration of $1x10^9$ CFU/dose.

Geometric mean titres of vibriocidal antibody against classical Inaba on Days 1, 11, 29, 91, and 181 are depicted for the Immune Sub-study Population of the challenge trial, lot consistency trial, and older adult trial in Figure 7. Vibriocidal antibody levels in vaccine recipients increased rapidly following vaccination, reaching a peak at Day 11 when GMTs ranged from 3555 in the challenge trial to 9922 in the lot consistency trial. While GMT levels had declined at Day 29 and Day 181, in each Phase 3 trial vaccine recipients attained a significantly higher GMT than placebo recipients at each post-vaccination time point through Day 181.



Note: For classic Inaba V. cholerae in Challenge trial, only 3-Month Challenge group is measured at Day 91, and only Non-challenge group is measured at Day 181.

Figure 5 Time course of vibriocidal geometric mean titre (95% CI) against Classical Inaba V. cholera – Immune substudy population.

Serum vibriocidal Antibody responses to other biotypes and serotypes of V. cholerae in adults and older adults

Vibriocidal antibody seroconversion through Day 11 against all three heterologous vaccine strains assessed for placebo and vaccine recipients in the Immunogenicity Evaluable Populations in the challenge and older adult trials is shown in Table 27 (fold rise) and Table 28 (titre). Interestingly, seroconversion against the Inaba serotypes was similar in younger and older adults while seroconversion against the Ogawa serotypes was lower in older adults when compared to younger adults.

Table 27 Vibriocidal 4-fold rise through Day 11, All cholera strains – Immunogenicity Evaluable Population

Cholera Strain	Challenge PXVX0200 N=94	Older PXVX0200 N=291	Total Placebo N=201
Classical Inaba [95% CI]	90.3% [82.4%, 95.5%]	90.4% [86.4%, 93.5%]	2.9% [1.7% 4.7%][a]
El Tor Inaba [95% CI]	91.4% [83.8%, 96.2%]	91.0% [87.1%, 94.1%]	4.5% [2.1%, 8.5%]
Classical Ogawa [95% CI]	87.1% [78.5%, 93.2%]	73.2% [67.7%, 78.2%]	2.5% [0.8%, 5.8%]
El Tor Ogawa [95% CI]	89.2% [81.1%, 94.7%]	71.4% [65.8%, 76.5%]	5.6% [2.8%, 9.7%]

Note: Statistics describe the cumulative number and percentage of subjects who had at least a 4-fold rise in titer over the titer measured by Day 1.
a The classical Inaba seroconversion rate and CI were calculated from 544 placebo subjects with analyzable results pooled across all four trials in the ISE.

Table 28 Vibriocidal Geometric Mean Titre on Day 11, All Cholera Strains – Immunogenicity Evaluable Population

Cholera Strain	Challenge PXVX0200 N=94	Older PXVX0200 N=291	Total Placebo N=201
Classical Inaba [95% CI]	4313 [2873, 6476]	4282 [3344, 5484]	71 [63, 81][a]
El Tor Inaba [95% CI]	6898 [4370, 10889]	4929 [3912, 6209]	59 [46, 74]
Classical Ogawa [95% CI]	2324 [1519, 3554]	1235 [944, 1617]	71 [56, 89]
El Tor Ogawa [95% CI]	2239 [1492, 3358]	1120 [863, 1453]	60 [48, 76]

a The classical Inaba seroconversion rate and CI were calculated from 544 placebo subjects with analyzable results pooled across all four trials in the ISE.

Classical Inaba Serum Vibriocidal Antibody Seroconversion and Age

Immune response is known to decline with age. The effect of age on the immune response to the vaccine was assessed primarily through analysis of titres of vibriocidal antibodies against classical Inaba V. cholerae.

Since the older adult trial was designed specifically to enrol subjects from an age range that did not overlap with the age range for the challenge and lot consistency trials, the classical Inaba modelling results shown in Table 32 and Table 33 do not cleanly separate the effect of age on immune response from effects due to other differences between the trials. However, despite the confounding between the trial-to-trial and age

covariates, the summaries in these two tables clearly suggest that both classical Inaba vibriocidal titres and the proportion of classical Inaba seroconverters drop with age.

Post-hoc analyses were performed to develop a deeper understanding of the effect of age on immune response. These supporting analyses used only the data from the lot consistency and older adult trials because these two trials utilized the same concentration ($1x10^9$ CFU/dose) of vaccine, whereas the lot used in the challenge trial had a different concentration ($5x10^8$ CFU/dose). Since the older adult and challenge trials differ in age range and vaccine release concentration (CFU/dose), it is not possible to tell whether differences in immunogenicity between the trials were due to vaccine release concentration or age.

Figure 8 depicts the geometric mean of the fold-increase in the titre of vibriocidal antibody against classical Inaba and associated 95% confidence interval as a function of age. Locally weighted smoothers (lowess) of the age-specific geometric means are provided for each of the two trials. This graph indicates that fold-increase in vibriocidal titre declined steadily with age but remained above the level that defines seroconversion for all ages studied.

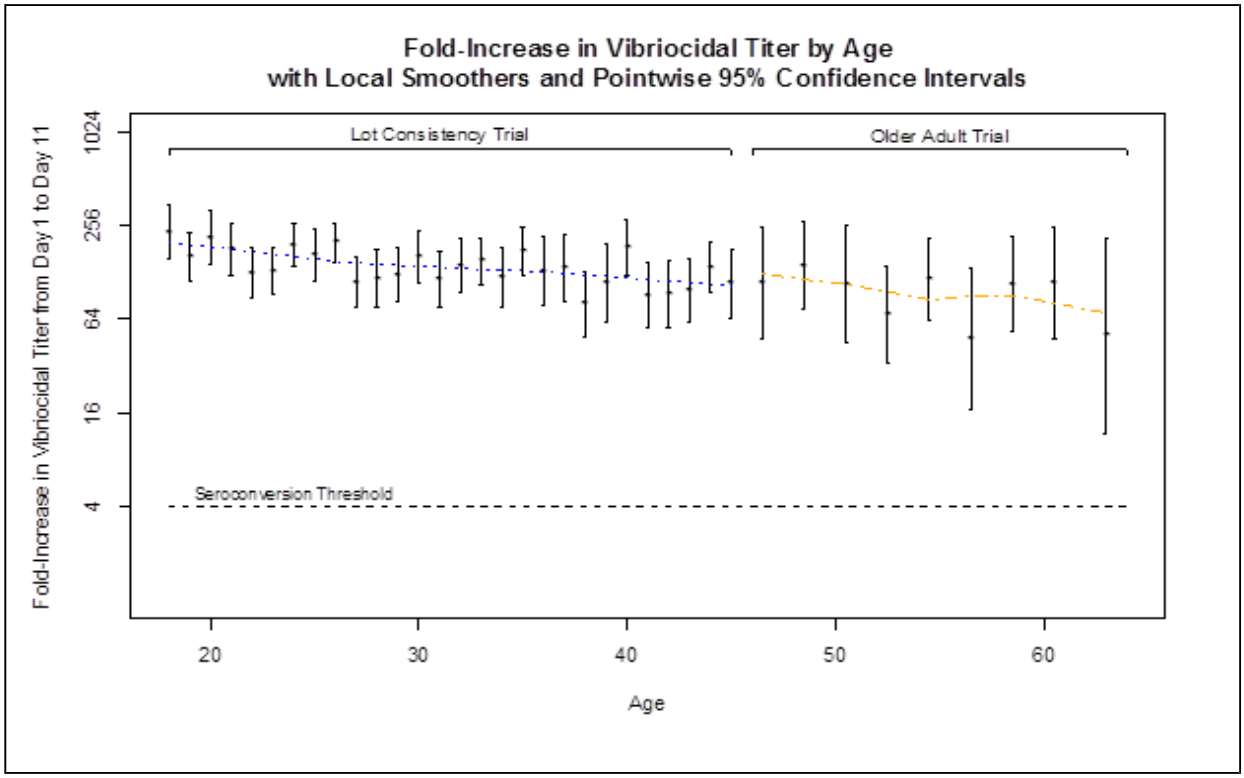


Figure 6 Fold rise in vibriocidal titre by age with local smoothers and pointwise 95% confidence intervals (Post-hoc analysis)

As for fold rise in vibriocidal antibody titre from Day 1 to Day 11, the observed proportion of classical Inaba vibriocidal antibody seroconverters on Day 11 decreased with age ($p = 0.0136$). The summary presented in Table 29 divides the age range into seven non-overlapping categories to facilitate insight into the magnitude of the decline of seroconversion with age. As shown in the table, the proportion of seroconverters fell by about 8 percentage points between the ages of 20 and 60, or about two percentage points per decade.

Table 29 Classical Inaba Seroconversion and Age – Immunogenicity Evaluable Population

	Lot Consistency Trial	Older Adult Trial

Age Range	18-24	25-31	32-38	39-45[a]	46-52	53-59	60-64
n	828	775	574	510	135	109	47
Seroconversion %	95%	93%	94%	92%	92%	90%	87%
95% CI on Seroconversion %	[93%, 96%]	[91%, 95%]	[91%, 95%]	[90%, 95%]	[86%, 96%]	[83%, 95%]	[74%, 95%]

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

The other set of results in Table 30 is from a comparison between older adults and the younger adults in the challenge trial. Like the comparison to the lot consistency trial, the comparison to the challenge trial also showed good agreement between age groups. Specifically, classical Inaba antibody seroconversion in older adults was 0.1 percentage points higher than in younger adults with a 95% CI of [-6.8%, 7.0%].

Table 30 Bridging Analysis: Difference between older and younger adults in Classical Inaba Cumulative Vibriocidal Seroconversion Through Day 11 – Immunogenicity Evaluable Population

Statistic	Challenge PXVX0200 N=94	Lot PXVX0200 N=2688	Older PXVX0200 N=291
N Analysable[a]	93	2687	291
Seroconverted through Day 11[b]	90.3%	93.5%	90.4%
Bridging Analysis			
Difference between Older and Challenge [95% CI][c]	0.1% [-6.8%, 7.0%]		
P-value[d]	1.000		
Difference between Older and Lot [95% CI][c]		-3.1% [-6.7%, 0.4%]	
P-value[d]		0.0491	

Note: A subject is considered to have seroconverted through a visit if they achieve a titre at or prior to that visit that is at least 4-fold higher than their Day 1 titre.
a N Analysable is the number of subjects with any analysable samples available between Day 1 and the indicated visit.
b 95% CIs of seroconversion rate are based on Clopper-Pearson method.
c Wald confidence limits for the seroconversion rate difference between older and younger adults.
d Fisher exact Test between older and younger adults.

Bridging analysis between studies in adults and children >6 and <18 years of age

The PXVX-VC-200-006 clinical study was conducted using a randomized, placebo-controlled, double-blind, single-crossover design with two treatment groups across 3 of which Cohorts 1 and 2 discussed in this report are shown in Figure 9. The primary endpoint of the study was Seroconversion Rate at Day 11: the proportion of subjects achieving seroconversion of serum vibriocidal antibody (SVA) against the classical Inaba biotype of V. cholerae at Day 11 following one dose of PXVX0200, defined as a 4-fold or greater rise over baseline Day 1 SVA titre. The secondary endpoint was seroconversion of SVA against V. cholerae at Day 29 for all subjects and additionally at Days 91 and 181 for Cohort 1 subjects. Interim data from the PXVX-VC-002-006

clinical study addresses bridging of seroconversion and immunogenicity between adults in the Lot consistence clinical study PXVX-VC-200-004 and children >6 to <18 years.

Table 31 shows that healthy males and non-pregnant females between the ages of 2 and <18 years who had no previous history of cholera were recruited. A total of 559 participants >2 years to <18 years of age were enrolled, however, for this application data was provided for 381 participants aged >6 to <18 years. The groups were divided according to age ranges >12 to <18 years of age (Cohort 1) and >6 to <12 years of age (Cohort 2).

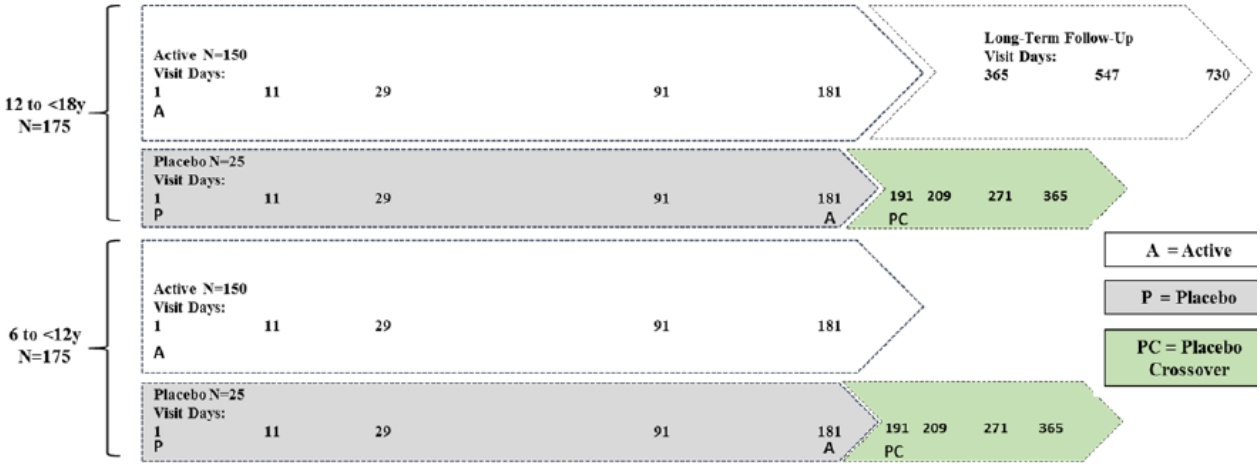


Figure 7 PXVX-VC-200-006 Study Design showing only Cohorts 1 and 2.

Table 31 PXVX-VC-200-006 Study Treatments by Cohort and Treatment Group

Cohort	Age (years)	Treatment Group	N	Day 1 Treatment (blinded)	Day 181 Treatment (Placebo crossover)
1	12 to <18	Active	150	PXVX0200	None
		Placebo-Crossover [a]	25	Placebo	PXVX0200
2	6 to <12	Active	150	PXVX0200	None
		Placebo-Crossover [a]	25	Placebo	PXVX0200
3[a]	2 to <6	Active	210	PXVX0200	None
		Placebo-Crossover [a]	35	Placebo	PXVX0200
		Total	**595**		

a Analysis of Cohort 3 as well as crossover experience with PXVX0200 for the Day 181 treatment are not included in the interim report since they are not included in the indication.

Of the 381 subjects, 52.0% of subjects were male and 48.0% were female. 65.1% of the enrolled subjects were white, 24.9% were black or African American, 1.3% were Asian, 0.5% were American Indian or Alaska Native, and 8.1% were multiple races. The median age was 12.0 (range 6 to <18) years, with each age

group represented. Other than age (and growth associated with age), the demographic and other baseline characteristics of the treatment groups and cohorts were similar.

PXVX0200 used for dosing in the vaccine group of the paediatric study had a potency range of approximately 8.9 x 10^8 CFUs to 7.7 x 10^8 CFUs. By comparison, the PXVX0200 product used in the 004 adult study maintained a potency range of approximately 1.4 x 10^9 CFUs to 9.0 x 10^8 CFUs.

The Bridging Population comprised all subjects in the PXVX-VC-200-006 Immunogenicity Evaluable Population along with subjects vaccinated with PXVX0200 in the Immunogenicity Evaluable Population from the adult PXVX0200 lot consistency trial PXVX-VC-200-004. These were participants that had received the vaccine dose, and had SVA results at Day 1 and Day 11 within the required window.

The two primary objectives were met for both Cohorts 1 and 2; the paediatric age groups were non-inferior to the 004 adult study subjects in their rate of seroconversion and achieved the minimum 70% seroconversion rate required to show success.

In summary, both primary and secondary immunogenicity data show:

- At Day 11, 98.8% and 96.6% of vaccine recipients in Cohorts 1 and 2, respectively, had seroconverted. These rates met the non-inferiority criterion in comparison to the adult Bridging Population and were significantly higher than the Placebo seroconversion rates (0% and 4.2%, respectively).
- By Day 29, the cumulative seroconversion rate was 99.4% for Cohort 1 and remained 96.6% for Cohort 2. Again, these rates were significantly higher than the respective Placebo seroconversion rates (0% and 8.3%, respectively). These findings show that, overall, 98.1% of the pediatric PXVX0200 subjects reached the level associated with efficacy as defined for the adult Bridging Population.
- The geometric mean fold-increase across both cohorts showed a sizeable increase in vibriocidal antibody production as well; at Day 11, the geometric mean fold-increase in antibodies was 245.3 times baseline and at Day 29 the mean fold-increase was 67.9. By Days 91 and 181, mean fold-increase values were 9.9 and 5.7. As a comparison, the mean fold increase for both Placebo groups was approximately 1.0(no mean change over baseline). This decline from Day 11 to Day 181 is consistent with previous studies of PXVX0200 and also consistent with a shift toward gastrointestinal antibody production (Kollaritsch 2000).

The impact of baseline titre on SVA seroconversion

Since seroconversion of vibriocidal antibodies against classical Inaba is so closely linked to protection against moderate/severe cholera, the behaviour of said seroconversion as a function of Day 1 vibriocidal titre was explored graphically to provide further insight into the implications of different possible values of the Day 1 titre. Figure 10 is based upon the data from the vaccine recipients in the lot consistency trial and depicts the logistic regression curve for the model with seroconversion at Day 11 as the response variable and Day 1 titre as the sole covariate.

Also shown in the figure are the raw, model-free estimates of seroconversion with corresponding 95% confidence intervals for each value of the Day 1 titre that was observed in at least 40 vaccine recipients. The figure illustrates that Day 1 titre has an appreciable impact upon the proportion of seroconverters only at relatively high pre-vaccination titres that occur infrequently in a cholera-naïve population. For example, after stratifying the vaccine recipients into groups based upon their Day 1 titre, it is observed that the raw

conditional estimate of seroconversion exceeds 90% in each group for which the Day 1 titre is ≤640, and that 95% of the vaccine recipients have Day 1 titres ≤ 640 and fall into one of these high-converting groups.

In summary, Figure 10 shows that the higher the baseline titre, the potential to achieve seroconversion (4-fold increase from baseline) is reduced.

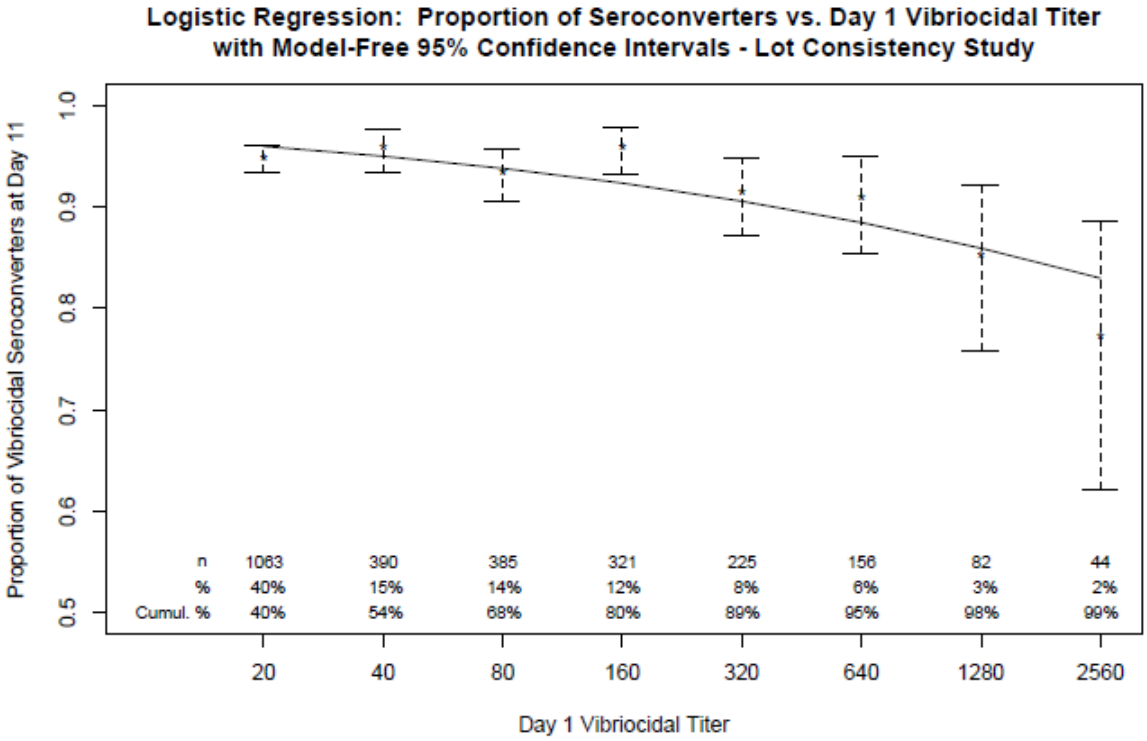


Figure 8 Logistic Regression: Proportion of seroconverters versus Day 1 Vibriocidal titre with model-free 95% confidence intervals – Lot Consistency Trial

Assessing potential associations between race and SVA response to Classical Inaba in PXVX0200 clinical studies

White race was associated with higher serum classical Inaba vibriocidal antibody titre at Day 11 compared with black or African American (p=0.0005). The geometric mean ratio comparing Day 11 titres between whites and black or African Americans was estimated to be 1.300 [1.122, 1.506] from the linear model that combined data across all trials and adjusted for other covariates including baseline titre. The largest difference between the two races was observed in the challenge trial.

The higher absolute titres observed in whites did not confer a statistically significant advantage in seroconversion – the immune marker used for immunobridging – over black or African Americans (p=0.5482). In fact, blacks or African Americans had a slight numerical advantage in seroconversion over whites in the lot consistency and older adult trials, which together accounted for almost 97% of the 3067 subjects included in the logistic regression analysis.

Although the number of subjects tested was smaller, the pattern of significantly higher titres in whites was repeated for the other three vibriocidal antibody strains. In contrast to the classical Inaba assay, the advantage among whites in titre was large enough in the El Tor Inaba and classical Ogawa assays to lead to a significant edge in seroconversion (p=0.0044 and 0.0104, respectively).

Assessing potential associations between sex and SVA response to Classical Inaba in PXVX0200 clinical studies

Men exhibited significantly higher Day 11 classical Inaba vibriocidal antibody titres than women (p<0.0001) although the difference was not large in absolute terms; specifically, the geometric mean ratio of men's titres to women's was 1.157 [1.020, 1.312]. That is, men's classical Inaba vibriocidal tiers were about 15.7% higher than women's. The difference in titre between men and women was not large enough to yield a significant difference in seroconversion (p=0.2353).

Similar trends, with men generally reaching higher titres than women, emerged from the analyses of the heterologous vibriocidal strains but none of the differences were significant except for El Tor Ogawa (p=0.0460). No significant differences in seroconversion were observed among the heterologous strains.

Assessing potential associations between blood type and SVA response to Classical Inaba in PXVX0200 clinical studies

Blood type O has previously been associated with more severe V. cholera infection. It was therefore important to assess the potential impact of this blood type on disease outcome following vaccination.

Vaccine recipients with blood type O had significantly higher classical Inaba vibriocidal titres (p=0.0421) than vaccine recipients with non-O blood types, but the difference was modest, similar in magnitude to the difference between sexes. Specifically, the geometric mean ratio of blood type O to non-O was 1.138 [1.005, 1.288]. The difference in GMTs was not large enough to produce a significant difference in seroconversion (p=0.1835).

B-cell memory responses and duration of protection - Bridging Analysis Comparing anti-O1 LPS IgA Memory B-cells between Older and Younger Adults in all Phase 3 Trials

An exploratory immunogenicity objective in the challenge, lot consistency, and older adult trials was to characterize the memory B cell response in order to determine whether memory B cells collected on Days 91 and 181 could provide information about the persistence of protection.

The Challenge study had shown a statistically significant association between the post-vaccination/pre-challenge percentage of anti-O1 LPS IgA memory B cell percentage on Day 91 and total post-challenge diarrhoeal volume in vaccine recipients (Spearman correlation = -0.39, p = 0.02, n = 33), suggesting that IgA memory B cell levels are associated with outcome.

The mean and median percentages and median fold-rises of anti-O1 LPS IgA memory B cells among all IgA memory B cells on Days 1, 91, and 181 were measured for vaccine recipients in the Immune Sub-study Populations from the challenge, lot consistency, and older adult trials. Significant increases from day 1 to day 181 were noted in the Challenge (median 2.1 fold increase [95% CI: 1.2, 6.6] p=0.001, n= 22) and older adults (median 2.0 fold increase [95% CI: 1.5, 4.1] p=0.0009, n= 34) , but not for the Lot consistency immune substudy population (median 0.9 fold increase [95% CI: 0.5, 1.9] p=0.8831, n=23). However, in a pooled analysis of the immune substudy populations LPS-specific IgA percentages increased by 1.5-fold (p=0.0012, n=93) between Day 1 and Day 91 and exhibited a similar increase of 1.6-fold (p <0.0001, n = 79) between Day 1 and Day 181.

Additional bridging analyses then compared anti-O1 LPS IgA memory B cells in adults 46-64 years of age with younger adults 18-45 years of age from both the challenge and lot consistency trials. The anti- O1 LPS IgA memory B cell percentage was 0.08 [0.01%, 0.18%] percentage points higher in vaccine recipients from the older adult trial compared with those from the challenge trial on Day 91, and 0.12 [0.03%, 0.19%] points

higher at Day 181. The analogous differences between vaccine recipients from the older trial and those from the lot consistency trial were -0.03 [-0.15, 0.08] points at Day 91 and 0.03 [-0.06, 0.12] points at Day 181.

The estimates from the logistic regression analysis with anti-O1 LPS IgA memory B cell outcome as the response identified baseline titre as an independent predictor of both Day 91 and Day 181 anti-O1 LPS IgA memory B cell percentage. In these models, higher baseline percentage was associated with higher post-vaccination anti-O1 LPS IgA memory B cell percentages.

Summary of main studies

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 32 Summary of Efficacy for trial PXVX-VC-200-300: serum vibriocidal antibody responses

Title: 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 cholerae O1 El Tor Inaba 10 Days or 3 Months after Vaccination		
Study identifier	Protocol number PXVX-VC-200-003	
Design	Multicenter Randomised Double-blind Placebo-controlled Efficacy Phase 3 Clinical Study	
	Duration of main phase: Date of first enrolment: Date of last completed:	10.5 months 13 September 2013 28 July 2014
Hypothesis	Demonstrate that the lower 95% confidence bound on the protective efficacy (PE) of a single dose of PXVX0200 is $\geq$30% following a challenge with virulent V. cholerae O1 El Tor Inaba 10 days and 3 months post-vaccination.	
Treatments groups	PXVX0200 10-Day challenge	PXVX0200 single administration n=35 Challenge with V. cholera O1 El Tor Inaba 10 days post-vaccination
	PXVX0200 3-Month challenge	PXVX0200 single administration n=33 Challenge with V. cholera O1 El Tor Inaba 3-months post-vaccination
	Placebo 10-day challenge	Placebo single administration n=33 Challenge with V. cholera O1 El Tor Inaba 10 days post-vaccination

	Placebo 3-month challenge		Placebo single administration n=33 Challenge with V. cholera O1 El Tor Inaba 3-months post-vaccination
	Placebo - not challenged (combined)		Placebo single administration n=63
Endpoints and definitions	Co-Primary endpoint	Efficacy	Demonstrate that the lower 95% confidence bound on the protective efficacy of a single dose of PXVX0200 is $\geq$30% following a challenge with virulent V. cholera O1 El Tor Inaba 10 days post-vaccination and 3-months post-vaccination. Associated primary end point: The occurrence of moderate or severe diarrhoea ($\geq$3L purge) Comparator: pooled placebo recipients challenged at 10 days or 3-months Success: The lower, two side 95% confidence bound on protective efficacy must be >30% in both groups.
	Secondary	Evaluate impact of vaccination on disease severity	• Incidence of diarrhoea of any severity • Incidence of faecal shedding • Peak concentration of wild type V. cholera in stool
	Tertiary	Evaluate pre-challenge immune response	Serum vibriocidal antibody (SVA) and serum IgG cholera toxin geometric mean titre (GMT) at all available pre-challenge time-points. Percentage participants who demonstrate a 4-fold rise in SVA prior to challenge
		Evaluate post-challenge immune response	SVA responses post-challenge Titre and 4-fold rise in SVA post-challenge

	Exploratory	Correlate immune response and disease severity	Correlate SVA with disease severity
Database lock	Not provided		
Results and Analysis			

Analysis description	**Primary Analysis**			
Analysis population	Intention to Treat (ITT) - All randomised participants that received vaccination			
Primary endpoint	**Treatment group**	**PXVX0200 10-Day Challenge**	**PXVX0200 3-Month Challenge**	**Combined placebo**
	Number of participants	n=35	n=33	N=66
	No. qualifying diarrhoea	30 (87.5%)	18 (54.5%)	5 (7.6%)
	Mild: <3L diarrhoea	3 (8.6%)	11 (33.3%)	22 (33.3%)
	Moderate: >3L – 5L diarrhoea	1 (2.9%)	2 (6.1%)	11 (16.7%)
	Severe: >5L diarrhoea	1 (2.9%)	2 (6.1%)	28 (42.4%)
	Attack rate (moderate/severe)	2 (5.7%)	4 (12.1%)	39 (59.1%)
	Protective efficacy (PE) **Lower 95.1% CI**	**90.3%** **[62.7%]**	**79.5%** **[49.9%]**	
Secondary endpoint	Attack rate mild diarrhoea <3L	5 (14.3%)	15 (45.5%)	61 (92.4%)
	Protective efficacy	84.5%	50.8%	
	95% CI	[67.0%, 100.0%]	[33.6%, 66.8%]	

Shedding challenge CFU/g	Attack rate severe diarrhoea (>5L)	1 (2.9%)	2 (6.1%)	28 (42.4%)
	Protective efficacy	93.3%	85.7%	
	95% CI	[56.2%, 100.0%]	[46.2%, 100.0%]	
	Days Mean ± SD	0.9±1.20	2.1±1.41	3.3±1-01
	Days Median (Min-Max)	0.0 (0.0 – 4.0)	2.0 (0.0 – 4.0)	3.0 (0.0 – 5.0)
	p-value Median CFU/g p-value	<0.0001 0 <0.0001	<0.0001 135500 <0.0001	31500000
Tertiary Day 11 GMT (95% CI)		**PXVX0200 Challenge combined N=94**	**Placebo challenge combined n=102**	**P-value**
	Classical Inaba	4313.4 (2873.2, 6475.6)	64.8 (47.8, 87.9)	<0.0001
	El Tor Inaba	6894.4 (4370.1-10889.3)	63.1 (44.6-89.1)	<0.0001
	Classical Ogawa	2323.6 (1519.2-3553.9)	94.0 (67.4-131.1)	<0.0001
	El Tor Ogawa	2238.6 (1492.3-3358.2)	71.5 (50.6-101.1)	<0.0001
N (%) showing 4-fold rise Classical Inaba	Day 8	75 (79.8%)	2 (2.0%)	<0.0001
	Day 11	84 (89.4%)	2 (2.0%)	<0.0001
	Day 29 #	85 (90.4%)	2 (2.0%)	<0.0001
	Day 91 #	85 (90.4%)	2 (2.0%)	<0.0001
	Day 181 §	85 (90.4%)	2 (2.0%)	<0.0001
		PXVX0200 10-day challenge n=35	**Combined placebo n=66**	
Post-challenge	GMT (95% CI) 28 days post-challenge	2460 (1523.3, 3974.5)	17409.1 (13827.6, 21918.2) (n=64)	<0.0001

Post-challenge SVA titre	GMT (95% CI) 181 days post-challenge	295.6 (183.1, 477.2)	2031.9 (1464.7, 2828.8) (n=63)	<0.0001
		PXVX0200 3-month challenge n=33	**Combined placebo n=66**	
	GMT (95% CI) 28 days post-challenge	1646.9 (1112.2, 2438.7)	17409.1 (13827.6,21918.2) (n=64)	<0.0001
	GMT (95% CI) 181 days post-challenge	223.9 (148.2, 338.3)	2031.9 (1464.7, 2828.8) (n=63)	<0.0001
Post-challenge 4-fold rise SVA		**PXVX0200 10-day challenge n=35**	**Combined placebo n=66**	
	28 days post-challenge n (%)	2 (5.7%)	64 (97.0%)	<0.0001
	Day 181 post-challenge n (%)	2 (5.7%)	66 (100%)	<0.0001
Post-challenge 4-fold rise SVA		**PXVX0200 3-month challenge n=33**	**Combined placebo n=66**	
	28 days post-challenge n (%)	20 (60.6%)	64 (97.0%)	<0.0001
	Day 181 post-challenge n (%)	20 (60.6%)	66 (100%)	<0.0001
Exploratory		Mod/Severe Diarrhoea in Seroconverters n(%)	Mod/Severe Diarrhoea in non-Seroconverters n(%)	Rate of protection among seroconverters % 95% CI
	Vaccine Day 11 Challenge (n=35)	1/33 (3%)	1 / 2 (50%)	97% (84%, 100%)
	Vaccine 3-month Challenge (n=33)	1/29 (3%)	3 / 4 (75%)	97% (82%, 100%)

	All vaccine recipients (n=68)	Diarrhoeal volume (median) 0.0L Seroconverters (n=62)	Diarrhoeal volume (median) 6.8L Non-Seroconverters (n=6)	P=0.001 (Wilcoxon Rank Sum Test)
Notes	# Day 29 and Day 91 results from subjects challenged at 10 days were not reported in this table. § Day 181 results from challenged subjects not shown in this table			

Table 33 Summary of efficacy for Phase 1 trial PXVX-VC-200-002

Title: A Phase 1 Randomized, Double-Blind Placebo-Controlled Study to Evaluate the Safety and Immunogenicity of the Live Oral Cholera Vaccine Candidate PXVX0200 Vibrio cholerae O1 Serotype Inaba Vaccine Strain CVD 103-HgR			
Study identifier	PXVX-VC-200-002		
Design	Phase 1, randomised, double-blind, placebo-controlled two-centre study in healthy young adult subjects 18–50 years old to evaluate the safety and immunogenicity of one oral dose of live oral cholera vaccine. Immunogenicity was assessed with serum vibriocidal and anti-CT antibody responses measured before and 10, 14, and 28 days post-vaccination. To assess vaccine shedding and transmission, subjects were allocated to providing a fresh stool sample or having a rectal swab performed before vaccination and either on Days 1, 3, and 7 or on Days 2, 4, and 7 following vaccination. The possibility of transmission to household contacts (HHCs) residing with the study subject was also evaluated: HHCs were evaluated for shedding of PXVX0200 on Day 7 and for vibriocidal seroconversion on Day 28.		
	Duration of main phase: Duration of run-in phase: Duration of extension phase:		28 days not applicable not applicable
Hypothesis	Superiority		
Treatments groups	Vaccine group		single oral vaccine dose of 4.43×10^8 55 subjects randomised
	Placebo group		single oral dose of lactose 11 subjects randomised
Endpoints and definitions	Co-Primary endpoint	Ab titre	vibriocidal antibody titre on Days 10, 14, and 28 post-vaccination
	Co-Primary endpoint	SVA rate	seroconversion by vibriocidal antibody titre on Days 10, 14, and 28 post-vaccination
Database lock	07 January 2013		

Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	Per protocol Day 28			
Descriptive statistics and estimate variability	Treatment group	Vaccine	Placebo	
	No of subjects	55	11>	
Effect estimate per comparison	Co-Primary endpoint	Comparison groups	Vaccine	Placebo
		Ab titre	1231.6	45.4
		95 % CI	749.7, 2023.3	15.4, 133.4
		P-value	<0.001	
		SVA rate	88.9	0.0 %
		95 % CI	77.4–95.8 %	0.0–28.5 %
Notes	The large majority of those who seroconverted – 45 out of 48 – had done so by Day 10. None of the 11 placebo recipients seroconverted. The difference between groups was statistically significant ($p < 0.001$ adjusted for 3 simultaneous comparisons at different time points. GMT among vaccine recipients attained a peak of 3,024.9 by Day 10, and was sustained through Day 14. GMT had fallen to 1,231.6 by Day 28.			

Table 34 Summary of efficacy for Phase 3 trial PXVX-VC-200-004

Title: 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		
Study identifier	Phase 3 PXVX-VC-200-004	
Design	Phase 3, randomised, double-blind, placebo-controlled three-lot consistency study in healthy adult subjects 18–45 years old at 19 investigational sites in the United States and 6 investigational sites in Australia.	
	Duration of main phase: Duration of run-in phase: Duration of extension phase:	11 days / 6 months not applicable not applicable
Hypothesis	Equivalence	
Treatments groups	Lot A	single oral vaccine dose of 1×10^9 CFU 927 subjects randomised
	Lot B	single oral vaccine dose of 1×10^9 CFU 933 subjects randomised
	Lot C	single oral vaccine dose of 1×10^9 CFU

			935 subjects randomised
	Placebo		single oral dose of physiological saline 351 subjects randomised
Endpoints and definitions	Primary endpoint	SVA	Serum vibriocidal antibody measured at Day 11 – Equivalence criteria: The GMT of each lot must be within ±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 endpoint	Seroconversion rate	Seroconversion rate by Day 11
Database lock	23 February 2015		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Immunogenicity evaluable population		

Descriptive statistics and estimate variability	Treatment group	Lot A	Lot B	Lot C	Placebo
	No of subjects	892	887	909	334

Effect estimate per comparison	Primary endpoint	Comparison groups	Lot A vs. B and C, B vs. C
		Lot A:B	0.92
		95 % CI	0.78; 1.08
		Lot B:C	1.02
		95 % CI	0.87; 1.20
		Lot A:C	0.94
		95 % CI	0.80; 1.10
	Secondary endpoint	Comparison groups	All lots vs. placebo
		Seroconversion rate	94 % vs. 4 %
		95 % CI	93 %; 94 % vs. 2 %; 7 %
		P-value	<0.0001
Notes	14 (4 %) subjects in the placebo group seroconverted is most likely due to dosing errors or mistakes in the sample handling. Sensitivity analyses were performed, allowing for worst case scenario. Even if the placebo seroconverters were changed for vaccine non-converters among vaccine recipients who received vaccine from Lot B who had the greatest Day 11 GMT among the 3 lots and giving weight to the comparison against Lot A with the smallest GMT, the study result would not be affected.		

Table 35 Summary of efficacy for Phase 3 trial PXVX-VC-200-005

Title: 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				
Study identifier	PXVX-VC-200-005			
Design	Phase 3, randomised, double-blind, placebo-controlled study to evaluate the safety and immunogenicity of the cholera vaccine candidate in older adults 46–64 years of age and to bridge to the efficacy established for younger adults in the challenge study 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			
	Duration of main phase: Duration of run-in phase: Duration of extension phase:	8 months not applicable not applicable		
Hypothesis	Non-inferiority			
Treatments groups	Vaccine groups (older adults bridged to 2688 younger adults in study 004)	single oral vaccine dose of 1×10^9 CFU 299 subjects randomised		
	Placebo group	single oral dose of physiological saline 99 subjects randomised		
Endpoints and definitions	Co-Primary endpoint	Difference	seroconversion by classical Inaba vibriocidal antibody at Day 11 in older adults was non-inferior to seroconversion at Day 11 in younger adults (from study 004) by having the lower bound of the two-sided 95 % CI on the difference in seroconversion between older and younger adults greater than -10 percentage points	
	Co-Primary endpoint	Sero-conversion rate	the lower bound of the two-sided 95 % CI on seroconversion by classical Inaba vibriocidal antibody at Day 11 was greater than 70 % in older adults	
Database lock	15 January 2015			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	Bridging analysis population Day 11			
Descriptive statistics and estimate variability	Treatment group	Older adults	Younger adults	Placebo
	Number of	291	2688	99

	subjects			
Effect estimate per comparison	Co-Primary endpoint	Comparison groups	Older vs. younger	
		Difference	-3.1 %	
		95 % CI	-6.7 %; 0.4 %	
	Co-Primary endpoint	Comparison groups	Older vs. younger	
		Seroconversion rate	90.4 % vs. 93.5 %	
		95 % CI	86.4 %; 93.5 %	
		P-value	0.0491	
Notes	–			

Table 36 Summary of efficacy for Phase 4 trial PXVX-VC-200-006 (Interim Report)

Title: A Phase 4 Two-Arm, Randomized, Double-blind, Single-crossover, Placebo-controlled Study to Assess the Safety and Immunogenicity of VAXCHORA (Cholera Vaccine, Live, Oral) in Children 2 to <18 Years of Age			
Study identifier	PXVX-VC-200-006 (Interim Report)		
Design	Phase 4 two-arm, randomised, double-blind, single-crossover, placebo-controlled safety and immunogenicity study in children 2 to <18 years of age		
	Duration of main phase: Duration of run-in phase: Duration of extension phase:	181 days not applicable not applicable	
Hypothesis	Non-inferiority		
Treatments groups	Cohort 1, ages 12 – <18	single oral vaccine dose of 1 × 10^9 CFU 191 subjects randomised	
	Cohort 2, ages 6 – <12	single oral vaccine dose of 1 × 10^9 CFU 190 subjects randomised	
	Placebo group	single oral dose of physiological saline 99 subjects randomised	
Endpoints and definitions	Co-Primary endpoint	Sero-conversion rate, bridged	Seroconversion rate at Day 11 in pediatric subjects was non-inferior to the seroconversion rate at Day 11 in previously studied adult subjects between the ages of 18 and 45 years. Endpoint: the proportion of subjects achieving seroconversion of SVA against the classical Inaba biotype of *V. cholerae* at Day 11 following one vaccine dose, defined as a 4-fold or greater rise over baseline Day 1 SVA titer.
	Co-Primary endpoint	Sero-conversion rate	Seroconversion rate in pediatric subjects was greater than or equal to 70 % with 98.3 % confidence. Endpoint: the proportion of subjects

			achieving seroconversion of SVA against the classical Inaba biotype of *V. cholerae* at Day 11 following one vaccine dose, defined as a 4-fold or greater rise over baseline Day 1 SVA titer.			
Database lock	24 May 2018					
Results and Analysis						
Analysis description	**Primary Analysis**					
Analysis population and time point description	Adult bridging population Day 11					
Descriptive statistics and estimate variability	Treatment group	Adults	Cohort 1	Cohort 2		
	No of subjects	2688	160	148		
Effect estimate per comparison	Seroconversion rate	Comparison groups	Adults	Cohort 1	Cohort 2	
		Seroconversion rate	93.5 %	98.8 %	96.6 %	
		98.3 % CI	92.3; 94.6	94.4; 99.7	91.0; 98.8	
Notes	The seroconversion rates were significantly higher than the placebo group's seroconversion rates (0 % and 4.2 %, in corresponding age cohorts, respectively).					

2.5.3. Clinical studies in special populations

No clinical studies have been carried out in individuals aged 64 years of age and over.

2.5.4. Supportive studies

The clinical trials conducted with Orochol by SSVI/Berna (Bern, Switzerland) aimed to evaluate vaccine immunogenicity, efficacy, safety and transmissibility to close contacts and the environment. Studies were performed in non-immune adult volunteers from industrialised countries such as North America and Switzerland.

Separate studies were also performed to evaluate the immunogenicity and safety among healthy adults and children (including infants) residing in countries where cholera was endemic. Study populations ranged from healthy adults and adolescents (16–56 years of age) to children (2–9 years of age), and infants (3–17 months of age). Vaccinees were tested for shedding of CVD 103-HgR to determine the probability of releasing the organisms into the environment. Unvaccinated close contacts were monitored for seroconversion due to vaccine spread. Two field efficacy trials were conducted: in Jakarta (Richie 2000) and Pohnpei (Calain 2004).

Generally, only healthy subjects were included in the Orochol studies with age ranges from healthy adults and adolescents (16–56 years of age) to children (2–9 years of age) and infants (3– 17 months of age), though one study evaluated safety and immunogenicity in HIV+ adults in Mali. Although vibriocidal titres

were somewhat lower in HIV+ adults, compared to HIV- adults, no safety concerns were identified (Perry 1998).

Immunogenicity was evaluated by measuring serum vibriocidal (antibacterial) and anti-toxin antibody titres as both types of antibodies are associated with protection (Levine 1981, Glass 1985a). However, there was increasing evidence that antibacterial immunity is more important in cholera (Levine 1981, Levine 1979, Levine 1988b), therefore, serum vibriocidal antibodies became recognised as the best immune marker correlating with protection against cholera. This was confirmed in the PXVX-VC-200-003 study conducted by PaxVax using PXVX0200 (PaxVax Report PXVX-STAT-VIB-003).

In the Orochol/Orochol E clinical trials, volunteers received vaccine doses ranging from 108 to 1010 CFU of live attenuated CVD 103-HgR bacteria. The marketed specification for Orochol was 2-10x108 CFU/dose and for Orochol E was 2-10x109 CFU/dose. Placebo recipients were given a single dose of 5x108 CFU of inactivated E. coli K12. Blood samples were collected prior to and on Days 10, 21 and 28 after administration of a single dose of the vaccine. Serum vibriocidal antibody titres against homologous (Inaba) or heterologous (Ogawa) serotypes were measured in a vibriocidal assay in vitro using complement. Seroconversion was defined as a ≥ 4-fold rise in vibriocidal antibody titres. Additionally, serum levels of IgG to cholera toxin were measured by enzyme-linked immunosorbent assay (ELISA). Seroconversion of antitoxin antibodies was defined as a ≥4-fold rise in titre or an increase of net optical density of ≥0.2 (CSL 2000).

In early open-label studies in the US, high seroconversion rates for vibriocidal antibodies were observed after the administration of a single 3-5x108 CFU dose of CVD 103-HgR (94 %-100 % of volunteers had a ≥ to 4-fold increase in Inaba vibriocidal antibody titres, Levine 1988a). The antitoxin response was similar to the vibriocidal response with 83 %–100 % of vaccinees seroconverting. Further studies were performed in the US and Switzerland and confirmed that in previously non-immune individuals from industrialized countries CVD 103-HgR is a potent immunogen, stimulating a significant rise in Inaba vibriocidal antibodies in 81–97 % of vaccinees (Kotloff 1992, Tacket 1999, Cryz 1990, Cryz 1992, Cryz 1995b). A slightly lower but good seroconversion rate (67 %) was observed in a study performed in US military personnel in Panama wherein 40 % of participants had elevated levels of baseline immunity to V. cholerae O1 (Taylor 1999).

Compared with seroconversion for Inaba vibriocidal antibodies, slightly lower rates were observed for heterologous Ogawa vibriocidal antibodies (53 %–68 %) (Cryz 1990, Cryz 1993, Taylor 1999). The studies also showed that a single 5x108 CFU dose elicited significant rise in serum antitoxin levels (51–100 %). The cholera antitoxin response was measured using an ELISA with serum tested at a 1:50 final dilution (Cryz 1990). These results support the use of CVD 103-HgR to generate an immune response against V. cholerae O1 in persons from developed countries where cholera is not endemic.

Studies were also conducted in subjects from countries with endemic/epidemic cholera. Unlike the US and Switzerland, seroconversion rates were generally lower in individuals who were administered 5x108 CFU dose of the vaccine. Two Thai trials were conducted, one showing higher seroconversion rates (92 %) (Migasena 1989) and the other showing lower rates (25–33 %) (Su Arehawaratana 1992). The latter study was in Thai soldiers. Further investigation showed a direct correlation between socioeconomic status of the recipients and the proportion with vibriocidal seroconversion was related to poor living conditions and sanitation. Two possible explanations were hypothesized: exposure to V. cholerae causing the subjects to exhibit elevated baseline titres of vibriocidal antibodies which do not increase dramatically in response to a 5 x 108 CFU dose; and increased levels of small intestinal microflora in lower socioeconomic group subjects. This was also shown in studies conducted in Peru (Gotuzzo 1993). These findings are consistent with studies with other vaccines, particularly orally administered vaccines, which suggest that antibody responses and efficacy are diminished in vaccinees in developing countries, leading to a need for higher doses in those countries (Levine 2010).

Accordingly, a 10-fold higher dose was used in clinical trials in cholera-endemic countries. Using this higher dose (subsequently marketed as Orochol E; specification 2 x 109 to 1 x 1010 CFU/dose), significantly greater rates of Inaba vibriocidal antibody seroconversion were observed. Thus, use of PXVX0200 in indigenous populations for prevention of endemic or epidemic cholera will require formulations with higher dosages. Therefore, it would not be appropriate to evaluate efficacy of the current PXVX0200 formulation/dosage (specification 5 x 108 to 2 x 109 CFU/dose) aimed at persons from non-endemic countries as a preventative vaccine for endemic cholera.

The protective efficacy of Orochol was assessed in a series of studies in healthy adult subjects in the US and Switzerland. The subjects received a single, oral dose of 3–5 x 108 CFU of CVD 103-HgR and were subsequently challenged with pathogenic wild-type V. cholerae O1 of homologous (classical) or heterologous (El Tor) biotypes. Challenge organisms were administered at a dose which normally elicits diarrhoea. Controls received an initial dose of placebo (5 x 108 CFU inactivated E. coli K12) or were unvaccinated prior to challenge. Post challenge, the subjects were monitored for the onset of or protection from V. cholerae - induced diarrhoea. Diarrhoea was defined as the passage of 2 or more unformed stools of ≥200 ml or a single stool of ≥300 ml over a 48-hour period. Moderate cholera cases were those who passed at least 3 l of diarrheal stool over the study period. Severe cases were those who excreted at least 5 l.

The challenge studies in North American and Swiss volunteers demonstrated that vaccination conferred significant protection against diarrhoea due to cholera. Cholera-induced diarrhoea was prevented in 100 % of individuals challenged with the classical V. cholerae O1 serotype Inaba (Tacket 1992). Protection was observed as early as 8 days after vaccination and continued until 6 months post vaccination (Tacket 1992).

In subjects who were challenged with the heterologous El Tor biotype, the majority of the vaccinees (54–80 %) were protected from cholera diarrhoea at 1–3 months post-vaccination (Levine 1988a, Tacket 1999). In vaccinees who developed diarrhoea, the disease was mild. For a more clinically meaningful evaluation of the efficacy of a cholera vaccine it is important to look at the severity of the disease. Orochol provided 95 % protective efficacy against moderate and 92 % protective efficacy against severe diarrhoea caused by cholera.

An ambitious placebo-controlled study in 67,508 subjects was conducted in Jakarta between July 1993 and December 1997 to examine the protective efficacy of Orochol against endemic V. cholerae O1 El Tor (Richie 2000). The vaccine did not confer significant protection over the 4-year follow-up (13.5 % efficacy), but did have a modest efficacy in blood group O recipients (45 %). Unfortunately, too few cases (7 total: 5 in controls, 2 in vaccinees, giving a point estimate of efficacy of 60 %) occurred in the first four months of follow-up after vaccination to allow a valid comparison with the previous studies using adult volunteers.

This field trial was carried out in a crowded urban setting where the annual incidence of cholera prior to the trial had been > 1 case per 1000 for several years and where there was very high community participation in the trial. Following the large-scale field trial, the incidence of cholera rapidly fell. One explanation for the disappointing results is that the live vaccine led to widespread indirect protection (herd immunity) as well as direct protection that caused the incidence to plummet even in the control group and removed the ability to detect vaccine efficacy. This scenario has been reported in relation to a re-analysis of the field trial of oral inactivated cholera vaccine in Bangladesh in the 1980s and it may also apply to the Indonesia trial of CVD 103-HgR (Ali 2005).

In 2000, an outbreak of V. cholerae O1 El Tor cholera in Pohnpei, Micronesia led the government there, with the support of the WHO, to request a donation of 48,000 doses of Orochol E. This was used in a vaccination campaign covering 47 % of the total population in Pohnpei State (estimated 14,587 persons vaccinated). A

retrospective evaluation of the outcome of vaccination was then conducted. Of the subsequent 344 evaluable cases of cholera, 50 had been vaccinated more than 10 days before illness and 294 were non-vaccinated. The overall estimated protective efficacy was 79.2 % (95 % CI: 71.9-84.6 %) (Calain 2004). This result was more consistent with the data from cholera challenge studies than with the result from the trial in Jakarta, and demonstrated that a single dose of CVD 103-HgR given promptly at a suitable dose (Orochol E) can be effective in curtailing cholera outbreaks in endemic areas.

In addition to immunogenicity and protective efficacy, the safety of Orochol was also assessed in many of the clinical trials. Vaccinees were monitored for adverse reactions 7–9 days after the ingestion of 108 to 1010 CFU of CVD 103-HgR. Successful attenuation of CVD 103-HgR would enable administration of the vaccine without eliciting diarrhoea in vaccinees. This was confirmed in numerous trials where up to 1010 CFU did not elicit diarrhoea in vaccinees at a rate greater than the inactivated E. coli K12 used as placebo. Other adverse reactions such as fever, abdominal pain, and vomiting were also monitored. CVD 103-HgR did not exhibit significantly higher rates of these AEs compared to placebo (bacteriological media). AEs were transient and mild and possibly occurred due to the sodium bicarbonate buffer.

Since Orochol contained the recombinant CVD 103-HgR bacterial strain, transmissibility was monitored to evaluate the possible release of organisms into the environment; therefore, the excretion in stools of vaccinees was monitored. The strain contained in the vaccine was minimally excreted with a geometric mean number of 200 V. cholerae per gram of stool (Levine 1988a). Data from clinical trials have demonstrated that not more than 30 % of V. cholerae CVD 103-HgR recipients excrete detectable numbers of the modified bacteria, with excretion lasting for a maximum of 7 days (Levine 1988a, Cryz 1995, Kotloff 1992, Simanjuntak 1993, Lagos 1999). Given that the infectious dose of cholera is up to 108 CFU, (except in individuals who produce less stomach acid, such as young children, the elderly and those who take antacids, who may be infected by approximately 104 CFU [Kitaoka 2011]), mean shedding of approximately 2.6 x 104 CFU of vaccine bacteria (assuming an average stool weight of 128 g [Rose 2015]) by a vaccine recipient is unlikely to translate into an infection in another person. A dose of 105 CFU of virulent V. cholerae was used in volunteer challenge studies, but was given in conjunction with buffer to neutralize gastric acid and therefore improve the possibility of a successful infection.

Transmission to close contacts was monitored in one Indonesian study where pairs of children in the same household were given either vaccine or placebo. The vaccine strain was not recovered from placebo recipients indicating no transmission had occurred from vaccinees to controls.

CVD 103-HgR was isolated from only 1 out of 174 other family contacts (Simanjuntak 1993). This is particularly relevant since the study was performed in a cholera-endemic country in a paediatric population where faecal spread is high (ages 24–59 months), living in conditions of poor sanitation. A similar study with pairs of children in the same household conducted in Chile (Lagos 1996) also demonstrated a low rate of shedding and transmissibility, with 5 % of unvaccinated contacts shown to shed vaccine strain bacteria.

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. This study was undertaken in an area of Indonesia where cholera is endemic (Simanjuntak 1993). 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.

2.5.5. Discussion on clinical efficacy

Vaxchora (PXVX0200) is a live attenuated vaccine based on V. cholerae serogroup O1, Classical biotype, serotype Inaba. PXVX0200 is indicated for active immunisation against disease caused by Vibrio cholerae serogroup O1 in adults and children aged 6 years and older, and should be used in accordance with official recommendations. The PXVX0200 clinical development program was designed to demonstrate safety and efficacy of the PXVX0200 vaccine as a new entity. Data derived from previous studies of the Orochol vaccine could be considered supportive.

Design and conduct of clinical studies

Design and conduct of clinical studies were considered overall acceptable.

A phase 3 randomized, double-blind, placebo controlled (1:1 ratio) clinical study (PXVX-VC-200-300) showed that immunisation with PXVX0200 provided protective efficacy against challenge with wild type V. cholerae O1, El Tor biotype, serotype Inaba in adults aged 18-45 years. The co-primary objective/endpoint was to demonstrate that the lower 95% confidence bound on the protective efficacy of a single dose of PXVX0200 was ≥30% following challenge 10 and 90 days post-vaccination respectively.

Although 197 participants were randomised in the PXVX-VC-200-300 challenge study, the participants were ultimately divided into six groups of with a minimum of 27 and a maximum of 36 participants per group. The efficacy evaluation was based on the groups that were challenged with V. cholera, which consisted of 35/33 (vaccine/placebo) participants challenged 10 days post-vaccination and 33/33 (vaccine/placebo) participants challenged 3-months post-vaccination. The group size for the efficacy evaluation was therefore small, but sufficient for the statistical analysis. Protective efficacy data from these groups was used as a basis to bridge immunogenicity data from a large Lot consistency trial (PXVX-VC-200-004) in adults having the same age range as the challenge study (18-45 years) with analysable samples for baseline and Day 11 in 3021 participants. Since the large Lot consistency study showed comparable (non-inferior) seroconversion frequency (93.5%) compared to the challenge study (90.3%) at Day 11, subsequent clinical studies in older adults (46-64 years) and children (>6-18 years) bridged their immunogenicity seroconversion frequencies to this study. In contrast to the challenge study which used a dose of 5×10^8 CFU, the Lots consistency study and the studies in older adults used a higher dose of PXVX0200 (1×10^9) which may have led to greater antigen exposure following PXVX0200 replication. The study in children was a phase 4 study and used the dose range in children >6 years as defined in the FDA approved Vaxchora SmPC (4×10^8 to 2×10^9 CFU).

Serum vibriocidal antibodies (SVA) seroconversion was predefined in the study protocol as >4-fold increase over baseline. Both SVA geometric mean titre (GMT) and fold-increase in titre from baseline were measured. Day 11 post-vaccination showed the highest GMT and fold increase over baseline in vaccinees but not placebo. Only two of 62 seroconverting Vaxchora recipients developed moderate cholera after challenge: 1 of 33 at Day 11 and 1 of 29 at Day 91. Based on this near 1:1 relationship between seroconversion and protection, along with the fact that approximately 90% of Vaxchora recipients seroconverted, SVA seroconversion (>4-fold increase in titre from baseline) at Day 11 was used as the measure to correlate immunogenicity to disease severity and for establishing an immunobridge between populations.

Seroconversion rates at Day 11 exceeded 90 % in all clinical studies, and notably after single oral dose. In the Challenge study (003), 94 % of subjects had seroconverted by Day 11 and 88 % by Day 91 88 %. The Protective Efficacy at Day 10 was 90.3 % and 79.5 % at Month 3. Comparing seroconverters with non-seroconverters, 97 % were protected in the challenge at both time points. For non-seroconverters, 50% were protected at Day 11 challenge and 25% protected in the 3-month challenge group.

None of the clinical studies measured mucosal IgA antibodies in the intestinal tract induced following vaccination. This would have been an appropriate parameter since the site of cholera pathogenesis is the intestinal tract. However, it is acknowledged it may be challenging to collect these samples adequately.

Memory B-cell responses are considered as markers to address the duration of protection. The ELISPOT assay used to assess the reactivation of B-cell memory responses producing IgG and IgA antibodies to lipopolysaccharide (LPS) from V. cholerae (Inaba) and cholera toxin B (CTxB), respectively was qualified but not validated. The assays used mainly frozen peripheral blood lymphocytes (PBMC) as a source of memory B-cells. Upon thawing, it is anticipated that thresholds for cell recovery and viability would be needed to allow for valid assay results. No criteria for recovery or viability upon thawing were used, but the cells were found to have acceptable viability.

The efficacy clinical study recruited healthy adults. Inclusion criteria ensured that participants had not previously been exposed to V. cholerae, and did not have any immunosuppressive condition or gastrointestinal disease that could impact on immunogenicity or disease severity, which is acceptable as this is a live vaccine which needs to replicate in the gut to be effective. This would have excluded participants with known autoimmune conditions. The use of the vaccine is contraindicated in individuals with congenital immune deficiency or receiving immunosuppressive drugs or treatments. At present, vaccination is recommended to be postponed for persons with acute gastrointestinal disease until after recovery. The SmPC section 4.4 additionally reflects that the degree of protection and the effects of vaccination in individuals with chronic gastrointestinal disease are unknown.

Participants who were not allergic to antibiotics used to treat V. cholerae infection after challenge were selected. This is important in that the vaccine is not completely protective and antibiotic treatment may still be needed in addition to rehydration.

The dose and choice of the challenge strain of V. cholerae was based on the dosage used previously for Orochol (Tacket 1999). The challenge strain was not homologous to Vaxchora (Vibrio cholerae serogroup O1, biotype classical, serotype Inaba), but corresponded to a different biotype (Vibrio cholera serogroup O1. Biotype El Tor serotype Inaba, strain (N16961)). Although the challenge strain may not represent currently circulating strains, it is acceptable as a heterologous challenge since it represents the El Tor biotype, rather than the classical biotype of the vaccine. Vaccinated individuals may be exposed to either biotype when travelling to endemic regions. However, neither the vaccine, nor the challenge V. cholera included the Ogawa serotype which is also part of serogroup 01 and included in the Vaxchora indication. Serological analyses showed lower seroreactivity to the Ogawa serotypes suggestive of lower protection against these serotypes, which are also prevalent in cholera endemic areas.

During the challenge part of the study, participants were in-patients and were monitored over a 10 day period or until disease-free. Oral hydration was started once diarrhoea was apparent (grade 3 stool) and antibiotics were given to all subjects 96 hours post-challenge (or sooner if they experienced cumulative diarrhoeal volume of >5 L) according to the protocol. Both oral rehydration and antibiotics represent standard treatment for cholera, and may therefore have influenced the efficacy primary endpoint which was protection against moderate/severe diarrhoea. Moderate diarrhoea was a cumulative stool output of >3L over the 10 day in-patient period post-challenge, and severe diarrhoea was a cumulative stool output >5L. However for six of these 10 days, participants were treated with antibiotics. No viable challenge V. cholerae was shed 6 days post-challenge in the placebo group. The placebo group shed higher levels of challenge V. cholera in stool (median peak of 3.15×10^7CFU/g over 10 days) than the vaccinated group (median peak of 1.355×10^5 CFU/g over 10 days). The lack of shedding from 5-6 days post-challenge cannot be attributed to immune responses induced by the vaccine.

Based on previous results with Orochol, PXVX0200 should not be taken concurrently with anti-malaria medication chloroquine. Based on data from Orochol, it is apparent that this is not a feature of other anti-malarial drugs. No clinical studies are included that assess the concomitant use of PXVX0200 with particularly oral vaccines such as vaccines protecting against typhoid fever and polio. Data from Orochol are cited in the SmPC, which is acceptable as they are relevant for PXVX0200.

There is no clinical data available from persons living in cholera endemic regions, and use in persons coming from cholera endemic countries is not planned. This would include travellers and tourists (of all ages and their children), aid workers and military personnel. Currently, the WHO position paper (2017) regarding cholera vaccination has the following recommendation for international workers and travellers: "Vaccination is not generally recommended for long-term or short-term travellers to cholera-affected countries, but should be guided by specific travel risks". Whilst for healthcare workers, the following is recommended: "OCV (oral cholera vaccine) should be considered for emergency and relief workers who are likely to be directly exposed to cholera patients or to contaminated food or water, particularly those staying in areas with poor access to health-care facilities. Other health-care workers are generally not at special risk of cholera." Persons in non-endemic regions may also be exposed to V. cholerae through occupation or through contaminated food/water. The vaccine could therefore be used in regions within the EU that may be subject to a cholera outbreak.

Information reflecting the fact that no data are available in persons living in cholera-affected areas or in individuals with pre-existing immunity to cholera has been included in section 4.4 of the SmPC because the efficacy of Vaxchora in these individuals has not been established (see also section 3.7.2). The historical experience with CVD 103-HgR suggests that a higher dose may be necessary in this population. However, this gap in knowledge is not immediately relevant for a European population since cholera is not endemic in any region within the EU. In lack of such data, the vaccine is only recommended in travellers.

Efficacy data and additional analyses

The co-primary objective and endpoint of the challenge study (PXVX-VC-200-300) was to demonstrate that the lower 95% confidence bound on the protective efficacy of a single dose of PXVX0200 was ≥30% following challenge at 10 and 90 days post-vaccination respectively. The study showed that participants challenged 10 days post-vaccination achieved a protective efficacy against moderate/severe diarrhoea of 90.3% [95% CI: 82.4%, 95.5%] whilst participants challenged 3-months post-vaccination showed a protective efficacy of 79.5% [95% CI: 49.9%, 100.0%]. The lower CI was 62.7% for participants challenged 10 days post-vaccination and 49.9% for participants challenged at 3-months post-vaccination. The study thereby met its co-primary objectives/endpoints.

Given the enrichment aiming at approximately 60 % of subjects with blood type O (as they may be predisposed to develop cholera disease of a greater severity than those with blood group A and/or B antigens), it is acknowledged that efficacy irrespective of blood type stratum was shown to be similar (among subjects with blood type O only, the 10-Day protective efficacy (PXVX0200 vs combined placebo) was 84.8 % (lower 95.1 % CI bound 50.2 %) with a 3-Month protective efficacy of 78.4 % (lower 95 % CI bound 44.0 %); among subjects with non-O blood types only, the 10-Day efficacy was 100.0 % (lower 95 % CI bound 48.1 %) and the 3-Month PE was 83.5 % (lower 95 % CI bound 20.5 %)).

In the Challenge Study (PXVX-VC-200-003), the combined placebo group served as comparator in all analyses. Seen as some evidence that the use of a combined placebo group is appropriate is the observation that the attack rate of post-challenge moderate/severe diarrhoea in placebo recipients in the 10-day

challenge and 3-month challenge respectively have been found to be very similar: 60.6 % (20/33) in the 10-day challenge group compared with 57.6 % (19/33) in the 3-month challenge group.

Cholera is an infection of the digestive tract mucosal membranes leading to diarrhoea. Protection was correlated to serum vibriocidal antibodies (SVA) assay, which detect functional antibodies that bind complement resulting in lysis of V. cholera (vibriolysis). As such, SVA assay detects predominantly serum IgM antibodies which are associated with primary immune responses, and peak between 7- 14 days after antigen exposure. SVA seroconversion (pre-defined as a >4-fold increase from baseline) at Day 11 post-vaccination was considered to be a more reliable immune marker correlating with protection compared to SVA geometric mean titre (GMT) alone, because it took into consideration baseline levels and corresponded to the time when SVA levels were at their peak. However, two individuals that seroconverted nevertheless experienced moderate diarrhoea (>3L cumulative stool volume over 10 days). It is not clear if a different increment in SVA from baseline would correlate better with protection.

PXVX0200 vaccination did not completely prevent diarrhoea following challenge with virulent V. cholerae. The protective efficacy against mild or worse diarrhoea was 84.5% for those challenged 10-days post-vaccination, and 50.8% for those challenged 3-months post-vaccination. This indicates that protective efficacy waned over time.

There is currently no data in individuals >64 years of age. In the clinical study that included older participants, (aged 46-64 years of age – PXVX-VC-200-005), the seroconversion frequency and titre of SVA to Classical Inaba was observed to decline with advancing age (p=0.0136) as did the SVA titre (p<0.0001). Seroconversion rates in ages 46-52 were 92% [86%, 96%] and dropped to 87% [74%, 95%] in 60-64 year olds where the confidence interval was also wider. These observations may reflect immune senescence and/or the effect of pre-existing (baseline) cross-reactive antibodies since increased baseline levels of SVA reduced the potential for seroconversion (4-fold increase from baseline). Usually for vaccines, no upper age limit to the therapeutic indication is expected to be imposed unless there are specific concerns raised by the data in younger adults; it is considered sufficient to mention the limitations of the data in the SmPC. Cross-protection to other biotypes and serotypes of V. cholera showed that the frequency of seroconversion at Day 11 to the Ogawa serotypes (for both Classical and El Tor biotypes) was reduced compared to the Inaba serotypes in individuals aged 18-45 in the Challenge study (n=94) (Classical Ogawa 87.1% [78.5%, 93.2%]; El Tor Ogawa 89.2% [81.1%, 94.7%]) and especially so in participants 46-64 years of age (n=291) (Classical Ogawa 73.2% [67.7%, 78.2%]; El Tor Ogawa 71.4% [65.8%, 76.5%]. These data and information about the lack of data in participants >64 years of age is included in section 5.1 and 4.4 of SmPC and is considered acceptable.

The attenuated V. cholerae vaccine strain was not as effective as the virulent challenge strain in inducing SVA responses. Twenty eight days following challenge, the combined placebo group (n=66) had a SVA geometric mean titre of 17,409 compared to the 10 day challenge group (n=35), which had a GMT of 2460 (p<0.0001), and the 3-month challenge group (n=33) a GMT of 1646.9 (p<0.0001) at the same time point. Similarly, the percentage of participants with a 4-fold rise in SVA 28 days post-challenge was 5.7% for the 10 day challenge group and 60.6% for the 3-month challenge group compared to placebo where 97% participants showed a 4-fold increase in titre. The post-challenge SVA against different biotypes and serotypes was similarly greater in the placebo group compared to the vaccine group. Again, responses against the Ogawa serotypes were lower than those against the Inaba serotypes as was observed for pre-challenge SVA antibodies. For the placebo group, the challenge corresponded to their first exposure to V. cholerae. The potential mechanisms underlying these observations and the implications of these findings with respect to the potential for re-vaccination (booster immunisations) have been discussed by the Applicant, where re-boosting

is not considered to increase SVA. No re-boosting studies are planned and the interval for potential re-vaccination remains unknown as described in the SmPC.

The duration of protection provided by PXVX0200 is not known and no studies have been carried out to determine when re-vaccination (booster immunisations) would be required. This is of particular importance for aid workers who may stay in endemic regions beyond three months, or may return a year or so later. Furthermore, the lower SVA response post-challenge amongst vaccinees raises the question as to whether SVA (which detects predominantly serum IgM) represents a suitable parameter for measuring secondary immune responses and if remaining immunity could prevent a secondary response. Scientific advice in 2012 recommended a second study with challenge at 6-months, but this could be done in the post-approval period. In a 2017 scientific advice to the Applicant, the CHMP maintained that the duration of protection could not be concluded based upon currently available data and that a challenge study at six months post-vaccination would provide the best possible data on the duration of protection. However, the CHMP acknowledged that a challenge study is an artificial situation and may not entirely reflect field exposure to cholera. The CHMP therefore concluded that it was acceptable not to conduct a challenge study at six months post vaccination. The uncertainties with regards to duration of protection will be described in the SmPC. Earlier work with Orochol inferred duration of protection in a revaccination study where participants were re-vaccinated 2.5 and 3.5 years later. While the duration of protection beyond 6 months against challenge with wild-type V. cholerae remains unknown, the Applicant considers that individuals with pre-existing immunity to V. cholerae are not likely to be further boosted after re-immunisation with CVD 103-HgR (Orochol). This is because pre-existing immunity will remove the live attenuated vaccine used to boost immunity, thereby reducing its potential for effect.

A fold-increase from Day 1 to Day 91 in the percentage of anti-LPS IgA memory B cells was found to be associated with total diarrhoeal volume (Spearman correlation=-0.56, $p<0.001$, $n=33$) in the challenge study. However, B-cell memory responses were based on an ELISPOT assay that was qualified but not validated. The assay was based on frozen cells and thresholds for cell recovery and viability were not provided, no criteria for assay validity. The findings are therefore preliminary. An increase in IgA B-cell memory responses to O1 lipopolysaccharides (LPS) from baseline to day 91 showed some correlation with total post challenge stool volume in the 3-month challenge group study. Significant increases in anti-O1 LPS IgA were also observed from baseline to day 91 and day 181 respectively when pooling eligible samples from all clinical studies, however these participants were not challenged. It is therefore not possible to correlate these findings to efficacy.

The frequency of SVA seroconversion to V. cholerae Classical Inaba was non-inferior in older adults <64 years when compared to younger adults (18-45 years) in the Lot consistency study, and slightly higher in children >6 – 18 years. A non-inferior frequency of seroconversion could suggest similar protective efficacy, however the frequency of seroconversion declines with advancing age. The higher frequency of seroconversion in children could reflect the lack of prior exposure to cross-reactive antigens from other enteric bacteria.

Baseline characteristics for the challenge clinical study showed a greater number of black/African American participants compared to white participants. However, these groups were more balanced in subsequent pooled analyses assessing immunogenicity and the frequency of seroconversion. Combining data from all studies, whites were associated with a higher serum vibriocidal antibody titre to classical Inaba at Day 11 compared to black or African American ($p=0.0005$), but this did not provide any statistically significant advantage in seroconversion.

In the challenge study there was a greater number of men compared to women participants, although in subsequent studies were more balanced. Men exhibited approximately 15.7% higher Day 11 SVA titres against classical Inaba than women ($p<0.0001$), although the difference was not large enough to yield significant differences in seroconversion ($p= 0.2353$).

Vaccine recipients with blood type O had significantly higher classical Inaba vibriocidal titres ($p=0.0421$) than vaccine recipients with non-O blood types, but the difference was modest, similar in magnitude to the difference between sexes. Specifically, the geometric mean ratio of blood type O to non-O was 1.138 [1.005, 1.288]. The difference in GMTs was not large enough to produce a significant difference in seroconversion ($p=0.1835$).

2.5.6. Conclusions on the clinical efficacy

The clinical efficacy data show that a single administration of PXVX0200 (5×10^8 CFU) induces a protective effect against moderate/severe diarrhoea of 90.3% upon challenge 10 days post-vaccination and 79.5% at 3-months post-vaccination in adults 18-45 years of age.

A pre-defined serum vibriocidal antibody (SVA) seroconversion rate (>4-fold increase in titre from baseline) represented an immune marker that correlated with protection to support immunobridging to younger and older age groups. SVA were detected using a functional assay involving complement-mediated bacteriolysis. Bridging analysis showed that SVA seroconversion rates in adults 18-45 years from a large Lot consistence clinical trial (n=3146) were non-inferior to seroconversion rates in the Challenge study. Seroconversion rates in older adults (46-64 years of age) and children (6-18 years of age) were found to be non-inferior to seroconversion rates in the Lot consistency study. However, SVA seroconversion rates to the Ogawa serotype were consistently low when compared to the Inaba serotypes. Seroconversion rates declined with advancing age, and no data is available for individuals over the age of 64 years. Lack of data in individuals over 64 years is included in section 5.1 of the SmPC. No major safety concerns were observed that could be considered to prevent the use of the vaccine in the elderly.

The drop in protection against disease from challenge 10 days post-vaccination to 3 months post-vaccination is a concern. While 85.7% of subjects had no qualifying diarrhoea when challenged 10 days post-vaccination, the number decreased to 54.5% in participants challenged 3 months post-vaccination.

The duration of protection is currently not known.

In conclusion, Vaxchora has been shown to be immunogenic and its efficacy in preventing cholera has been documented in well-controlled vaccination and challenge clinical trial settings.

2.6. Clinical safety

2.6.1. Patient exposure

A total of 3235 adults have been exposed to Vaxchora (55 subjects exposed in a phase I study and 3180 subjects in phase 3 studies). The safety database consists mainly of subjects from the Lot consistency trial (PXVX-VC-200-004, n=2789). In addition, 328 children aged 6-18 years, were exposed in a phase 4 study. In all cases, Vaxchora was administered as a single dose.

An overview over clinical studies and overall exposure to Vaxchora, can be found in the table below.

Table 37 Overview of Clinical Studies and Overall Exposure to Vaxchora

Type of Trial	Trial No. Lit. ref.	Objectives of the Trial	Trial Design and Type of Control	Safety Followup Duration	Country	Age Range/years	Number Enrolled *(Number of Subjects who Received Study Vaccine)*
Phase 1	PXVX-VC-200-002 Chen 2014	Safety and immunogenicity	Randomized, double-blind, placebo-controlled	6 months	US	18 to <51	66 *(55 vaccine, 11 placebo)*
Challenge Phase 3	PXVX-VC-200-003 Chen 2016	Demonstrate protection from live cholera challenge	Randomized, double-blind, placebo-controlled	6 months	US	18 to <46	197 *(95 vaccine, 102 placebo)*
Lot Consistency Phase 3	PXVX-VC-200-004 McCarty 2018	Demonstrate clinical lot consistency	Randomized, double-blind, placebo-controlled	6 months	US and Australia	18 to <46	3146 *(2789 vaccine, 350 placebo)*
Older Adults Phase 3 46 to <65	PXVX-VC-200-005	Safety and immunogenicity	Randomized, double-blind, placebo-controlled	6 months	US	46 to <65	398 *(296 vaccine, 99 placebo)*
Paediatric Phase 4 Cohorts 1 and 2 only: 6 to <18	PXVX-VC-200-006 (interim report)	Safety and immunogenicity	Randomized, double-blind, placebo-controlled	6 months	US	6 to <18	381 *(328 vaccine, 50 placebo)*
					Applicant-sponsored Studies Total Subjects Exposed:		*3563 Vaxchora*
Phase 2 in Mali vs Shanchol	CVD 51000 Sow 2017	Safety and immunogenicity	Randomized, double-blind, placebo-controlled, placebo cross-over	6 months	Mali	18 to <46	150 *(98 Vaxchora, 50 Shanchol, 2 placebo only)*
					Overall Total Subjects Exposed:		*3661 Vaxchora*

Pooling strategy for safety analyses

The Applicant-sponsored studies in adults (4 studies) were pooled to a total of 3235 subjects who received Vaxchora for integrated safety analyses. Integrated safety analyses of the adult trials do not include subjects included in the CVD 51000 trial published by Sow et al. 2017. CVD 51000 was an investigator-sponsored study, and the observed adverse event profile was consistent with that seen in the other trials of Vaxchora.

The Applicant provided furthermore the Interim Clinical Study Report for the phase 4 paediatric trial PXVX-VC-200-006, which contains safety analyses for 328 subjects who received Vaxchora included in cohorts 1 (12 - < 18 years) and 2 (6- < 12 years) [cohort 3 (2 - < 6 years) was not included in the analysis]. The data cut-off for the paediatric study is 19 July 2018.

2.6.2. Adverse events (AEs)

Safety assessment of the vaccine included evaluation of solicited AEs (reactogenicity) as well as unsolicited AEs (treatment-emergent AEs). Solicited AEs were assessed Day 1-7 following vaccination. Unsolicited AEs were assessed through day 29, but serious AEs (SAEs) were assessed through day 181.

Solicited adverse events in adults

Solicited AEs were reported by about 50% of vaccine recipients and about 46% of placebo recipients. Tiredness, headache and various gastrointestinal disorders were reported at a frequency ≥1/10 (very common) in both the vaccine and placebo groups. Of the specified symptoms, only diarrhoea occurred at a significantly increased frequency compared to placebo (3.63% in adult vaccine recipients vs. 1.63% in placebo recipients; p=0.0140). Most AEs were mild, occurred within 1-3 days following vaccination and decreased progressively thereafter. In children solicited AEs were reported by about 60% of the subjects both in vaccine and placebo recipients.

Table 38 Reactogenicity Signs and Symptoms by Highest Reported Severity – Safety population (adults)

Signs and Symptoms	PXVX0200 N=3235	Placebo N=562	P-value[a]
No. of Subjects Assessed	3177	553	
Subjects who Reported Any Reactogenicity [b]	1591 (50.08%)	253 (45.75%)	0.0652
95% CI[c]	[48.32%, 51.83%]	[41.54%, 50.01%]	
Tiredness[d]	953 (30.00%)	163 (29.48%)	0.8405
95% CI[c]	[28.41%, 31.62%]	[25.70%, 33.47%]	
Mild	571 (17.97%)	93 (16.82%)	
Moderate	360 (11.33%)	63 (11.39%)	
Severe	22 (0.69%)	7 (1.27%)	
Potentially Life-Threatening	0	0	
Headache[d]	882 (27.76%)	144 (26.04%)	0.4390
95% CI[c]	[26.21%, 29.35%]	[22.43%, 29.91%]	
Mild	579 (18.22%)	87 (15.73%)	
Moderate	287 (9.03%)	53 (9.58%)	
Severe	16 (0.50%)	4 (0.72%)	
Potentially Life-Threatening	0	0	
Abdominal Pain[d]	582 (18.32%)	94 (17.00%)	0.4736
95% CI[c]	[16.99%, 19.71%]	[13.96%, 20.39%]	
Mild	385 (12.12%)	65 (11.75%)	
Moderate	185 (5.82%)	28 (5.06%)	
Severe	12 (0.38%)	1 (0.18%)	
Potentially Life-Threatening	0	0	
Nausea/Vomiting[d]	554 (17.44%)	86 (15.55%)	0.2990
95% CI[c]	[16.13%, 18.80%]	[12.63%, 18.85%]	
Mild	404 (12.72%)	62 (11.21%)	
Moderate	141 (4.44%)	22 (3.98%)	
Severe	9 (0.28%)	2 (0.36%)	
Potentially Life-Threatening	0	0	
Lack of Appetite[d]	495 (15.58%)	93 (16.82%)	0.4488
95% CI[c]	[14.34%, 16.89%]	[13.79%, 20.20%]	
Mild	346 (10.89%)	71 (12.84%)	
Moderate	137 (4.31%)	19 (3.44%)	
Severe	12 (0.38%)	3 (0.54%)	
Potentially Life-Threatening	0	0	

Signs and Symptoms	PXVX0200 N=3235	Placebo N=562	P-value[a]
Diarrhea[d]	115 (3.62%)	9 (1.63%)	0.0140
95% CI[c]	[3.00%, 4.33%]	[0.75%, 3.07%]	
Mild	70 (2.20%)	5 (0.90%)	
Moderate	21 (0.66%)	2 (0.36%)	
Severe	23 (0.72%)	2 (0.36%)	
Potentially Life-Threatening	1 (0.03%)	0	
Fever[d,e]	22 (0.69%)	6 (1.08%)	0.2915
95% CI[c]	[0.43%, 1.05%]	[0.40%, 2.35%]	
Mild	10 (0.31%)	2 (0.36%)	
Moderate	9 (0.28%)	3 (0.54%)	
Severe	2 (0.06%)	1 (0.18%)	
Potentially Life-Threatening	1 (0.03%)	0	

Note: The number of subjects assessed is the number of subjects who completed a memory aid following vaccination in PXVX-VC-200-002 (Phase 1), PXVX-VC-200-003 (Challenge), PXVX-VC-200-004 (Lot), and PXVX-VC-200-005 (Older).

Note Percentages are based on the number of subjects in PXVX-VC-200-002 (Phase 1), PXVX-VC-200-003 (Challenge), PXVX-VC-200-004 (Lot), and PXVX-VC-200-005 (Older). The denominator is the number of subjects assessed.

[a] P-value is from a Fisher's exact test comparing the frequency of the corresponding reactogenicity sign or symptom between the vaccine and placebo recipients.

[b] Counts subjects who report reactogenicity of any severity at any time from Day 1 through Day 8 following vaccination.

[c] Exact confidence interval is for the proportion of subjects in the given treatment arm who recorded the reactogenicity event(s) on the memory aid.

[d] Subjects are counted at most once at the highest severity level reported for the corresponding sign or symptom at any time within 8 days following vaccination.

[e] Fever was defined as a body temperature greater than 100.4°F measured by oral thermometer.

Unsolicited adverse events in adults

Overall, for the one Phase 1 (through Day 181) and three Phase 3 trials (through Day 29), AEs were reported by 23.7% of vaccine recipients and 27.4% of placebo recipients and were mostly mild in severity. The three most common AEs with an incidence in the range of 2% (range of 2.1% to 3.2%) in both vaccine and placebo groups were headache, fatigue, and upper respiratory tract infection. No meaningful differences were observed between vaccine and placebo recipients in the incidence of AEs by PT or grouped by SOC. Differences in the incidence of AEs were not assessed for statistical significance.

Table 39 Treatment-related Unsolicited Adverse Events in Decreasing Order of Incidence (Adult safety population)

Preferred Term[a] [n, (%)]	PXVX0200 N=3235	Placebo N=562
Number of subjects with at Least One Vaccine-Related AE	255 (7.9)	48 (8.5)
Fatigue	39 (1.2)	14 (2.5)
Flatulence	30 (0.9)	5 (0.9)
Headache	28 (0.9)	6 (1.1)
Abdominal pain	20 (0.6)	3 (0.5)
Decreased appetite	18 (0.6)	5 (0.9)
Dizziness	13 (0.4)	2 (0.4)
Pain	12 (0.4)	0
Nausea	10 (0.3)	4 (0.7)
Constipation	10 (0.3)	2 (0.4)
Abdominal distension	8 (0.2)	3 (0.5)
Dyspepsia	8 (0.2)	2 (0.4)
Oropharyngeal pain	8 (0.2)	0
Abnormal faeces	7 (0.2)	1 (0.2)
Diarrhoea	6 (0.2)	4 (0.7)
Arthralgia	6 (0.2)	1 (0.2)
Cough	6 (0.2)	1 (0.2)
Abdominal pain upper	6 (0.2)	0
Back pain	5 (0.2)	1 (0.2)
Insomnia	5 (0.2)	1 (0.2)
Neck pain	5 (0.2)	1 (0.2)
Abdominal discomfort	5 (0.2)	0
Dry mouth	5 (0.2)	0

Note: Percentages are based on the number of subjects in PXVX-VC-200-002 (Phase 1), PXVX-VC-200-003 (Challenge), PXVX-VC-200-004 (Lot), and PXVX-VC-200-005 (Older). The denominator represents the total number of subjects who received treatment combined from all trials. Only treatment-emergent adverse events are presented. Challenge-emergent adverse events are excluded.

Note: Subjects are counted once within each preferred term. Preferred terms are listed in order of descending incidence in all vaccine recipients, then all placebo recipients, and remaining ties are ordered alphabetically.

[a] All adverse event terms were coded using MedDRA dictionary version 15.0.

In adults, the incidence of subjects with at least one unsolicited AE considered to be treatment-related was similar for the placebo group (8.5%) and the vaccine group (7.9%). The most frequent events by SOC were different gastrointestinal disorders, reported in 3.7% of subjects both in the vaccine- and placebo- group.

For the paediatric population, the incidence of subjects with at least one unsolicited AE considered to be treatment-related, was higher for the vaccine group (14.6%) than for the placebo group (10.0%). Treatment related AEs in the group of solicited AEs occurred in high frequency in the paediatric population (47.3 % (V) vs. 44.0 % (P)). Abdominal pain (24.1 % vs 16.0 %), Lack of Appetite (17.7 % vs 12.0 %) and Vomiting (3.4 % vs 0 %) occurred more often in the vaccination arm than in the placebo arm. For both solicited and unsolicited AEs, data indicate that frequencies of AEs were in general higher for children than for adults. Also for children, the most frequent events by SOC were Gastrointestinal disorders such as abdominal pain, pyrexia, fatigue, headache and decreased appetite. These were more commonly observed in children compared to adults. Diarrhoea was more often observed in adults compared to children.

The Tabulated summary of adverse reactions in section 4.8 of the SmPC is based on a combination of the solicited and unsolicited AEs.

2.6.3. Serious adverse event/deaths/other significant events

One death (suicide) occurred on day 85 in a vaccine recipient and was not considered to be related to the vaccine. Serious adverse events through Day 181 were uncommon. The reporting incidence of SAEs in adults was 0.6% for vaccine- and 0.5% for placebo-recipients. None of these events were considered to be related to vaccine or placebo. In the paediatric trial, no serious adverse events considered to be vaccine-related were reported.

2.6.4. Laboratory findings

Clinical safety laboratory evaluations were performed at screening and Day 7 in the Phase 1 study and at screening in the challenge and older adult studies to ensure that subjects were healthy and eligible for study participation. No trends between pre-vaccination values on Day 1 and post-vaccination values on Day 7 were observed in the clinical safety laboratory evaluations in the Phase I trial.

No clinical safety laboratory evaluations were performed in the paediatric trial.

For the adult population shifts in pre and post vaccination vital signs (i.e. BP, Pulse, respiratory rate, temperature) were evaluated. Abnormal vital signs were graded using the Tables for Laboratory Abnormalities from Section III, Table B of the FDA Guidance for Industry, Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, [US FDA 2007]. No meaningful post-vaccination worsening was observed relative to placebo for vital signs or physical findings in the adult population. However, a few cases of hypokalaemia were noted in the vaccination arm.

For the paediatric trial, vital signs were listed for each of the individuals for screening and for Day 1 (time point of inclusion). No post-vaccination vital signs were generated in this study.

2.6.5. Safety in special populations

Elderly patients

No data are available for patients ≥64 years.

Pregnant women

No animal data is available as the vaccine bacteria do not replicate in animal toxicology models. Pregnant women were excluded from the clinical trials. In the trials, 10 vaccine recipients were found to be pregnant following vaccination. Each pregnancy was followed to term and resulted in a healthy baby. One case was lost to follow-up. No safety signal has been detected so far.

Immunocompromised subjects

No data are available as immunocompromised subjects were excluded from the clinical trials. Vaxchora is contraindicated in individuals with congenital immune deficiency or receiving immunosuppressive drugs. The protection afforded by Vaxchora may be reduced in HIV-infected individuals, but this use is not contraindicated.

Vaccine shedding and potential transmission to household contacts

Shedding of the vaccine organism into stools of the vaccine recipients and potential transmission to their household contacts (HHCs) was studied in the Phase I trial (CSR PXVX-VC-200-002) during Day 1 to 7 following vaccination. Shedding reached a peak on day 7 when it was detected in 7.3% (95% CI 2.0-17.6) of

vaccine recipients. Cumulatively through to Day7 post-vaccination, shedding of vaccine virus occurred in a total of 11.3 % (95% CI 4.2-22.6) of vaccine recipients. Potential transmission of the vaccine organism from vaccine recipients to their HHCs was monitored via the detection of either shedding and/or serum vibriocidal antibodies in HHCs. No transmission to household contacts could be detected.

The duration of shedding of the vaccine strain is unknown. However in a study of CVD 103-HgR in Mali (Perry 1998), bacteriological test to detect vaccine virus shedding were taken the day of vaccination, then daily for the first 4 days and again on 6th and 12th days after dosing. The vaccine strain was not isolated from the co-pro-cultures of any subjects. In a cholera treatment study (Lindenbaum 1967), 94 untreated control patients shed *V. cholerae* for a mean duration of 5.8 days with a range of 1-13 days, which supports the Applicant's statement that shedding will be unlikely after day 14.

2.6.6. Safety related to drug-drug interactions and other interactions

No interaction studies have been performed for the product with other vaccines or other medicinal products. However, some data and clinical experience have been generated with other similar vaccines, which can be applicable to Vaxchora.

The vaccine is acid-labile and is administered with a buffer. Eating and drinking should therefore be avoided for 60 minutes before and after oral ingestion of Vaxchora, as this may interfere with the protective effect of the buffer.

Vaxchora should not be administered concomitantly with systemic antibiotics active against V. cholerae since these agents may prevent to some degree bacteria multiplication, which is necessary in order to induce a protective immune response.

Concomitant use of immunosuppressive drugs is contraindicated as with any live vaccine.

Concomitant use of chloroquine was shown to decrease the immune response to the earlier vaccine Orochol and the Orochol package insert advised to start chloroquine prophylaxis no sooner than 1 week after administration of Orochol. Although interaction studies with PXVX0200 have not been conducted, a warning has been included in the SmPC of Vaxchora against concomitant administration with chloroquine based on the experience with Orochol.

The Orochol SmPC included a warning that the vaccine should not be administered directly together with Vivotif (typhoid vaccine) as the enteric coating of the drug might be damaged by Orochol. For Orochol it was suggested that Orochol and Vivotif should be separated by 8 hours. Studies of concomitant vaccination with oral polio vaccine and yellow fever vaccine (YF 17D) demonstrated that these vaccines did not suppress the immune response to Orochol and that the immune response to YF 17D was also not suppressed by Orochol.

A formal interaction study between Vaxchora and Vivotif has not been performed. However, the Applicant has assessed to what extent a separation of concomitantly administered Vaxchora and Vivotif is necessary based on the nature of the buffer used in Vaxchora and on data from published literature. The current marketed formulation of Vivotif (typhoid vaccine, live, oral, Ty21a) consists of enteric coated capsules, and it is possible that the buffer used with Vaxchora vaccine could adversely affect the protective effect of the enteric coating of Vivotif. It is estimated that the buffer of Vaxchora rapidly interacts with the acid in the stomach allowing a return to normal pH values within a time frame of two hours. The SmPC is therefore suggesting a separation time of two hours between the administration of Vivotif and Vaxchora, which is supported.

2.6.7. Discontinuation due to adverse events

In the adult pooled data, only three individuals discontinued the study due to AEs after having received the vaccine. Thus, discontinuation rates in the adult study pool are very low and acceptable.

Of the 378 children having received the vaccine, at study day 181 ten individuals (8 receiving the vaccine (V) and 2 receiving placebo (P)) were lost to follow up, and for 10 additional individuals (8 (V)/2 (P)) parents withdrew the allowance to continue study participation.

Overall discontinuation rates are acceptable.

2.6.8. Post marketing experience

The product was licensed in US in 2016, and a total of 70,041 doses have been distributed until September 2018. According to the Applicant no new safety issues have been identified so far. However, there are in total 16 post marketing reported cases, and eight of these consist of various forms of medication errors (drug administration errors). These concern instances where the subject has eaten/drunk at inappropriate time points, administration of expired product and administration of product without buffer.

In addition, the product may be stored incorrectly (product should be stored in a refrigerator) and the shelf-life following reconstitution of the vaccine is short. In the US the vaccine is prepared and administered in a healthcare setting. In the EU the vaccine will be prepared and self-administered and preparation of the vaccine and self-vaccination increase the risk of medication error, which can lead to vaccine failure.

The Applicant has submitted a Vaxchora Usability Study Report, rev 1 dated 02 Aug 2019 and a Memorandum regarding Vaxchora Risk Benefit Analysis. In the Usability study 21 different user errors have been studied in 18 subjects who were responsible for preparing their own medication, of which 13 were characterized as "critical use errors". Only one of these critical user errors was considered having a "high risk": the risk of touching face or eyes during reconstitution, which could potentially lead to infection that could require antibiotics. The expert report concludes that there is an inherent tendency of humans to subconsciously touch their face and that no further changes to the packaging or labelling could further reduce this risk level.

2.6.9. Discussion on clinical safety

A total of 3235 adults and 328 children/adolescents (6 - <18 years) were exposed to the vaccine and included in the safety database. No data are currently available for children < 2 years, but a study is ongoing in children 2-<6 year. No data are available for subjects > 64 years. The size of the database is considered acceptable and in line with available guidance.

Safety assessment of the vaccine included evaluation of solicited AEs (reactogenicity) as well as unsolicited AEs (treatment-emergent AEs). Solicited AEs were assessed Day 1-7 following vaccination. Unsolicited AEs were assessed through day 29, but SAEs were assessed through day 181. Solicited AEs were reported by about 50% of the vaccine recipients and about 46% of the placebo recipients. Tiredness, headache and various gastrointestinal disorders were reported at frequency ≥1/10 (very common) in both the vaccine and placebo groups. Of the specified symptoms, only diarrhoea occurred at a significantly increased frequency compared to placebo (3.63% in adult vaccine recipients vs. 1.63% in placebo recipients; p=0.0140). Most AEs were mild or moderate, occurred within 1-3 days following vaccination and decreased progressively thereafter.

Overall in adults, for the one Phase 1 (through Day 181) and three Phase 3 trials (through Day 29), unsolicited AEs were reported by 23.7% of vaccine recipients and 27.4% of placebo recipients and were mostly mild in severity. In adults, the incidence of subjects with at least one related unsolicited AE was similar between the placebo group (8.5%) and the vaccine group (7.9%). The most frequent events by SOC were different gastrointestinal disorders, reported in 3.7% of subjects both in the vaccine- and placebo-group.

Serious adverse events through Day 181 were uncommon. The reporting incidence in adults was 0.6% for vaccine- and 0.5% for placebo-recipients. None of these events were considered related to vaccine or placebo. One death (suicide) occurred on day 85 in a vaccine recipient and was not considered related to vaccine.

Shedding of the vaccine organism into stools of the vaccine recipients reached a peak on day 7 post-vaccination when it was detected in 7.3% (95% CI 2.0-17.6) of vaccine recipients. No transmission to household contacts could be detected. Duration of viral shedding with Vaxchora remains unknown, however data from the medical literature support the assumption that shedding will be unlikely after day 14. The low frequency of shedding (7.3% of participants) may be related to the development of serum vibriocidal antibodies by most individuals, which will control infection by day 10 post vaccination. The SmPC and the PL include a warning that there is a potential for transmission of the vaccine strain to non-vaccinated close contacts, which is acceptable.

Concerning use of the product during pregnancy and lactation, data is very limited. Following licensure in US, a pregnancy registry was established as a post-marketing commitment to FDA to track women who were pregnant at the time of Vaxchora administration. As data during pregnancy is very limited, use during pregnancy is categorized as missing information in the RMP. Cases from EU will be included in the US pregnancy registry and should be reported in upcoming PSURs.

No interaction studies have been performed for Vaxchora. The vaccine is acid-labile and is administered with a buffer. Eating and drinking should therefore be avoided for 60 minutes before and after oral ingestion of Vaxchora, as this may interfere with the protective effect of the buffer. Concomitant administration of immunosuppressive drugs is contraindicated. The SmPC also warns against concomitant administration of Vaxchora with systemic antibiotics active against V. cholera and chloroquine, since these agents may diminish the immune response based on previous data and clinical experience.

Based on post-marketing data from the US (a total of 70,041 doses have been distributed from launch in 2016 until September 2018), no new safety issues have been identified so far. However, there are in total 16 post marketing reported cases, and eight of these concern various forms of medication errors e.g. the subject has eaten/drunk at inappropriate time points, administration of expired product and administration of product without buffer. Additionally, the vaccine may be stored incorrectly (the vaccine should be stored in a refrigerator). Based on the initial data, it was considered that the shelf-life of the reconstituted vaccine was too short in the context of a self-administration, which would increase the risk of medication error potentially leading to vaccine failure. In addition, considering that even under administration in a healthcare setting as in US, medication errors are reported, it was deemed necessary to include medication error in the RMP as an important potential risk.

During the procedure, the Applicant has presented new improved stability data that minimises the risk of medication errors to an acceptable level so that self-administration could be considered acceptable. Detailed information on how to prepare and administer the vaccine is provided in the SmPC and PL. Additionally, educational material for HCPs and vaccine users will be prepared as reflected in Annex II of the Marketing

Authorisation. Finally, based on additional data presented by the Applicant during the procedure, the risk of serious allergic reactions with the product is anticipated to be very low. Handling possible serious allergic reactions outside a healthcare setting is considered adequately described in the SmPC and PL. Allergic reactions to vaccine components and to previous use of Vaxchora are listed as contraindications in the SmPC. In addition, the PL states that a doctor should be contacted immediately if the following serious side effects occur, e.g. serious allergic reactions causing swelling of the face or throat and hives, itchy rash, breathlessness and/or a drop in blood pressure and fainting.

For all these above-mentioned reasons, it is considered that the risks raising from self-administration are adequately minimised and self-administration is acceptable and can be advantageous for the traveller.

From the safety database all the adverse reactions reported in clinical trials and post-marketing have been included in the Summary of Product Characteristics.

Assessment of paediatric data on clinical safety

A clinical trial was conducted in 374 children age 6 to <18 years (of which 50 received placebo). Solicited AEs were reported by about 60% of the subjects both in vaccine as well as placebo recipients. The incidence of subjects with at least one related unsolicited AE was higher for the vaccine group (14.6%) than for the placebo group (10.0%). In children like in adults, the most frequent events by SOC were gastrointestinal disorders. For both solicited and unsolicited AEs, data indicate that frequencies were in general higher for children than for adults. AEs such as abdominal pain, pyrexia, fatigue, headache and decreased appetite were more commonly observed in children compared to adults [headache (36.0% vs 28.3%), fatigue (37.9% vs 30.2%), abdominal pain (32.6% vs 18.4%), vomiting (5.3% vs 0.2%), decreased appetite (22.4% vs 15.7%) and pyrexia (2.5% vs 0.8%). Diarrhoea was more often observed in adults compared to children.

As in adults, most AEs were mild or moderate, occurred within 1-3 days following vaccination and decreased progressively thereafter.

In the paediatric trial, no serious adverse events considered to be vaccine-related, were reported.
From the safety database all the adverse reactions reported in paediatric clinical trials have been included in the Summary of Product Characteristics.

2.6.10. Conclusions on the clinical safety

Safety data from >3000 adults as well as >300 children above 6 years were presented. The most common adverse reactions are fatigue, headache and various gastrointestinal disorders which were reported with the same incidence among vaccine and placebo recipients (ranging from 15 to 30%). Only diarrhoea occurred at a significantly higher incidence with the vaccine compared to placebo. No serious adverse events considered to be related to the vaccine have been reported.

It is proposed that the vaccine can be prepared and administered by the user, which entails a risk of medication errors. However, the Applicant has presented new stability data that minimise such risk. It is therefore considered that the Applicant has justified how the risks of medication errors of bigger concern (vaccine failure) can be taken care of when the vaccine is self-administered.

In addition, risk minimisation measures are reflected in Annex II of the Product Information, including preparing and distributing a HCP guide and a user guide.

The risk of serious allergic reactions is anticipated to be very low based on the presented data. In case of

such an unexpected event, adequate information regarding handling of the event is given in the PL.

As data during pregnancy is very limited, use during pregnancy is categorized as missing information in the RMP. Cases from the EU will be included in the US pregnancy registry and should be reported in upcoming PSURs.

2.7. Risk Management Plan

Safety concerns

Important identified risks	None
Important potential risks	Medication errors
Missing information	Use during pregnancy

Pharmacovigilance plan

Study (study short name, and title) Status (planned/on-going)	**Summary of objectives**	**Safety concerns addressed**	**Milestones (required by regulators)**	**Due dates**
Category 3 - Required additional pharmacovigilance activities				
The PXVX-VC-200-PR, VAXCHORA Pregnancy Registry: an observational prospective study of the safety of VAXCHORA exposure on pregnant women and their offspring. Ongoing	The objective of the VAXCHORA Pregnancy Registry is to evaluate pregnancy outcomes in women immunized with VAXCHORA vaccine within 28 days prior to conception or at any time during pregnancy.	preterm birth, low birth weight, spontaneous abortions, stillbirths. Other pregnancy outcomes will be collected including major congenital malformation.	Annual report	-Annual report -Data reviewed on an on-going basis as a part of signal detection and reported with the PSUR
			Five-year summary report	Submitted to EMA 5 years after start of registry (continuation to be determined at that time)

Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Important identified risks		
None		
Important potential risks		
Medication errors	**Routine risk minimisation measures:** • SmPC sections 4.2, 6.3, 6.4, 6.6 • PL section 2, 3, 5 • Medicinal product subject to medical prescription **Additional risk minimisation measures:** Additional risk minimisation measures will include a patient guide containing key messages and administration highlights. Also, a health care professional's guide (checklist) for assisting the provider with instructing patients will be provided.	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** None **Additional pharmacovigilance activities:** None proposed
Missing information		
Use during pregnancy	**Routine risk minimisation measures:** • SmPC sections 4.6 • PL section 2 • Medicinal product subject to medical prescription **Additional risk minimisation measures:** No risk minimisation measures	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** None **Additional pharmacovigilance activities:** The PXVX-VC-200-PR VAXCHORA Pregnancy Registry observational study was initiated in September 2016 and is ongoing.

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.0 of 27 January 2020 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 10 June 2016. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The applicant declared that *V. cholerae* live, attenuated strain CVD 103-HgR has not been previously authorised in a medicinal product in the European Union.

The CHMP, based on the available data, considers *V. cholerae* live, attenuated strain CVD 103-HgR to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Vaxchora [Cholera vaccine (recombinant, live, oral)] is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Cholera is an acute and potentially fatal toxigenic diarrhoeal illness caused by the bacterium Vibrio cholerae. Humans are the only host for V. cholerae. The vibrio predominantly associated with epidemic cholera is V.

cholerae serogroup O1. O1 vibrios contain an enterotoxin (cholera toxin) which is responsible for causing diarrhoea.

V. cholerae O1 is divided into two biotypes, Classical and El Tor. Both biotypes contain two major serotypes, Inaba and Ogawa. Worldwide, V. cholerae O1 El Tor is currently the predominant biotype.

3.1.2. Available therapies and unmet medical need

Cholera can be successfully treated with prompt and adequate replacement of lost fluid and electrolytes. Antibiotics may shorten the course of the diarrhoea and/or diminish the severity of the illness, to be used in addition to rehydration.

In Europe, Australia, New Zealand, and Canada, an inactivated cholera vaccine is available for travellers to epidemic and endemic areas from 2 years of age (Dukoral), and therefore fulfils to some extent the medical need for travellers in Europe. The development of efficacious cholera vaccines is important also to combat drug resistance which for instance is a frequent occurrence in cholera-endemic areas and can complicate the treatment of cholera and increase treatment costs.

Currently, the WHO position paper (2017) regarding cholera vaccination has the following recommendation for international workers and travellers: "Vaccination is not generally recommended for long-term or short-term travellers to cholera-affected countries but should be guided by specific travel risks". Whilst for healthcare workers, the following is recommended: «OCV (oral cholera vaccine) should be considered for emergency and relief workers who are likely to be directly exposed to cholera patients or to contaminated food or water, particularly those staying in areas with poor access to health-care facilities. Other health-care workers are generally not at special risk of cholera. »

Orochol was another vaccine against cholera available from 1993 until it was withdrawn for commercial reasons in 2003. Orochol included the same V. cholerae strain as Vaxchora.

3.1.3. Main clinical studies

The main study was a randomized, double-blind, placebo-controlled Phase 3 challenge trial PXVX-VC-200-300 where safety and efficacy as compared with placebo following challenge at 10 days or at 3 months post-vaccination in healthy volunteers aged 18 to 45 years. Participants were given a single dose of Vaxchora (Classical, Inaba) at a dose of 5 x 108 CFU. The primary efficacy endpoint was the occurrence of moderate or severe diarrhoea (≥3.0 L cumulative purge) post-challenge with virulent V. cholerae O1 El Tor Inaba 10 days (Day 11) and 3 months post-vaccination (Day 91) in two separate challenges. The co-primary objectives of the study were to demonstrate that the lower 95% confidence bound on the protective efficacy was ≥30% at both time points. Meeting the objective at both the 10-Day and 3-Month Challenges was required to achieve success for the trial.

Immunogenicity was based on serum vibriocidal antibody (SVA), and the main parameter was seroconversion measured at Day 11 and predefined as a 4-fold increase in SVA titre from baseline. There was a statistically significant association between fold-rise in SVA from Day 1 to Day 11 and total post-challenge diarrhoeal volume (Spearman's r=-0.72; p<0.001). Only 2 of 62 (3%) of seroconverting vaccine recipients developed moderate/severe cholera after challenge. Based on this near 1:1 relationship between seroconversion and protection, along with the fact that approximately 90% of vaccine recipients seroconverted, serum vibriocidal seroconversion at Day 11 was used as immunological marker for inferring efficacy in other age groups which could not be included in the challenge trial.

Studies PXVX-VC-200-004, PXVX-VC-200-005, PXVX-VC-200-006 were considered pivotal and assessed immunogenicity and safety of one single dose of vaccine. All studies were randomized, double-blind and placebo controlled. Immunogenicity data from the Lot consistency study PXVX-VC-200-004 in adults 18-45 years was used to bridge to older adults up to 64 years of age (study PXVX-VC-200-005) as well as to children >6 to <18 years of age (study PXVX-VC-200-006).

3.2. Favourable effects

In the main clinical efficacy study (PXVX-VC-200-300), the co-primary objectives and endpoints of the study were met in the ITT group with a protective efficacy of 90.3 % at Day 10 post-vaccination (lower 95% CI bound 62.7%) and of 79.5% at 3-Month post-vaccination (lower 95% CI bound 49.9%). As a secondary endpoint, protective efficacy against diarrhoea of any severity (mild or worse) was 84.5% (95% CI, 67.0%-100.0%) at day 10 post-vaccination and 50.8% (95% CI, 33.6%-66.8%) at month 3 post-vaccination.

Overall seroconversion rate for the Challenge study (PXVX-VC-300-200) in participants aged 18-45 was 90.3% [82.4, 95.5] (n=93). For the Lot consistency study in the same age group, Day 11 seroconversion rate was 93.5% [92.5, 94.4] (n=2687). In older adults the Day 11 seroconversion rate was 90.4% [86.4, 93.5] (n=291). In paediatric patients the seroconversion rate in children aged 6-18 was 97.7% [95.4, 98.9]. Although the seroconversion rate and SVA titres were higher in the lot consistency study with younger adults (age 18-45) compared to the study with older adults (aged 46-64) and in the challenge study (age 18-45), the differences in seroconversion rates satisfied the predefined requirement for non-inferiority.

The key favourable effects were the robust protective efficacy of 90% against moderate and severe diarrhoea at 10 days post-vaccination and the non-inferior seroconversion rates to the Classical Inaba biotype and serotype of the vaccine in all other age groups tested (children >6YOA and older adults <64YOA), which allowed to infer efficacy in these age groups. The immunobridging strategy was further strengthened by the fact that seroconversion correlates with protection.

3.3. Uncertainties and limitations about favourable effects

Vaxchora will not protect against disease caused by the O139 serogroup of V. cholerae, and therefore the indication is limited to the O1 serogroup. As with other vaccines, the protective efficacy induced by the vaccine is not 100% and thus normal hygiene measures are essential to prevent the disease.

The main uncertainties lie in the duration of protection because protective efficacy was reduced from 90.3% at 10 days post-vaccination to 79.5% at 3-months post-vaccination. Similarly, protective efficacy against mild or worse diarrhoea was reduced from 84.5% at 10-days post-vaccination to 50.8% at 3-months post-vaccination. Duration of protection after 3 months is not known and consequently no recommendation can be made at this time on when re-vaccination should be carried out. Furthermore, the potential effects of residual immunity from the primary vaccination on the efficacy of booster vaccination is not known. This is particularly relevant for health care workers living in cholera affected regions for long periods of time or travelling back and forth to cholera affected regions over time.

Protective effect was determined in participants 18-45 years of age, and this was bridged to individuals aged 45-64 and >6 – 18 years of age based on seroconversion rates. However, it is not known whether other factors may affect the efficacy of Vaxchora in these older and younger populations.

SVA seroconversion rates at Day 11 have mainly focussed on responses to the vaccine strain, Classical biotype, Inaba serotype. Seroconversion rates at Day 11 were similar for the El Tor biotype, Inaba serotype

(Challenge study 91.4% [83.8, 96.2] n=94; Older adults 91% [87.1, 94.1] n=291). However, responses to the Ogawa serotypes were lower (Challenge study seroconversion day 11 to Classical Ogawa 87.1% [78.5, 93.2]; El Tor Ogawa 89.2% [81.1, 94.7]; Older Adults seroconversion day 11 Classical Ogawa 73.2% [67.7, 78.2], El Tor Ogawa 71.4% [65.8, 76.5]. The lower seroconversion rates to the Ogawa serotype, particularly in older adults coupled with the observation that seroconversion rates decline with both time and age, remains a concern.

No data is available in adults >64 years of age. In subjects 46-50 years of age the seroconversion rate was 92% [86%, 96%], whereas the seroconversion rate in subjects aged 60-64 years was 87% [74%, 95%] 10 days post-vaccination. However older participants (aged 46-64) showed overall non-inferior seroconversion rates at 10-days post-vaccination vs. young adults, for whom efficacy was demonstrated. Since protection wanes over time, the protective efficacy will likely be lower vs. young adults at 3 months post-vaccination.

A limitation of the immune correlate of protection analyses is that SVA titres -and thus seroconversion rates- are an immunological marker that is only indirectly coupled to protection because it measures immune responses in serum and not at the site of infection, i.e. the intestinal tract.

As a live attenuated vaccine that needs to infect the digestive tract in order to raise protective immunity, concomitant use of Vaxchora with antibacterial agents is not advisable.

It is uncertain how efficacy may be affected in subjects with gastrointestinal disease, or autoimmune conditions affecting the intestinal tract such as coeliac disease. Use of immunosuppressive medication or the presence of an immunosuppressive condition could also attenuate the immune response to the vaccine, and these risks are reflected in the Product Information.

The use of Vaxchora was not studied in subjects previously exposed to cholera (e.g. from previous travel or consequently to residency in cholera endemic areas). The historical experience with CVD 103-HgR suggests that a higher dose may be necessary in this population. However, this gap in knowledge is not immediately relevant for a European population since cholera is not endemic in any region within the EU. In lack of such data, the vaccine is only recommended in travellers.

The concomitant use of Vaxchora with other travel vaccines or drugs was not studied. Vaxchora should not be administered together with oral typhoid vaccine or anti-malaria chloroquine as their respective efficacy may be diminished.

3.4. Unfavourable effects

The safety database was based on four studies in adults (one phase 1 and three phase 3 studies) which were pooled. In addition, interim data from a phase 4 study in children have been submitted. A total of 3235 adults and 328 children/adolescents (6 - <18 years) are included in the pooled safety database. Safety data are available from 562 adults and 50 children/adolescents who received placebo. Safety assessment of the vaccine included evaluation of solicited AEs (reactogenicity) as well as unsolicited AEs (treatment-emergent AEs). Solicited AEs were assessed Day 1-7 following vaccination. Unsolicited AEs were assessed through day 29 and SAEs were assessed through day 181. Solicited AEs were reported by about 50% of the vaccine recipients and about 46% of the placebo recipients in adults. In adults, the incidence of subjects with at least one related unsolicited AEs was also comparable between the placebo group (8.5%) and the vaccine group (7.9%).

Based on solicited AEs, the most common adverse reactions are fatigue, headache and various gastrointestinal disorders (frequency ≥1/10) which were reported with the same incidence among vaccine-

and placebo-recipients. Only diarrhoea occurred at a significantly higher incidence with the vaccine (3.6%) compared to placebo (1.6%). Based on related unsolicited AEs, the most common adverse reactions by SOC were different gastrointestinal disorders, reported in 3.7% of subjects both in the vaccine- and placebo group. Most adverse reactions are mild, occur within 1-3 days following vaccination and reversible.

In children, solicited AEs were reported by about 60% of the subjects both in vaccine and placebo recipients. This incidence is higher than the corresponding incidence reported in adults (about 50% in vaccine and about 46% in placebo recipients). Furthermore, in the paediatric population the incidence of subjects with at least one related unsolicited AE was higher for the vaccine group (14.6%) than for the placebo group (10.0%). Also for children the most frequent events by SOC were gastrointestinal disorders. Comparing children with adults, the reported incidence of subjects with at least one vaccine-related unsolicited AE, was higher for children (14.6%) than for adults (7.9%). Corresponding incidences for the placebo-group were 10.0 % (children) and 8.5% (adults).

No serious adverse events or deaths considered to be related to the vaccine have been reported.

Shedding of the vaccine strain was evaluated in the first 7 days post-vaccination in a phase I study of 53 healthy adult vaccine recipients. Vaxchora was shed in the stools of 11.3% of vaccine recipients cumulatively through to Day7 post-vaccination. During daily visits in the 7 days post-vaccination, the proportion of subjects with shedding was highest on day 7 (7.3%). No transmission to household contacts could be detected. The duration of shedding of the vaccine strain is unknown, but in a study with CVD 103-HgR in Mali (Perry 1998), no vaccine shedding was seen at days 12 post-immunisation.

3.5. Uncertainties and limitations about unfavourable effects

A major limitation of the safety database is that no data are available for older adults > 64 years of age.

No interaction studies have been performed with other medications or vaccines.

There is a risk of medication errors. For instance, the vaccine is acid-labile and should be administered with a buffer. Thus, it is important that eating and drinking should be avoided for 60 minutes before and after administering the vaccine. Furthermore, the vaccine should be kept refrigerated. It was also considered that the sort shelf-life of the reconstituted vaccine could also give rise to medication errors. The product was licensed in the US in 2016, and there have been post-marketing reports of medication errors even though the vaccine is administered in a health care setting. In EU it is proposed that the vaccine is prepared and administered by the user, which can increase the risk of medication error. In the worst case, medication errors may lead to vaccine failure.

However, additional stability data indicate that the shelf-life of the vaccine at room temperature and the reconstituted vaccine are longer than previously anticipated. Based upon the data submitted, storage periods should be limited to 12 hours at 25°C or less before reconstitution and 15 minutes after reconstitution to minimise the risk of medication error. Further, detailed instructions on how to prepare and administer the vaccine is given in the SmPC and PL. In addition, a HCP guide and user guide will be provided to ensure adequate information on the risks and on related minimisation measures. These measures are reflected in Annex II of the Marketing Authorisation.

The risk of serious allergic reactions is anticipated to be very low based on the data assessed. The PL adequately informs on how serious allergic reactions should be handled in case of such an unexpected event, including a description of the symptoms of serious allergic reactions and the recommendation to contact a physician without delay, should they occur.

3.6. Effects Table

Table 40 Effects Table for Vaxchora in prevention of disease caused by *Vibrio cholerae* serogroup O1 in adults and children from the age of 6 years.

Effect	Short description	Unit	Vaccine	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
Protective efficacy against Cholera at day 10 post-challenge	Prevention of Moderate to Severe Diarrhoea Following Challenge with V. cholerae O1 El Tor Inaba (ITT)	Protective Efficacy % [95% CI][a] Number of subjects with moderate or severe diarrhoea	90.3% [62.7%, 100.0%] 2 (5.7%) N=35	39 (59.1%) Combined Placebo 10 Day or 3 Month Challenge N=66	Success criterion: the lower, two-sided 95% confidence bound on protective efficacy must be ≥30%	Study PXVX-VC-200-300
Protective efficacy against Cholera at 3 Months post-challenge		Protective Efficacy % [95% CI] Number of subjects with moderate or severe diarrhoea	79.5% [49.9%, 100.0%] 4 (12.1%) N=33			
Unfavourable Effects (adults)[b]						
Fatigue	Solicited Unsolicited	% of reporters	30.00% 1.2%	29.48% 2.5%	Solicited AEs were assessed Day 1-7 post vaccination. Unsolicited AEs were assessed through day 29. AEs were reported at comparable frequency between the placebo and the vaccine groups	Pooled safety dataset of one phase 1 and three phase 3 Studies PXVX-VC 200-002, 200-003, 200-004 and 200-005
Headache	Solicited Unsolicited		27.76% 0.9%	26.04% 1.1%		
Abdominal pain	Solicited Unsolicited		18.32% 0.6%	17.00% 0.5%		
Nausea/ Vomiting	Solicited Unsolicited		17.44% 0.3%	15.55% 0.7%		
Lack of appetite	Solicited Unsolicited		15.58% 0.6%	16.82% 0.9%		

Abbreviations:

ITT: intention to treat population

Notes:

a) Efficacy was calculated as [(Attack rate in placebo group – Attack rate in vaccine group)/Attack rate in placebo group]*100

b) Only the most frequently reported adverse reactions following Vaxchora administration are listed above; for the full safety profile please refer to the SmPC. Children showed a similar safety profile to adults.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The proposed indication is supported by clinical efficacy data generated in a human challenge study in healthy individuals aged 18-45 years, which shows a protective efficacy against moderate/severe disease of 90.3% at 10 days post-vaccination and 79.5% at 3-months post-vaccination. Seroconversion rates based on SVA was found to correlate with protection and was used as an immunological marker to infer efficacy in older adults (45-64 years) and children (>6-18 years) based on non-inferior immune responses to young adults for whom efficacy was demonstrated. However, it is not known whether efficacy will be comparable in these different age groups over time.

SVA seroconversion rates at Day 11 have mainly focussed on responses to the vaccine strain, Classical biotype, Inaba serotype. Seroconversion rates at Day 11 were similar for the El Tor Biotype, Inaba serotype but lower to the Ogawa serotypes in both the Classical and El Tor biotypes. These observations cast some uncertainty as the protective efficacy may be reduced in some endemic regions where the Ogawa serotype may predominate. In addition, the protective effect 3-months post-vaccination was lower compared to 10-days post-vaccination. This is particularly concerning for individuals 60-64 years of age who have been shown to have reduced seroconversion rates compared to younger adults (46-50 years). Furthermore, no data was generated in individuals above 65 years of age.

The duration of efficacy is unknown beyond three to six months, and therefore the most appropriate time interval for re-vaccination is not known. This is concerning for aid workers who may be residing in cholera affected areas for longer periods, or who return to cholera affected areas after an interval of time. No booster studies have been carried out so the effect of residual immune responses following primary infection on the efficacy of a booster dose is not known. This is particularly important for live vaccines that replicate in vivo.

In general, the vaccine was safe and well tolerated. No serious adverse events were observed. However, as a live attenuated vaccine that infects the digestive tract, it is uncertain how efficacy may be affected in participants with gastrointestinal disease, or autoimmune conditions affecting the intestinal tract such as ulcerative colitis and coeliac or Crohns disease.

3.7.2. Balance of benefits and risks

Vaxchora is a live attenuated oral vaccine to protect against disease in adults and children over the age of 6 years. The vaccine was developed as a travel vaccine and was tested in healthy individuals not living in endemic areas. Participants with previous cholera episodes, or who travelled to cholera endemic areas within the previous 5 years were excluded from clinical studies. Thus, vaccine efficacy cannot be inferred in people with natural immunity to V. cholerae. Vaxchora is therefore only indicated for individuals who live in non-endemic areas who travel to endemic areas. This limitation of the dataset was reflected only in section 4.4 of the SmPC instead of as a restriction of indication in section 4.1, since there are no regions in the EU where cholera is endemic, so such use of the vaccine is not foreseen within the EU. Official recommendations should be followed.

The vaccine has been tested in a sufficient number of subjects and was well tolerated. The challenge clinical study to demonstrate efficacy met its primary endpoints showing robust efficacy in young healthy adults.

The duration of protection is not known at this stage and therefore the timing for re-vaccination is not known. This remains an uncertainty and no recommendation can be made at this stage, impacting especially some

categories of individuals who may need to travel to and reside in cholera endemic regions for longer periods (more than three months). The effect of previous exposure to Vaxchora and the consequently developed immunity on vaccine efficacy of a booster dose is not known.

The vaccine will not be 100% effective against all cholera biotypes and subtypes. Vaccinees should nevertheless develop a milder disease course compared to non-vaccinated individuals.

Based on improved stability data provided by the Applicant, the risk for medication errors associated with self-administration is reduced to an acceptable level provided the recommendation to minimise potential storage and reconstitution errors are followed. The risk of serious allergic reactions is anticipated to be very low, and it is considered that they can be adequately handled when the vaccine is self-administered.

Overall, the favourable effects are considered to outweigh the unfavourable effects of vaccination with Vaxchora.

3.8. Conclusions

The overall B/R of Vaxchora is positive provided that the conditions identified in the recommendation section are complied with.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Vaxchora is favourable in the following indication:

Vaxchora is indicated for active immunisation against disease caused by Vibrio cholerae serogroup O1 in adults and children aged 6 years and older.
This vaccine should be used in accordance with official recommendations.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Official batch release

In accordance with Article 114 Directive 2001/83/EC, the official batch release will be undertaken by a state laboratory or a laboratory designated for that purpose.

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and

any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Additional risk minimisation measures

Prior to the launch of Vaxchora in each Member State the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational programme, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The educational programme is aimed at minimising the risk of medication errors during the reconstitution and use of the product.

The MAH shall ensure that in each Member State where Vaxchora is marketed, all healthcare professionals and patients/carers who are expected to prescribe and use Vaxchora have access to/are provided with the following educational package:

- Physician educational material
- Patient information pack

Physician educational material:

- The Summary of Product Characteristics
- Guide for healthcare professionals
- Patient Guide
- **Guide for healthcare professionals key messages:**

  o That there is an important risk of medication errors during the reconstitution and use of Vaxchora,

  o The patients/caretakers should be informed about and follow the reconstitution instructions as advised

- The healthcare professionals should council the patients and their caretakers on how to reconstitute and administer Vaxchora
- Detailed description of the administration procedures of Vaxchora

The patient information pack:

- Patient information leaflet
- A patient/carer guide
- **Patient/carer guide key messages:**
  - That it is important that Vaxchora is reconstituted and administered as instructed
  - Detailed description of the modalities used for the self-administration of Vaxchora
  - The importance of reporting medication errors.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that *V. cholerae* live attenuated strain CVD 103-HgR is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0381/2018 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.