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Safety and Immunogenicity of Single-Dose Live Oral Cholera Vaccine Strain CVD 103-HgR, Prepared from New Master and Working Cell Banks

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Currently, no cholera vaccine is available for persons traveling from the United States to areas of high cholera transmission and who for reasons of occupation or host factors are at increased risk for development of the disease. A single-dose oral cholera vaccine with a rapid onset of protection would be particularly useful for such travelers and might also be an adjunct control measure for cholera outbreaks. The attenuated *Vibrio cholerae* O1 vaccine strain CVD 103-HgR harbors a 94% deletion of the cholera toxin A subunit gene (*ctxA*) and has a mercury resistance gene inserted in the gene encoding hemolysin A. We undertook a phase I randomized placebo-controlled two-site trial to assess the safety and immunogenicity of a preliminary formulation of CVD 103-HgR prepared from new master and working cell banks. Healthy young adults were randomized (5:1 vaccinees to placebo recipients) to receive a single oral dose of ~4.4×10^8 CFU of vaccine or a placebo. Blood serum vibriocidal and cholera toxin-specific IgG antibodies were measured before and 10, 14, and 28 days following vaccination or placebo. Excretion of the vaccine strain in the stool was assessed during the first week postvaccination. A total of 66 subjects were enrolled, comprising 55 vaccinees and 11 placebo recipients. The vaccine was well tolerated. The overall vibriocidal and anti-cholera toxin seroconversion rates were 89% and 57%, respectively. CVD 103-HgR is undergoing renewed manufacture for licensure in the United States under the auspices of PaxVax. Our data mimic those from previous commercial formulations that elicited vibriocidal antibody seroconversion (a correlate of protection) in ~90% of vaccinees. (This study has been registered at ClinicalTrials.gov under registration no. NCT01585181.)

Cholera remains a public health problem among the least-privileged subpopulations in many developing countries. When cholera invades immunologically naive underprivileged populations in so-called "virgin soil" epidemic areas, as occurred recently in Haiti (1), the attack rates can be exceedingly high. Whereas the overall risk of becoming infected with cholera is not high among travelers from industrialized countries who visit developing countries (2), for certain types of individuals (e.g., providers of emergency aid during cholera outbreaks, as in Haiti) (3), hosts with known risk factors for cholera gravis (e.g., hypochlorhydria, O blood type, and cardiac or renal disease), and those who will not have ready access to clinical care, immunization against cholera is both indicated and prudent (4). Regrettably, no cholera vaccine is presently available for travelers from the United States.

Two oral cholera vaccines that contain inactivated *V. cholerae* are licensed and available in some countries. Dukoral (Crucell) contains inactivated *V. cholerae* O1 of different serotypes and biotypes in combination with the B subunit of cholera toxin. Shanchol (Shantha Biotechnics) contains inactivated *V. cholerae* O1 and O139 organisms. Currently, Dukoral is mainly used to immunize travelers from industrialized countries, whereas Shanchol is expected to be utilized for the control of cholera in developing-country populations. Notably, both Dukoral and Shanchol require the ingestion of at least two vaccine doses. The recommended immunization schedule for Dukoral is two doses administered 1 to 2 weeks apart (3 doses for pediatric subjects 2 to 6 years of age), while the recommended schedule for Shanchol is two doses administered 2 weeks apart. Two-dose vaccines are particularly amenable for preemptive use in areas for cholera to mitigate the extent of impending seasonal cholera in high-risk segments of the population and to help control epidemic disease, including in newly affected areas, where local logistics support the delivery and monitoring required for correctly administering two spaced doses of the vaccine.

For travelers from industrialized countries or from nonendemic areas of a developing country that are not endemic for cholera who must travel on short notice to areas of intense cholera transmission, an oral cholera vaccine that rapidly confers protection after a single dose would be particularly advantageous, as well as highly practical (5). A cholera vaccine with these characteristics would also be useful for reactive mass vaccination to control cholera in explosive unsettled "virgin soil" epidemics and in other unsettled developing-country venues where the administration of more than one dose is impractical, if not daunting (6).

CVD 103-HgR is a live attenuated *V. cholerae* serogroup O1 serotype Inaba strain in which 94% of the gene encoding the A (ADP-ribosylating) subunit of cholera toxin (CT) is deleted and only the nontoxic immunogenic B (binding) subunit of CT is synthesized (7–9). In addition, a mercury resistance gene has been inserted into the *hlyA* gene of CVD 103-HgR, thereby inactivating

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the hemolysin A locus and providing a phenotypic marker that is unique to this vaccine strain (7–9).

Blood serum vibriocidal antibodies, which constitute the best correlate of protection against cholera (10–12), have been used extensively to monitor the immunogenicity of oral cholera vaccines (7, 13–17). A single oral dose of an industrial formulation of CVD 103-HgR containing $\sim 5 \times 10^8$ CFU (subsequently commercialized as Orochol and Mutacol; Swiss Serum and Vaccine Institute, Berne, Switzerland) was well tolerated and elicited blood serum vibriocidal antibody seroconversion in ~92 to 97% of adult North American subjects (5, 7, 14, 18), ~72 to 85% of whom also exhibited rises in blood serum IgG cholera antitoxin levels. A formulation containing $\sim 5 \times 10^9$ CFU (Orochol E) prepared for use in developing countries was shown to be well tolerated and immunogenic in diverse adult and pediatric populations (19–23), including in infants as young as 3 months of age (24) and in adults infected with HIV (25).

The ability of a single dose of CVD 103-HgR to prevent cholera in North American adults (a study proxy for travelers) was documented in a series of experimental challenge studies (5, 7, 14, 26, 27). Two separate challenge studies at 10 days and 8 days after ingestion of the vaccine demonstrated a rapid onset of protection (26). Although CVD 103-HgR was a licensed cholera vaccine commercialized as Orochol (in Switzerland, New Zealand, Australia, and several other countries) and as Mutacol (in Canada) for the protection of travelers, the licensure process for Mutacol for the U.S. Food and Drug Administration was never completed, and the manufacturer ceased production in 2004.

In 2009, PaxVax, a U.S. manufacturer, acquired exclusive licensure rights to redevelop CVD 103-HgR. The small phase I clinical study described here (registered at ClinicalTrials.gov under registration no. NCT01585181) represents the first step of the clinical development program that will ultimately generate data on the safety, immunogenicity, and efficacy of CVD 103-HgR and the consistency of its manufacture. This phase I trial utilizes a vaccine generated from a pilot good manufacturing practice (GMP) fermentation prepared from a new master cell bank and working cell bank.

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MATERIALS AND METHODS

Vaccine and placebo. CVD 103-HgR is a live attenuated vaccine strain derived from the *V. cholerae* serogroup O1 serotype Inaba classical biotype wild-type parent strain 569B (7–9). The material was manufactured according to current GMPs (cGMPs) from a progenitor master seed obtained from the Center for Vaccine Development (CVD) of the University of Maryland School of Medicine. Aliquots of vaccine grown in culture medium were stabilized with sucrose and casein hydrolysate prior to filling into 3-ml glass vials that were then lyophilized. Each vial of lyophilized vaccine contained $\sim 4.4 \times 10^8$ CFU of vaccine organisms upon reconstitution. The vials of lyophilized CVD 103-HgR were stored at −60°C or colder until used. For this initial clinical trial, vials containing lyophilized vaccine were reconstituted with 1 ml of water and the contents transferred into 100 ml of bicarbonate buffer solution. The bicarbonate buffer solution was prepared by reconstituting the contents of a single-use buffer sachet (NextPharma, Göttingen, Germany) containing 2.5 g $NaHCO_3$ and 2 g lactose in 100 ml of water. The placebo consisted of 2 g of lactose from a single-use sachet reconstituted in 100 ml of water. Two study sites, the CVD in Baltimore, MD, and the University of Kentucky in Lexington, KY, participated. Nonbacteriostatic water was used to reconstitute the vaccine at CVD, while tap water was used at the Kentucky site.

Study design. We conducted a phase I randomized, double-blinded, placebo-controlled, two-center (Baltimore, MD, and Lexington, KY) trial. The study was approved by the institutional review board at each clinical site. Written informed consent was obtained from healthy adults 18 to 50 years of age who were screened for the absence of chronic medical conditions, immunodeficiencies, a history of recent foreign travel to a region where cholera is endemic, history of a prior cholera or enterotoxigenic *Escherichia coli* (ETEC) infection (natural infection or experimental challenge), or prior receipt of a cholera vaccine; the complete inclusion and exclusion criteria are published at http://clinicaltrials.gov/show/NCT01585181. In order to study the potential for transmission of the cholera vaccine, close household contacts (HHCs) of the study subjects were required to participate and completed a separate written informed consent.

The eligible subjects were randomly assigned in a 5:1 ratio to receive a single oral dose of cholera vaccine or placebo. The subjects fasted for 90 min before and after ingesting the vaccine or placebo. The vaccine was prepared and administered to each subject by unblinded research personnel who were not involved with clinical evaluations, adverse event assessment, or laboratory measurements. The subjects returned to the clinic on days 1 and 3 or days 2 and 4 (as randomly assigned) and on days 7, 10, 14, and 28 postvaccination/postadministration of placebo. The subjects were contacted by phone at 6 months after vaccination (day 180). Meanwhile, the HHCs who were present with study subjects on the day of vaccination returned to the clinic on days 7 and 28 and were contacted by phone at day 180.

Safety and clinical tolerability evaluation. For 7 days following vaccination, each subject recorded a daily oral temperature and the number of loose stools over each 24-h period. In addition, the presence and subjective severity of abdominal pain, nausea or vomiting, tiredness, headache, and loss of appetite were recorded as potential adverse events. In this study, as in previous ambulatory studies of the reactogenicity of CVD 103-HgR (14, 18, 26) and the cholera vaccine strain Peru-15 (28), the passage of four or more loose stools within 24 h constituted the key endpoint of diarrheal reactogenicity. The grading for fever, using oral temperatures, was mild for 100.5 to 101.1°F, moderate for 101.2 to 102°F, and severe for ≥102.2°F. Volunteers were instructed to grade the severity of the other solicited symptoms as mild (no interference with normal activities), moderate (some interference with normal activities), severe (prevents normal activities), or potentially life-threatening (requires an emergency evaluation or hospitalization). During the 28 days after vaccination, any changes in medical history, concomitant medication usage, and the occurrence of unsolicited adverse events (AEs) were recorded. The presence of any severe adverse event (SAE) was recorded through 6 months after vaccination. Clinical safety laboratory tests were performed before and 7 days after vaccination, including complete blood counts with differential, a comprehensive metabolic panel (also known as a Chem 12), and urinalysis.

Bacteriology evaluation. The subjects were randomly allocated into one of two groups to provide a fresh stool sample (or to have a rectal swab performed in the clinic if a stool was not passed) before vaccination and either on days 1, 3, and 7 or on days 2, 4, and 7 following vaccination. HHCs provided a fresh stool sample or had a rectal swab performed in the clinic on day 7 only. An aliquot of stool collected with a swab or the rectal swab specimen was immediately placed into Cary-Blair transport medium and maintained at room temperature until transported to the central microbiology laboratory (CVD, Baltimore, MD) for the culture of *V. cholerae* O1, as previously described (29). Briefly, the specimens were plated onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar plates (Eiken, Tokyo, Japan) both directly and after 8 to 12 h of enrichment in alkaline peptone water (APW) (Remel, Lenexa, KS). After overnight in-

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TABLE 1 Subject demographics

Characteristic	Data for patients in the indicated group				
	Placebo recipients (n = 11)	All vaccinees (n = 55)	UKY vaccinees (n = 40)	UMB vaccinees (n = 15)	All HHCs (n = 28)
Gender (n [%])					
Female	5 (45.5)	28 (50.9)	22 (55)	6 (40)	14 (50)
Male	6 (54.5)	27 (49.1)	18 (45)	9 (60)	14 (50)
Ethnicity (n [%])					
Non-Hispanic or Non-Latino	10 (90.9)	55 (100)	40 (100)	15 (100)	28 (100)
Hispanic or Latino	1 (9.1)	0	0	0	0
Race (n [%])					
Black/African-American	5 (83.3)	12 (21.8)	1 (2.5)	11 (73.3)	6 (21.4)
White	1 (16.7)	41 (74.5)	37 (92.5)	4 (26.7)	22 (78.6)
Asian	0	2 (3.6)	2 (5)	0	0
Other/unknown	0	0	0	0	0
Age (yr)					
Mean (SD)	25.4 (4.0)	30.8 (6.4)	28.8 (4.4)	36.1 (8.0)	32.6 (11.1)
Median	25.0	29.0	28.0	32.0	29.5
Minimum, maximum	21, 48	22, 36	21, 40	25, 48	18, 60
Blood type (n [%])					
O	4 (36.4)	21 (38.2)	16 (40)	5 (33.3)	ND[a]
Non-O	7 (63.6)	34 (61.8)	24 (60)	10 (66.7)	ND

[a] ND, not done.

cubation at 37°C, colonies from the TCBS plates that were suggestive of *Vibrio* organisms were tested biochemically and agglutinated with polyvalent Ogawa plus Inaba antiserum (Remel).

Immunology. Blood serum specimens were collected from vaccinees before and on days 10, 14, and 28 days following vaccination and tested for Inaba vibriocidal antibodies as previously described (30, 31). CT antibodies were measured by enzyme-linked immunosorbent assay (ELISA). Briefly, Immulon II plates were coated with 1 μg/ml of CT from *V. cholerae* Inaba 569B (List Biological Laboratories, Inc.) for 3 h at 37°C, washed with phosphate-buffered saline (PBS)-Tween (0.05%), and blocked overnight at 4°C with 10% nonfat dry milk in PBS. Blood serum samples diluted in PBS-Tween were added for 1 h at 37°C. Bound antibodies were detected with peroxidase-labeled goat anti-human IgG (Fcγ) antibodies (Jackson Immunoresearch). Endpoint titers were obtained through interpolation in the linear regression curve of a calibrated control and represent the inverse of the blood serum dilution that produces an absorbance value of 0.2 above the means of the blanks. Blood serum samples were collected from HHCs on the day of the corresponding vaccination and then at the day-28 visit, and vibriocidal antibodies were measured at that time. Seroconversion was defined as a ≥4-fold rise in the antibody titer compared to baseline.

Statistical analysis. Safety and seroconversion by Inaba vibriocidal antibodies were prespecified as co-primary objectives. The sample size was chosen to afford 99% power for detecting a significant difference in the seroconversion rate between the vaccine and placebo recipients using Fisher's exact test (with two-sided α of 0.05) and assuming 90% seroconversion in the vaccinees compared to 5% among the placebo recipients.

Vibriocidal seroconversion at days 10, 14, and 28 was summarized by the number and percentage of subjects who exhibited a 4-fold rise over baseline at each visit and by the cumulative total of subjects who seroconverted at any visit. The vibriocidal titers were summarized by the geometric mean titer (GMT) and 95% confidence interval at each of the same time points. Analogous statistics were used to summarize the anti-CT immunogenicity results.

TABLE 2 Fecal shedding of CVD 103-HgR

Study day	No. of subjects with positive stool by indicated day postvaccination/no. of total tested					Cumulative no. of subjects with any positive stool
	1	2	3	4	7	
Even day	0/24		1/24		3/24	6/54
Odd day		1/30[a]		1/30[a]	1/30[a]	

[a] One subject from this group failed to provide a stool sample for the assessment of fecal shedding.

Binary endpoints were compared using Fisher's exact test, while categorical endpoints with >2 categories were compared using exact chi-square tests. Between-group continuous endpoints were compared using Wilcoxon rank sum tests. Comparisons of continuous endpoints between two time points were also performed using Wilcoxon rank sum tests. All P values were derived from two-sided tests. Demographic and safety comparisons were not adjusted for multiplicity. Immunogenicity comparisons at days 10, 14, and 28 between vaccine and placebo and between the two study sites were performed simultaneously and adjusted for multiplicity using Hochberg's method (32).

RESULTS

Demographics. Sixty-six healthy adults were enrolled in the study, with an overall mean (standard deviation [SD]) age of 29.9 (6.4) years (range, 21 to 48 years). The study participants included 33 (50%) males and 33 (50%) females, among which 50 (76%) self-reported as white, 14 (21%) self-reported as black, and 2 (3%) self-reported as Asian (Table 1). Between the two sites, there were differences in the ages ($P = 0.002$) and races ($P < 0.001$) of the subjects. There were 28 HHCs associated with these study subjects. All 66 subjects (100%) completed the follow-up visits through day 18, and all 66 subjects completed the month-6 telephone call contact.

Excretion of vaccine. Shedding of the vaccine was detected in the specimens of 11% of the vaccinees (Table 2). No instances of transmission of the vaccine strain to HHCs were detected in the stool cultures.

Reactogenicity and adverse events. Of the three fever and diarrhea reactogenicity events reported, two occurred among vaccinees and the other occurred in a placebo recipient (Table 3). One vaccinee developed fever, as did one placebo recipient (who reached a temperature of >102.1°F). One of the 55 vaccinees (1.8%), versus 0 of the 11 placebo recipients, passed four or more loose stools within 24 h.

In addition to fever and diarrhea, there were 23 participants

TABLE 3 Reactogenicity within 7 days of receiving vaccine or placebo

Symptom	No. of subjects with symptom/total no. in group (%):		P
	Vaccine	Placebo	
Diarrhea[a]	1/55 (1.8)	0/11 (0.0)	0.37
Fever	1/55 (1.8)	1/11 (9.1)	0.31
Nausea/vomiting	4/55 (7.3)	1/11 (9.1)	1.0
Abdominal pain	10/55 (18.2)	3/11 (27.3)	0.68
Asthenia	6/55 (10.9)	0/11 (0.0)	0.58
Headache	8/55 (14.5)	2/11 (18.2)	0.67
Anorexia	3/55 (5.5)	1/11 (9.1)	0.53

[a] Characterized by ≥4 loose stools within a 24-h period.

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TABLE 4 Blood serum Inaba vibriocidal response

Category[a]	No. of vaccinees	Baseline GMT (95% CI)	No. (%) of vaccinees who seroconverted by any day, compared to baseline	No. of vaccinees (%) or GMT (95% CI) by day:		
				10	14	28
Seroconversion						
All placebo recipients	11		0	0	0	0
All vaccinees	54		48 (88.9)	45 (83.3)	48 (88.9)	44 (81.5)
UMB	15		15 (100)	15 (100)	15 (100)	15 (100)
UKY	39		33 (84.6)	30 (76.9)	33 (84.6)	29 (74.4)
Blood type O	20		17 (85)	16 (80)	17 (85)	16 (80)
Blood type non-O	34		31 (91.2)	29 (85.3)	31 (91.2)	28 (82.4)
GMT						
All placebo recipients	11	45.4 (15.4–133)		48.3 (17.0–137)	45.4 (15.4–133)	45.4 (15.4–133)
All vaccinees	54	53.7 (34.8–83.0)		3,025 (1,720–15,320)	3,025 (1,760–45,198)	1,232 (750–2,023)
UMB	15	20.9 (12.3–35.7)		7,410 (4,013–13,682)	6,160 (3,690–10,281)	2,229 (1,266–3,924)
UKY	39	77.2 (45.3–132)		2,143 (1,032–4,449)	2,301 (1,123–4,714)	981 (511–1,881)
O blood type	20	51.0 (23.8–109)		3,044 (985–9,406)	2,744 (948–7,939)	1,154 (469–2,840)
Non-O blood type	34	55.4 (31.8–96.7)		3,014 (1,565–5,802)	3,204 (1,693–6,062)	1,280 (688–2,383)

[a] UMB, University of Maryland School of Medicine, Baltimore; UKY, University of Kentucky.

with additional solicited adverse events over the 7 days following vaccination or placebo receipt (Table 3), with 16 AEs achieving a maximum subjective severity grading of mild and 7 as moderate. The moderate-grade subjective reactogenicity events were reported in the vaccinees and consisted of 1 report of abdominal pain, 1 report of nausea, 4 reports of asthenia, 1 report of headache, and 2 anorexia complaints.

Twenty-one subjects (32%) experienced at least one adverse event (unsolicited AE). Of the 33 total unsolicited AEs reported, 17 of the 55 (31%) vaccinees experienced 28 unsolicited events (22 mild, 4 moderate, and 2 severe), while 4 of the 11 (26%) placebo recipients experienced 5 events (4 mild and one severe). None of the severe AEs met the regulatory definition of serious, and all AEs were transient and resolved spontaneously. Only 2 of the 33 unsolicited AEs were deemed as being possibly related to vaccine: 1 episode of mild abdominal discomfort in a vaccinee and 1 episode of mild abdominal distension in a placebo recipient. Among the clinical safety laboratory tests performed, there were 15 abnormalities, 10 of which were mild and 5 of which were moderate; there were no severe-grade abnormalities, and all the laboratory test abnormalities were considered to be clinically insignificant.

Immunogenicity. The blood serum Inaba vibriocidal antibody seroconversion rates and the geometric mean titers (GMTs) and 95% confidence intervals observed before and on various days following vaccination (or ingestion of placebo) are summarized in Table 4. None of the 11 placebo recipients mounted a seroconversion of either blood serum vibriocidal or IgG cholera antitoxin antibodies. Among the recipients of CVD 103-HgR, the overall rate of vibriocidal antibody seroconversion was 89% (48 of 54 vaccinees experienced seroconversion at any time postvaccination). As expected, most (45 of 48 total) vibriocidal antibody seroconversions were evident by day 10 after vaccination, with the peak responses occurring between 10 and 14 days after vaccination. There was a trend toward better immune responses among the vaccinees from Baltimore than those from Kentucky (P = 0.335, 0.335, and 0.335 for seroconversions using Fisher's exact test adjusted for multiplicity and P = 0.099, 0.170, and 0.099 for GMTs using a pair-wise Wilcoxon rank sum test adjusted for multiplicity for days 10, 14, and 28, respectively). There was no difference in the vibriocidal antibody responses between the O and non-O blood type groups. The baseline GMT for subjects at the Kentucky site was higher than that for the Baltimore subjects (P = 0.043 by Wilcoxon rank sum test adjusted for multiplicity). Regression model analysis failed to identify a relationship between baseline titer and seroconversions (data not shown). No HHC subjects manifested a seroconversion of vibriocidal antibody.

Overall, 32 of 54 vaccinees (59%) manifested significant rises in blood serum IgG anti-CT (comparing baseline with either day 10, 14, or 28 postvaccination). The blood serum IgG cholera antitoxin antibody GMT and 95% confidence interval (CI) and the rates of seroconversion are summarized in Table 5. There was essentially no response among the placebo recipients. Although the single oral dose of vaccine elicited anti-CT IgG response as early as 10 days after vaccination ($P < 0.001$, Wilcoxon signed-rank test of log day 10 versus log baseline for all vaccinees), the anti-CT response continued to peak through day 28 after vaccination. While the anti-CT seroconversions only demonstrated a trend (P = 0.0.333, 0.333, and 0.333 for seroconversions using Fisher's exact test adjusted for multiplicity), the GMT was consistently higher among the vaccinees from Baltimore (P = 0.032, 0.032, and 0.027 using a pair-wise Wilcoxon rank sum test adjusted for multiplicity for days 10, 14, and 28, respectively). There was no difference in the anti-CT responses among those in the non-O and O blood type group subjects. The baseline anti-CT GMT for subjects at the Baltimore site was higher than for those at the Kentucky site (P = 0.027 by pair-wise Wilcoxon exact test with Hochberg correction).

DISCUSSION

This study marks a modest but critical first step of a renewed effort to bring the single-dose live oral cholera vaccine CVD 103-HgR back to production and to achieve licensure for use with travelers from the United States. The previous commercial formulation of CVD 103-HgR that was available for travelers from Europe, Aus-

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TABLE 5 Blood serum IgG ELISA anti-CT antibody responses

Category[a]	No. of vaccinees	Baseline GMT (95% CI)	No. of vaccinees (%) or GMT (95% CI) by day:		
			10	14	28
Seroconversion					
All placebo	11		0	0	0
All vaccinees	54		16 (29.6)	25 (46.3)	31 (57.4)
UMB	15		6 (40)	9 (60)	11 (73.3)
UKY	39		10 (25.6)	16 (41)	20 (51.3)
O blood type	20		7 (35)	9 (45)	12 (60)
Non-O blood type	34		9 (26.5)	16 (47.1)	19 (55.9)
GMT					
All placebo	11	188 (111–319)	210 (122–363)	206 (120–354)	198 (112–351)
All vaccinees	54	292 (222–384)	906 (559–1,470)	1,307 (809–2,111)	1,392 (887–2,185)
UMB	15	608 (319–1158)	2,637 (988–7,039)	3,591 (1,158–8,279)	4,036 (2,070–7,871)
UKY	39	220 (171–283)	601 (356–1,013)	886 (509–1,540)	924 (545–1,567)
O blood type	20	338 (210–542)	957 (457–2,003)	1,444 (694–3,004)	1,476 (709–3,074)
Non-O blood type	34	268 (189–380)	878 (453–1,702)	1,232 (639–2,376)	1,344 (737–2,452)

[a] UMB, University of Maryland School of Medicine, Baltimore; UKY, University of Kentucky.

tralia, and Canada had its manufacture discontinued based on business considerations. No cholera vaccine is currently licensed in the United States for travelers to areas at risk of disease, and CVD 103-HgR is intended to fill this niche.

Our safety and reactogenicity data are consistent with previous experiences with CVD 103-HgR in that it was well tolerated. Diarrhea is the most concerning adverse effect with the cholera vaccine. Using a rate of passage of four or more loose stools within 24 h as the key measure of diarrhea among ambulatory North American adults (14, 18, 26), we observed that this occurred in one of the 55 vaccinees (1.8%) and in none of the 11 placebo recipients (Table 3). This is very similar to the rates of passage of four or more loose stools among the recipients of the previous commercial formulation of CVD 103-HgR (3 of 176 vaccinees [1.7%]), as summarized in Table 6. Therefore, we confirm that the present formulation of CVD 103-HgR was no more reactogenic than the historical experiences with CVD 103-HgR.

This preliminary GMP formulation of CVD 103-HgR generated blood serum vibriocidal seroconversions in 89% of the vaccinees overall. However, there was some evidence that the vibriocidal responses were more robust in the Baltimore than in the Kentucky subjects. We consider three possible explanations for such a difference: (i) some of the Kentuckian adults may have had prior exposure to *V. cholerae* O1 through visits to the Gulf of Mexico, where there are endemic environmental foci (33, 34), (ii) demographic differences, (iii) and the type of source water used to reconstitute the vaccine. Among these, we conjecture that the source water was the most plausible explanations. Whereas the Baltimore site used sterile nonbacteriostatic water to reconstitute the lyophilized vaccine (as was done with previous CVD trials), the Kentucky site used tap water. Treated municipal drinking (tap) water in the United States conventionally involves multiple processes (e.g., flocculation, sand filtration, and chlorination) to remove bacteria and other contaminants that can be harmful for human consumption. As a result, residual chlorine and intentional fluorination are expected components of U.S. tap water. Therefore, although the previous Orochol labeling specified that tap water can be used as a diluent, we conjecture that residual chlorine or other unmeasured components in the Kentucky tap water might have slightly reduced the number of organisms in the live vaccine and thereby marginally reduced the vibriocidal immune response among the Kentucky site participants.

TABLE 6 Diarrhea reactogenicity and vibriocidal antibody responses, in comparison with the previous commercial formulation

Study/source	No. of subjects with diarrhea/ total no. vaccinated (%)[a]	Vibriocidal antibody		
		% of patients with ≥4-fold rise in titer	% of patients with titer of ≥1:2,560	Peak GMT
Current	1/55 (1.8)	89 (48/54)	71 (39/55)	3,025
Kotloff et al. (18)	1/94 (1.1)	97 (91/94)	67 (63/94)	2,656
Tacket et al. (26)	0/39 (0.0)	96 (27/28)	68 (19/28)	NA
Tacket et al. (14)	2/43 (4.7)	91 (39/43)	NA[b]	3,056
Combined data among U.S. subjects from publications with the previous formulation	3/176 (1.7)	95 (157/165)	67 (82/122)	ND[c]

[a] Diarrhea was defined as passage of four or more loose stools within 24 h, within 7 days of receiving CVD 103-HgR vaccine or placebo.
[b] NA, not available.
[c] ND, not done.

The historical immune response to a single 10^8-CFU oral dose of CVD 103-HgR among North Americans was characterized by vibriocidal antibody seroconversion rates of >90% (between 91 and 100%) and antitoxin responses of 51 to 87% (7, 14, 18, 26). In the present study, the immune responses from the Baltimore participants were consistent with those in prior historical experience. On the other hand, the responses among the Kentucky participants were robust and are predicted to provide protection against clinically relevant cholera-induced diarrhea.

Clinical trials in U.S. adults and children will document the safety and immunogenicity of the new $\sim 5 \times 10^8$-CFU PaxVax formulation of CVD 103-HgR. Similarly, experimental challenge studies in U.S. adult volunteers will document the efficacy of the vaccine in preventing clinically important cholera diarrhea, thereby providing evidence for its future use in travelers. However, these data from industrialized-country subjects will not be applicable for predicting the appropriate formulation and behavior of the vaccine in impoverished developing-country popula-

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tions. Extrapolating from the experience of previous manufacturer's formulations of CVD 103-HgR, it is expected that in developing-country populations, a one-log-higher number of organisms (i.e., $\sim 5 \times 10^9$ CFU, resembling Orochol E) will be required to achieve high rates of seroconversion of blood serum vibriocidal antibodies like those observed in industrialized-country subjects who ingested a formulation containing one-log-fewer CFU (19–25, 35). Factors that modulate immunogenicity in developing-country populations and contribute to the need for a higher dosage of CVD 103-HgR have been studied extensively (19–25, 35).

Two studies addressed the ability of a single dose of the 5×10^9-CFU formulation of CVD 103-HgR to prevent cholera in developing-country populations. One was a large-scale, randomized, placebo-controlled field trial in densely populated communities hyperendemic for cholera in North Jakarta, Indonesia (36), where 33,696 subjects 2 to 41 years of age received vaccine, while 33,812 subjects got the placebo. The North Jakarta communities that participated each had a crude annual incidence of confirmed cholera in the previous 4 years before the field trial of $\geq$0.8 cases/10^3 population; however, most trial participants resided in villages where the pretrial mean annual incidence had been >3 cases/10^3 in children age 1 to 4 years, >2 cases/10^3 in children age 5 to 14 years, and >1.2 cases/10^3 in adults age 15 to 44 years. Disappointingly, over 4 years of follow-up in the field trial, the overall vaccine efficacy observed was only 14% (36). Nevertheless, once the field trial was under way, there was a precipitous fall in the number of cases of cholera in these North Jakarta communities, suggesting that the widespread use of CVD 103-HgR had somehow impeded the transmission of cholera in that population. Based on the age-specific incidence rates observed before the trial and the demography of the placebo group, approximately 237 confirmed cases of cholera were expected to occur among the placebo recipients over 4 years of follow-up. Instead, only 50 cases of cholera were detected (~80% less than expected). Thus, the efficacy results were considered enigmatic.

A highly plausible explanation for the drastic reduction in cholera cases in the Jakarta trial accompanied by a low-point estimate of vaccine efficacy came subsequently when Ali et al. (37) reported a reanalysis of the first-year results of the large-scale randomized controlled field trial in Bangladesh that assessed the efficacy of three oral doses of the B subunit (BS)/inactivated whole-cell cholera vaccine (the precursor of Dukoral) or whole-cell vaccine alone, versus placebo (38). This innovative reanalysis examined vaccine efficacy in relation to the proportion of the target population in a geographically separated baris (patrilineally related clusters of households) that was vaccinated. The authors noted that as the proportion of eligible subjects who received the vaccine increased, the incidence of cholera dropped not only in the vaccine group but also in the placebo group (37). For example, when vaccine coverage in the baris was <28%, the incidence of cholera was 7.01 cases/10^3 placebo subjects and was 2.66 cases/10^3 vaccinated subjects, yielding a vaccine efficacy of 62% (95% CI, 23 to 82%). As vaccine coverage rose from 28 to 35% to 36 to 40% to 41 to 50%, the incidence of disease progressively fell among placebo recipients as well as among vaccine recipients, and the point estimates of vaccine efficacy ranged from 52 to 67%. When vaccine coverage among the baris exceeded 51%, the incidence of cholera among the placebo recipients, 1.47 cases/10^3, was only slightly lower than among vaccine recipients, 1.47 cases/10^3, yielding a point estimate of vaccine efficacy of only 14% (95% CI, −111 to 64%) (37); this nonsignificant level of protection resembled that observed in the CVD 103-HgR vaccine trial in North Jakarta (36). The seminal report by Ali et al. (37) brought attention to the powerful indirect protection that is conferred in a high-density population when a moderately high level of coverage is achieved with oral cholera vaccine.

Longini et al. (39) utilized the Bangladesh field trial data to model the impact of the use of an oral cholera vaccine in a population endemic for cholera prior to the expected seasonal onset of cholera disease. In this model, ~30% vaccine coverage of the population would drop the incidence of cholera ~76% in the overall population, while a vaccine coverage of ~50% would achieve a 93% overall reduction in cholera incidence, including a drop of 89% among unvaccinated subjects due to the powerful indirection ("herd") protection.

During the outbreak of cholera on Pohnpei Island in Micronesia, where logistics precluded the practical administration of a cholera vaccine requiring two doses, the World Health Organization (WHO) carried out a reactive mass immunization using a single oral dose of CVD 103-HgR (Orochol E) as an adjunct measure to control the epidemic; 45% of the island's population was vaccinated (6). WHO epidemiologists estimated vaccine effectiveness to be 79.2% (95% CI, 71.9 to 84.6%) in preventing cholera under field conditions (6).

This phase I trial provides evidence that a preliminary GMP formulation of CVD 103-HgR based on the new master and working cell banks results in a product that has the desired biological properties of being well tolerated, lack of transmission to contact controls, and robust immunogenicity, particularly with respect to eliciting vibriocidal antibodies, the key correlate of protection against cholera and of comparison with earlier formulations of CVD 103-HgR. Further clinical development has begun that utilizes a practical formulation of lyophilized CVD 103-HgR contained in sachets that is mixed with buffer and water. This formulation will be used to document the clinical acceptability, immunogenicity, efficacy, and consistency of the manufacture of CVD 103-HgR to generate the evidence base necessary for licensure of the vaccine by the U.S. Food and Drug Administration and other regulatory agencies.

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