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Vaccines for viral and bacterial pathogens causing acute gastroenteritis: Part I: Overview, vaccines for enteric viruses and *Vibrio cholerae*

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Abbreviations: GEMS, global enteric multi-center study; ETEC, enterotoxigenic *E. coli*; STEC, shigatoxin producing *E. coli*; VP, viral proteins; IS, intussusception; REST, rotavirus efficacy and safety trial; RR, relative risk, CI, confidence interval; LLR, Lanzhou Lamb Rotavirus vaccine; WHO, World Health Organization; VLP, virus like particle; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; SRSV, small round virus, ORF, open reading frame; MPL, monophosphoril lipid A; HBGA, histo-blood group antibodies; SAES, serious adverse events; VLPs, virus like particles, VRPs, virus replicon particles; CT, cholera toxin; CT-A cholera toxin A subunit; CT-B cholera toxin B subunit; LB, lower boundary; RecA, recombinase A; ASC, antibody secreting cell; HA/P, hemaglutinin protease; Ace, accessory cholera enterotoxin; Zot, zonula occludens toxin; LPS, lipopolysaccharide; Cep, core encoded pilus; VA1.3, vaccine attempt 1.3; ALA, aminolevulenic acid; RITARD; removable intestinal tie-adult rabbit diarrhea; MSH, mannose-sensitive hemaglutinin pilus; TCP, toxin co-regulated pilus.

Efforts to develop vaccines for prevention of acute diarrhea have been going on for more than 40 y with partial success. The myriad of pathogens, more than 20, that have been identified as a cause of acute diarrhea throughout the years pose a significant challenge for selecting and further developing the most relevant vaccine candidates. Based on pathogen distribution as identified in epidemiological studies performed mostly in low-resource countries, rotavirus, *Cryptosporidium*, *Shigella*, diarrheogenic *E. coli* and *V. cholerae* are predominant, and thus the main targets for vaccine development and implementation. Vaccination against norovirus is most relevant in middle/high-income countries and possibly in resource-deprived countries, pending a more precise characterization of disease impact. Only a few licensed vaccines are currently available, of which rotavirus vaccines have been the most outstanding in demonstrating a significant impact in a short time period. This is a comprehensive review, divided into 2 articles, of nearly 50 vaccine candidates against the most relevant viral and bacterial pathogens that cause acute gastroenteritis. In order to facilitate reading, sections for each pathogen are organized as follows: i) a discussion of the main epidemiological and pathogenic features; and ii) a discussion of vaccines based on their stage of development, moving from current licensed vaccines to vaccines in advanced stage of development (in phase IIb or III trials) to vaccines in early stages of clinical development (in phase I/II) or preclinical development in animal models. In this first article we discuss rotavirus, norovirus and *Vibrio cholerae*. In the following article we will discuss *Shigella*, *Salmonella* (non-typhoidal), diarrheogenic *E. coli* (enterotoxigenic and enterohemorragic), and *Campylobacter jejuni*.

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Introduction

Efforts to develop vaccines for acute diarrhea have been going on for more than 40 y with partial success. The need is evident as acute diarrhea has been one of the 3 leading causes of childhood mortality in the past decades,[1,2] and although declining, in part due to the incremental use of selected enteric vaccines, acute diarrhea continues, in 2014, to be a significant cause of morbidity and mortality. Current estimates suggest that every year nearly 1.7 billion episodes of acute diarrhea occur in children younger than 5 y of age, of which 36 million are severe, leading to nearly 700,000 deaths.[2,3] Barriers for the development of safe and effective vaccines suitable for successful introduction into National Immunization Programs are numerous. The myriad of pathogens, more than 20, that have been identified as causes of acute diarrhea throughout the years has been one of the biggest challenges to vaccine development. During the past 5 years, several studies, most of which have been performed in Africa and Asia, have attempted to identify, among other objectives, the most relevant pathogens associated with acute diarrhea, reviewed in O'Ryan et al.[4] The recent Global Enteric Multi-center Study (GEMS)[5] provided highly valuable information that, together with other studies from resource deprived settings,[6–8] allows the following conclusions on enteric pathogens causing moderate to severe acute diarrhea to be drawn: i) rotavirus is the leading cause of acute watery diarrhea in children under 2 y of age and the second leading cause in children 2 to 5 y of age; ii) *Shigella* and Enterotoxigenic *Escherichia coli* (ETEC) are the leading bacterial causes for all age groups, and *Shigella* causes an important proportion of bloody diarrhea episodes; iii) *Cryptosporidium* is the second most common cause of moderate-to-severe diarrhea (typically watery diarrhea) in children under 1 y of age and is the third

leading cause in children 1 to 2 y of age; iv) *Campylobacter* causes moderate-to-severe acute diarrhea, although less frequently than the above mentioned pathogens it is, in some areas, a common cause of mild diarrhea cases resulting from significant human-animal contact; v) *V. cholerae* is a common cause of moderate-to-severe acute diarrhea in endemic areas; vi) *Giardia*, while very common in children without diarrhea, plays a pathogenic role as a cause of acute diarrhea in young infants with a primary infection; vii) *Entoameba* is an uncommon cause of bloody diarrhea in most of the regions studied; and viii) the role of norovirus, second only to rotavirus as the most studied cause of childhood associated acute diarrhea in middle/high-income countries, is less clear in resource-deprived settings, as current data are conflicting.

It is easy to envision how the implementation of enteric vaccines would result in the accomplishment of several desirable goals, first and foremost, further decreasing diarrhea associated deaths, mainly in resource-deprived and low-income countries. Based on the pathogen distributions mentioned above, rotavirus, *Cryptosporidium*, *Shigella*, diarrheogenic *E. coli* and *V. cholerae* are the main causes of severe diarrhea and thus the main targets for vaccine development and implementation. Vaccination against norovirus is most relevant in middle/high-income countries and possibly in resource-deprived countries, pending a more precise characterization of disease impact. According to the age in which these different pathogens are most prevalent, vaccines for rotavirus, and possibly norovirus, should target infants, while vaccines for the other pathogens should target toddlers. However, preventing diarrhea associated death is not the only goal of vaccination. Implementation of such vaccines should have a significant positive impact on healthcare by decreasing diarrhea-associated hospitalizations, emergency room visits and outpatient clinic visits, in addition to the potential indirect benefit of reducing pathogen transmission. The above should result in a positive cost-effectiveness ratio, which should be the main argument for incorporation of one or more of these vaccines, once licensed, into National Immunization Programs globally.

This review will focus on the most relevant viral and bacterial pathogens causing acute gastroenteritis and will discuss, for each, the main epidemiological and pathogenic features, current licensed vaccines, and vaccines in both advanced and early stages of development. Our intent is to be comprehensive, but not to exhaustively review each and every vaccine candidate. Vaccines discussed are presented in the **Table 1** including stage of development, main comments and critical references. For reviews of specific vaccines we recommend: for rotavirus refs.[9–15] for norovirus refs.[16,17] for *Shigella* ref.[18] for *Salmonella* ref.[19] for ETEC ref.[20] for *V. cholerae* ref.[21] for STEC refs.[22,23] and for *Campylobacter jejuni* ref.[24] *Cryptosporidium* vaccines are still far down the road and will not be discussed here, we refer the reader to Mead.[25]

Rotavirus

Pathogen and disease overview

Rotavirus is the most common cause of moderate-to-severe acute diarrhea in children under 5 y of age worldwide, causing nearly 40% of diarrhea-associated hospitalizations in this age group with some country-to-country variations, most likely related to variations in hospitalization practices and/or differences in the ages of patients included in various studies. In comparison to middle/high-income countries, where moderate-to-severe episodes of rotavirus tend to only occur during a child's first infection episode and predominantly between 6 months and 3 y of age, in resource-deprived countries rotavirus can cause more than one moderate to severe symptomatic episode, the first of which tends to occur at a younger age, shortly after birth and up to 1 y of age.[26,27] The most recent estimate, from 2011, is that rotavirus causes nearly 400,000–500,000 deaths every year;[28,29] in 2004 it was estimated that rotavirus accounted for 2.3 million hospitalizations and 24 million medical visits.[30] The progressive introduction of rotavirus vaccines into National immunization Programs, currently a universal World Health Organization (WHO) recommendation, is rapidly changing the above described scenario.

Rotavirus is a triple-layered, non-encapsulated, double-stranded RNA virus, and the antigens that have traditionally been considered most relevant for induction of protective immunity are the outer capsid structural proteins VP7 and VP4, which protrude through the outer capsid and are critical for virus adhesion and penetration into the intestinal cell where the virus replicates and causes damage.[31,32] Antibodies against VP7 and VP4 neutralize the virus in tissue culture, neutralization that is specific to each VP7 and VP4 type (denominated serotype specific neutralization). Twelve VP7 or G types and 11 VP4 or P types have been detected from infected children worldwide, with over 40 different GP combinations detected at least once in children; in addition, rotaviruses, mostly with distinct G and P types, also infect animals. Despite this significant variability, only 5 GP combinations accounted for over 95% of disease cases over the past decades (G1P[8], G2P[4], G3P[8], G4P[8], and G9P [8]).[32–34]

Current vaccines licensed worldwide

Several vaccine candidates initiated development in the early 1980s, within a decade after the discovery of rotavirus. These candidates have included strains obtained from animals, which do not cause disease in humans (the first candidates were based on cow and monkey rotaviruses and more recently sheep rotavirus), and later from humans (attenuated both naturally or in the laboratory) or animal-human reassorted strains constructed in the laboratory. Vaccine candidates containing only one strain (animal or human, commonly called "monovalent") relied on the possibility of "heterotypic" protection, where one vaccine strain would protect against several, if not all, different serotypes affecting children. Vaccine candidates containing several strains (animal-human reassortants, called "multivalent"), including the main VP7 and/or VP4 antigenic types, based their development on the concept that protection was mostly "homotypic" (one vaccine strain protects only against this strain in future exposures), because in vitro tests indicated that neutralizing antibodies conferred by a specific VP7 or VP4 serotype did not cross-neutralize rotavirus strains harboring a different serotype. The idea that

Table 1. Licensed vaccines and vaccine candidates designed to prevent gastroenteritis caused by rotavirus, norovirus and *Vibrio cholerae*.

Pathogen	Vaccine (s)	Status*	Comment	Selected references
Rotavirus	*RotaTeq* ®/*Rotarix* ®	Worldwide License	Eight years post-licensure; worldwide distribution; demonstrated effectiveness. Both prequalified by WHO.	Giaquinto et al., 2011[10]; O'Ryan et al., 2011[13]
	Rotashield®	First licensed rotavirus vaccine in 1998 (USA); was withdrawn due to association with intestinal intussusception.	Currently in clinical trials using a 2-dose regimen beginning within the first 30 d of life demonstrating 64% efficacy for the first 12 months of life.	Armah et al., 2013[42]
	LLR®/*Rotavin-M1*®/*Rotavac*®	Restricted license	Used only in China/Vietnam/ India respectively; lack of robust effectiveness data.	Fu et al., 2012;[61] Dang et al., 2012;[62] Bhandari et al., 2014[65]
	RV3BB/UK reassortant	Early clinical development	Phase I or early phase II studies.	Danchin et al., 2013;[63] Luna et al., 2013[66]
	Subunit vaccines/Inactivated rotavirus vaccine	Early clinical development	Immunogenic in the BALB/c mice model.	Lappalainen et al., 2013;[67] Jiang et al., 2008[70]
Norovirus	Intramuscular vaccine candidate containing GI.1 and GII.4 VLPs	Advanced clinical development	Phase I adult challenge study completed, moving into phase IIb/III studies.	Treanor et al., 2014[118]
	P particle-based vaccines	Preclinical development	Considered as a norovirus vaccine as well as a delivery system for other antigens, such as rotavirus, influenza and hepatitis E; immunogenic in the mouse model.	Tan and Jiang, 2014[17]
	Trivalent vaccine including norovirus GII.4 and GI.3 VLPs and rotavirus rVP6	Preclinical development	Immunogenic in the BALB/c mouse model.	Tamminen et al., 2013[115]
	Multivalent alphavirus replicon particles (VRPs)	Preclinical development	Considered as a delivery system or adjuvant; immunogenic in a BALB/c mouse model.	LoBue et al., 2009[113]
V. cholerae	*Dukoral*®	Worldwide License	Licensed in 65 countries. Short-term protection and potential herd effect. Prequalified by WHO.	Taylor et al., 2000;[132] Ali et al., 2005[139]
	Shanchol®	Worldwide License	Prequalified by WHO. Demonstrated effectiveness.	Sur et al., 2011[134]
	mORCVAX®	Restricted License	Identical to *Shanchol*®. Distributed in Vietnam only.	Anh et al., 2007;[136] 2011[137]
	CVD-103HgR	Restricted License	Production as *Orochol*®/ *Mutacol*® stopped in 2004. New clinical studies are ongoing.	Chen et al., 2014[128]
	Peru-15 (CholeraGarde®)	Early clinical development	Safe and immunogenic. Efficacy evidenced in volunteers in the USA. A phase II trial in an endemic region is ongoing.	Cohen et al., 2002[141]; Qadri et al., 2007[143]
	V.cholerae 638	Early clinical development	Safe and immunogenic. Efficacy evidenced in volunteers in	García et al., 2005;[146] Diaz Jidy et al., 2010[147]

(*continued on next page*)

Table 1. Licensed vaccines and vaccine candidates designed to prevent gastroenteritis caused by rotavirus, norovirus and *Vibrio cholerae*. *(Continued)*

Pathogen	Vaccine (s)	Status*	Comment	Selected references
			Cuba. Phase I/II trials in endemic regions are required.	
	CVD 112	Early clinical development	Safety, immunogenicity and efficacy evidenced in phase II trials. No information about further trials.	Tacket et al., 1995[148]
	VA1.3 / 1.4	Early clinical development	Safe and immunogenic after phase I trial. Phase II trials suggested.	Mahalanabis et al., 2009;[149] Kanungo et al., 2014[150]
	IEM 108	Preclinical development	Prevent fluid accumulation in rabbit ligated loops	Liang et al., 2003[151]
	VCUSM2	Preclinical development	Prevent fluid accumulation in rabbit ligated loops and RITARD model	Ravichandran et al. 2006[152]
	TLP01	Preclinical development	Safe and immunogenic in rabbits and rats	Ledon et al., 2012[153]

*Status: Worldwide Licensed in an important number of countries in several continents; restricted license in one or few countries; Advanced clinical development (phase IIb/III); Early clinical development (phase I/II); Preclinical development in animal models

monovalent vaccines could work was supported by several studies of natural infections in children, which consistently showed that most children suffered only one moderate to severe rotavirus episode, regardless of the rotavirus serotypes circulating in the community in different years.[27,35] Broad protection conferred by a G1P[8] strain, for example, could be based on induction of humoral and/or cellular mechanisms targeting VP7 (basically protecting against G1 strains), targeting VP4 (G3, G4 and G9 strains harboring VP4 type P[8]), and possibly targeting other antigens such as VP6, the most abundant intermediate core antigen shared by most human strains, which despite not eliciting neutralizing antibodies could elicit other mechanisms of protection. More recently, other viral proteins, such as the nonstructural NSP4 protein, which seems to play a pathogenic role related to viral induced secretion of water and chloride, may also be antigenically similar among different GP serotype strains, potentially inducing cross protection; although this is still theoretical.[31] Importantly, due to the absence of well-established immune correlates of protection feasible for use as serological markers of protection, rotavirus vaccine trials have been, and continue to be based on clinical efficacy.

Rotashield®

This was the first vaccine licensed against rotavirus (1998), developed by researchers from the National Institutes of Health (NIH) in the USA and Wyeth Lederle. It is a quadrivalent vaccine containing a rotavirus strain obtained from a rhesus monkey (serotype G3P[3]) and 3 reassorted strains that use the rhesus strain as the backbone (10 genes from this strain) covered by a "human" VP7 protein (reassorted gene) of serotype G1, G2 and G4 homology. Thus, the vaccine was intended to protect against the 4 most common human VP7 serotypes, G1 through G4, assuming that the "simian" G3 would protect against "human" G3 strains. G9 strains were not epidemiologically relevant in the 1980s and early 1990s when this vaccine was developed, and therefore were not included at the time. Five field studies with this candidate on different continents that recruited 6,559 children showed that 3 doses of the vaccine were highly efficacious (ranging from 70 to 90%) in preventing moderate-to-severe rotavirus disease in infants.[36–40] Because G1P[8] was the predominant strain in all studies, protection against other serotypes was unclear at the time of licensure. Unfortunately, before the full impact of mass vaccination could be assessed, such as protection against non-G1 types, Rotashield® was withdrawn from the market because when used in the recommended 2, 4 and 6 month regimen, an association with intestinal intussusception (IS) at an attributable risk-level of ~1:11,000 was reported.[41] After several years of abandonment, the vaccine was re-evaluated in a 2-dose regimen in a placebo-controlled study enrolling nearly 500 neonates per arm from Ghana, with the first dose administered within the first 30 d of life and the second dose before 60 d of age. The rationale for this approach is based on the fact that most IS cases attributed to the vaccine occurred in older children receiving the first dose after 3 months, the age at which IS is most common. The overall efficacy of this 2-dose regimen against rotavirus of any severity during the first 12 months of life per protocol was 64% (95% CI 35–81).[42] No IS cases occurred, although the study was not powered to determine the risk for IS. Potential licensing and use of this vaccine under this newly proposed schedule would require further evaluation for efficacy and safety.

Rotateq®

First licensed in 2006 by Merck & Company, this pentavalent vaccine contains 5 reassorted strains that use a bovine rotavirus

strain (serotype G6P[5]) as the backbone. The five reassorted rotavirus strains in the vaccine are of the following serotypes: G1P[5], G2P[5], G3P[5], G4 P[5] and G6P[8], the latter of which was included in order to protect against the most common human VP4 type infecting children. Two phase II and 3 phase III studies (including the large multicenter *REST* trial) demonstrated that 3 doses of the vaccine, in schedules beginning at 6–12 weeks of age with subsequent doses given 4–10 weeks apart, was safe and highly efficacious against severe rotavirus infection using the Clark scale to evaluate disease severity.[43–46] The *REST* trial recruited nearly 70,000 infants from Europe and the USA, with the main goal of addressing the issue of IS, from which a subgroup of nearly 5,700 children were evaluated for efficacy.[46] IS occurred at a similar rate in vaccine and placebo recipients, 6 and 5 of nearly 34,000 subjects per group evaluated for serious adverse events developed IS within a 42 day window after any of the 3 doses (RR: 1.6, 95% CI 0.4–6.4). Vaccine efficacy against any rotavirus diseases was 74% (95% CI 67–80), against severe rotavirus disease (98%, 95% CI 88- 100), protection against rotavirus gastroenteritis requiring emergency room visits was 87% (95% CI 68–90) and against hospitalizations due to rotavirus gastroenteritis (96%, 95% CI 90 - 98). A significant number of post-licensure studies, using a variety of designs, have demonstrated high vaccine effectiveness against different outcomes (hospitalizations, emergency room visits and healthcare costs, among others), predominantly in industrialized countries.[10] As for *Rotarix*®, discussed further down, vaccine efficacy and effectiveness has proven to be lower in resource-deprived countries. Efficacy of *RotaTeq*®, administered at 6, 10 and 14 weeks of age, against severe rotavirus gastroenteritis in Africa (Ghana, Kenya and Mali) was 64% (95% CI: 40–79%) during the first year of life and 20% (95% CI: -16–44%) during the second year of life. Similarly, in resource-deprived Asian countries (Vietnam and Bangladesh), efficacy was 51% (95% CI: 13–73%) during the first year of life and 46% (95% CI: 1–71%) during the second year of life.[14,47] A comparable protection level (OR: 0.55; 95% CI 0.41–0.74) was estimated in a post-licensure study in Nicaragua.[48] Also, similar to *Rotarix*®, large post-licensure studies have identified a low risk of IS attributable to the vaccine, which is currently considered a "class effect" with an estimated 1.5 (95% CI 0.2–3.2) excess cases of IS per 100,000 vaccinated infants during the 21 day window after any vaccine dose.[49] The first dose of *Rotateq*® can be given as early as 6 weeks of age followed by 2 additional doses, each separated by at least 4 weeks.

Rotarix®

First licensed in 2006 by GlaxoSmithKline Biologicals, this human attenuated strain, obtained from a child with acute rotavirus gastroenteritis and attenuated through serial passages in cell cultures, is a G1P[8] strain. Vaccine efficacy in phase III trials have now been performed on most continents and have demonstrated protection against moderate-to-severe rotavirus gastroenteritis using the Vesikari score (and have lowered rotavirus associated hospitalizations) over a 2-year period ranging from 96% (94% against hospitalizations) in high-income countries in Asia, to 91% (96%) in Europe, 85% (85%) in Latin America, 72% (81%) in China, 59% (hospitalizations not evaluated) in South Africa and 38% (hospitalizations not evaluated) in Malawi.[12,50-54] Notably, in a large Latin American trial that enrolled over 63,000 children, it was demonstrated that the risk of IS within 31 d after vaccination was similar between vaccine and placebo recipients, RR -0.32/10,000 (95% CI: -2.91/10,000; 2.18/10,000); 6 IS cases in nearly 31,700 vaccinees and 7 IS cases in nearly 31,600 placebo recipients occurred with the 31 day window after any of the 2 vaccine doses.[11,55] Post-licensure studies have demonstrated that administration of this vaccine has had a significant impact in different regions. In case-control studies, effectiveness against rotavirus hospitalizations has ranged between 75% and 85%, with one outlying study, which was performed in an Australian indigenous population where the vaccine did not show significant protection.[13] In a recent case-control study in the USA, effectiveness against rotavirus diarrhea requiring emergency care or hospitalization reached 91% (95% CI: 80-95%) for *Rotarix*® and 92% (95% CI: 70-96%) for *RotaTeq*®; critically for *Rotarix*®, effectiveness against G2P[4] strains, the fully heterotypic strain, was 94% (95% CI: 78-98%).[56] Notably, vaccination has been associated with a nearly 40% reduction in all diarrhea-associated hospitalizations, regardless of etiological diagnosis.[57] In resource-deprived regions *Rotarix*® has been demonstrated to confer significant protection against different serotypes including G2, G3, G8 and G12.[58] The impact of *Rotarix*® vaccination in the reduction of gastroenteritis-associated deaths in children under 5 y of age has been reported in Mexico, Brazil and Panama with estimates ranging from 22 to 35% using different analytical methods.[13] As for *RotaTeq*®, vaccine efficacy and effectiveness is lower in resource-limited regions and a low-level risk for IS, at a 1:50,000-70,000, has been calculated based on post-licensure studies in Mexico and Brazil.[59]

In the USA the incidence risk is similar, with a recent estimate of 1.5 excess cases per 100,000 vaccine recipients after the first dose (95% CI: 0.2-3.2).[60] The first dose of *Rotarix*® can be given as early as 6 weeks of age, followed by one additional dose separated by at least 4 weeks.

Current vaccines with restricted license

Lanzhou lamb rotavirus vaccine (LLR)

This vaccine was developed by the Chinese Lanzhou Institute of Biological Products, based on a G10P[12] rotavirus strain obtained in 1985 from a local lamb with diarrhea and attenuated through serial passages.[61] This vaccine was licensed in China in 2000, despite lacking studies of clinical efficacy and safety. Nearly 30 million doses have been distributed to children under 5 y of age using a schedule that includes one dose annually for children 2 months to 3 y of age for a total of 4 doses before 5 y of age.[15,61] The same researchers have performed a series of case-control effectiveness studies over the past years, which have a number of limitations. The latest study suggests effectiveness against rotavirus hospitalization of around 6078%- (95% CIs ranging from 29% to 89%) in children 2-11 months, 12-23 months and 24-35 months receiving one vaccine dose within the year prior. Very few

children received more than one vaccine dose. Widespread use of this vaccine outside of China seems unlikely due to the lack of thorough pre-licensure and post-licensure evaluations and the curious vaccine schedule adopted. An additional candidate using a reassorted lamb strain providing G4 specificity, strain NF-R7, is also in development by the Shenzhen Kangtai Biological Products Company, China (although published data is not readily available).[15]

Rotavin-M1®

This vaccine is produced by POLYVAC-Vietnam and is similar to *Rotarix®* in that it is a G1P[8] attenuated strain obtained from a Vietnamese child. There is only one available published study on this vaccine that includes evaluations of different virus concentrations and doses in phase I adult-infant and phase II infant trials, aimed at identifying the best dose and schedule based on safety and immunogenicity.[62] The authors conclude that the schedule of 2 doses, beginning at 6 to 12 weeks of age of the higher concentration (only 0.3 logs higher than the lower dose), separated by 2 months provided the best results when compared to *Rotarix®* in terms of immunogenicity (similar seroconversion rates) and safety (similar adverse event profiles, non-severe). The third dose did not significantly increase anti-rotavirus IgG seroconversion rates or geometric mean titers; interestingly vaccine virus shedding was higher for *Rotarix®* (65%) than *Rotavarin-M1®* (44% to 48%) after the first dose. According to the authors, a multi-center study is in progress.

ROTAVAC®

This vaccine is based on the concept of using naturally occurring reassorted strains that infect newborns without causing symptoms. This approach has been advanced by Indian researchers from Bharat Biotech International, leading to *ROTAVAC®* being licensed recently in India, and also by Australian researchers from the Murdoch Children's Research Institute, who have a vaccine candidate that is in earlier stages of development (see below).[63] The fundamental idea behind this strategy is to provide less expensive vaccines in India, where rotavirus has a very high disease burden.[64] The 116E strain in this vaccine is a naturally occurring human-bovine reassortant strain of serotype G9P[11], which demonstrated nearly 90% seroconversion after an 8, 12, 16 week schedule in infants.[65] In a recent phase III trial of nearly 7,000 Indian infants, randomized 2:1 to receive vaccine or placebo at 6-7, 10 and 14 weeks of age, protection against severe rotavirus gastroenteritis as measured by a Vesikari score was >11 and against rotavirus hospitalizations was 56% (95% CI: 37-70%) at 12 months of age.[65] Protection against rotavirus infection of any severity was 35% (95% CI: 20-47%). Significant protection was demonstrated for circulating serotypes G2P[4], 61% (95% CI: 29-79%), and G12P[6], 69.1% (95% CI: 21-89%). Six IS cases occurred in nearly 4,500 vaccinees and 2 cases in nearly 2,300 placebo recipients, all after the third dose, suggesting that if the vaccine triggers cases of IS, it will probably be within the range of the "class effect" demonstrated for *Rotarix®* and *RotaTeq®*, but this will require future evaluation in phase IV trials. The sponsors of this vaccine are currently applying for WHO pre-qualification.[15]

Vaccine candidates in early stages of clinical development or pre-clinical development

Live attenuated neonatal strain RV3BB

This is G3P[6] strain recovered from asymptomatic newborns in Australia, aimed at neonatal immunization. In a recent phase I study, 5/9 infants showed an IgA or serum neutralizing antibody serconversion after a single dose, and 7/9 showed evidence of viral replication in stools. Thus, the vaccine take is high and further phase II studies are expected soon.[63]

UK reassortants

The Butantán Institute in Brazil is advancing a pentavalent reassortant vaccine, similar to *RotaTeq®*, with the UK bovine rotavirus strain as the backbone, reassorted with 5 human strains: G1, G2, G3, G4 and G9. In adult volunteers, a similar proportion of complaints and solicited symptoms were reported by vaccine and placebo recipients after the first dose (36% versus 30%) and serconversion rates after 3 doses were close to 60% for each of the 5 serotypes.[66]

Subunit vaccines

As discussed below, this strategy is based on recombinant particles intended for parenteral use. Researchers from the Vaccine Research Center at the University of Tampere in Finland have been leaders in this approach. Recombinant VP6, the most abundant protein component of the virus that structures the intermediate viral capsid, which auto assembles in a tubular structure, and a double-layered virus-like particle (VLP) are being evaluated in an animal model. These proteins have been shown to induce humoral, mucosal and cellular immune responses in BALB/c mice.[67] Researchers from Cincinnati Children's Hospital Medical Center and Baylor College of Medicine have also been pioneers in the subunit vaccine strategy for both rotavirus and norovirus, as described below. The focus of their current strategy is to increase immunogenicity and functionality of candidates by developing a method to structure large polyvalent complexes.[68]

Inactivated vaccines

This strategy, which aims to produce a low-cost vaccine that could possibly circumvent side effects associated with the use of live oral vaccines, is being developed by the Centers for Disease Control and Prevention (USA) and others; this vaccine is intended for intramuscular and/or intradermal use.[69] Using unique thermal conditions, rotavirus was able to be fully inactivated while inducing high titers of neutralizing antibodies in mice; the adjuvant alum hydroxide further enhanced the immune response.[70] Using micro needles in a skin patch induced a higher immune response than intramuscular injection in BALB/c mice, leading the authors to conclude that this may become an alternative strategy in the future.[69]

Norovirus

Pathogen and disease overview

The Norwalk virus was discovered in 1972 by applying electron microscopy to stools related to a large gastroenteritis outbreak, which occurred several years earlier in a school in the city of Norwalk, Ohio.[71] Following this breakthrough, numerous viruses similar in structure, but not antigenically cross reactive with the available immunologic assays at the time, were identified and named after the locality were they were first identified (*e.g.* Southampton, Hawaii, Lordsdale).[72,73] The increasing number of discovered viruses were grouped either into the so-called "small round viruses" (SRSV) or caliciviruses due to their cup-shaped appearance by electron microscopy. Biochemical and genomic sequencing analysis subsequently confirmed that the SRSVs and human viruses with typical calicivirus morphologic features belonged to the family Caliciviridae.[74] Within this family, viruses were categorized as human caliciviruses (viruses infecting mainly humans, which today include the genera *Norovirus* and *Sapovirus*) and animal caliciviruses (infecting mainly animals, which today include the genera *Lagovirus*, *Vesivirus* and *Nebovirus*). In contrast to rotavirus, permissive cell lines for culture of human caliciviruses were not obtained (and have not been obtained to date), and successful animal models were extremely difficult to develop and reduced basically to one pig model[75] and more recently a mouse model.[76] Nevertheless, studies of virus identification using electron microscopy in stools from individuals affected by water and/or foodborne outbreaks, as well as seroprevalence studies using available human caliciviruses and sera from different populations, hinted that these viruses were an important cause of gastroenteritis outbreaks worldwide affecting individuals of all ages.[72,77-81] A second breakthrough was the sequencing of the full genome of the Norwalk virus, followed by the synthesis of virus like particles, which opened the field for comparative genetic studies between different human caliciviruses as well as for antigenic comparability, antigen detection in stools and seroprevalence studies due to the possibility of synthesising large quantities of VLPs from human caliciviruses with differing gene sequences.[82,83] These advances, which have occurred over the last 40 years, have allowed us to epidemiologically characterize this infection today.

Norovirus has been the main target for vaccine development, as it has been associated with over 90% of *Calicivirus* associated gastroenteritis episodes. *Sapovirus* has been a significantly less common cause, which has been reported mostly in Japan, although detection in other regions is increasing.[84-86] Noroviruses have been associated with 4 clinical circumstances: food and/or waterborne gastroenteritis outbreaks, acute endemic gastroenteritis in children, acute endemic gastroenteritis in adults, and gastroenteritis in immunocompromised individuals.[87] Norovirus is currently recognized as the most common cause of etiologically evaluated food and/or water gastroenteritis outbreaks, accounting for 30-80% of such outbreaks; these outbreaks occur in diverse settings including ships, hotels, restaurants, schools, camps and healthcare facilities, among others, and can be associated with severe outcomes including death.[88-90] A number of studies from industrialized and low/middle- to middle-income countries identify norovirus as the second leading cause, after rotavirus, of acute endemic gastroenteritis in children, accounting for 10-20% of gastroenteritis-associated hospitalization and emergency room visits[91-93] and is becoming the leading cause in countries that have implemented rotavirus vaccination.[94,95] In adults, norovirus (and also *Sapovirus*) are gaining recognition as a significant cause of acute endemic (non-outbreak associated) gastroenteritis, especially in the elderly, in whom the disease can lead to severe dehydration, complications and death.[86,96,97] Among immunocompromised individuals, noroviruses can cause a more severe and/or prolonged gastroenteritis episode.[87,98,99] Overall, the most recent disease burden estimates for the USA (data is not readily available for other regions) suggest that noroviruses cause an average of 570-800 deaths, 56,000-71,000 hospitalizations, 400,000 emergency room visits, 1.7-1.9 million outpatient visits, and 19-21 million total illnesses per year.[100] Norovirus disease burden in resource-deprived countries is less clear. One recent study reported the presence of norovirus in 14% of hospitalized children under 5 y of age in Tanzania.[6] The results of the recent GEMS study conducted in 4 African sites, Bangladesh, India and Pakistan using an age-stratified, matched case-control design identified norovirus as a significant pathogen causing moderate-to-severe acute diarrhea in children under 5 y of age in some countries (Basse, The Gambia where it was associated with 9% of severe diarrhea cases in under 12 month olds and 24-59 month olds, and 5% in 0-11 month old children in Kolkata, India), but not in the others.[5] The death toll in the developing world has been estimated at 200,000 deaths per year.[93]

Noroviruses are structurally quite different than rotavirus. At about half the size, they have a single capsid and are single-stranded RNA viruses with 3 open reading frames (ORFs). ORF 1 encodes for a polyprotein cleaved into a set of nonstructural proteins during replication (including the RNA-dependent RNA polymerase), ORF 2 encodes the main capsid protein VP1, and ORF 3 encodes a minor structural capsid protein VP2,[71] In contrast, rotavirus harbors double-stranded RNA, which encodes for 11 genes located within a triple protein layer. Noroviruses are genetically and antigenically diverse, due to frequent point mutations and recombination events,[73,101] and are currently grouped into 6 genogroups, which have significant genetic/aminoacidic differences between each other, and over 25 serotypes which represent aminoacidic differences within a genogroup; within a serotype there are variants with less than 5% genetic/aminoacidic differences. It is worth noting that the current nomenclature is under constant revision.[87,102] Similar to rotavirus, only a few norovirus genogroups have been recognized in humans (G1, GII and GIV), of which GII, and specifically the GII.4 genotype, has been predominant over the past few years worldwide.[103] The original Norwalk virus was a G1 virus.

Vaccine development for noroviruses has been considered difficult for 2 main reasons. First, immunity to natural norovirus infections seemed short lived, not surpassing a few years, in adult volunteers challenged and rechallenged with Norwalk virus.[104] Second, the significant genetic variability, which was considered

a proxy for significant antigenic variability, suggested that a vaccine against one virus would not be broadly protective; antigenic cross-reactivity between genogroups was shown to be less than 5% and 5 to 10% between serotypes within the same serogroup.[105] The rotavirus experience has taught us that antigenic specificities observed at the laboratory level do not necessarily translate to what may happen in real life. The fact that the virual load used in the adult challenge studies was extremely high, surpassing by several logs the virus' infectious dose, suggests that results from these studies may have been misleading.[106] Current modeling studies suggest that protective immunity may last up to 8 y[107] Importantly, despite the broad genetic variability of noroviruses, only a few genogroups predominate, with type GII.4 causing over 70% of infections, similar to rotavirus, where 5 genotypes cause over 90% of disease cases with a geographic and temporal predominance of the G1P[8] serotype. An important epidemiological observation was obtained from a cohort study of newborns followed throughout their first 3 y of life with monthly stool testing for norovirus, just as with rotavirus, most children had several norovirus infections, mostly asymptomatic, and only a few had more than one symptomatic infection.[108] Symptomatic GII infections occurred only as the primary infection or when preceded by a non-GII infection; no child with a symptomatic GII infection had a previous GII infection, while 10 children with asymptomatic GII infections had previous GII infections. These data suggest that similar to rotavirus, a prior infection could be protective against new symptomatic episodes, opening an avenue for vaccine development. Because a few children with a symptomatic GII infection had a prior GI or nontypeable norovirus infection, it is probable that cross protection between genogroups is not complete.[108] Immunologic correlates of protection for norovirus infections, using inhibition of hemmaglutination and histo-blood group antigen (HBGA) blocking assays, are being developed and may prove helpful in evaluating new vaccine candidates.[109,110]

Live virus vaccines, which have proven highly successful for rotavirus, are not available for norovirus, due to the inability to culture the virus. Vaccine candidates, therefore, rely on the synthesis of VLPs or smaller particles.[16,111-115] Licensed norovirus vaccines are not yet available.

Vaccines in advanced stages of clinical development

Intramuscular vaccine candidate containing GI.1 and GII.4 VLPs

VLPs have proven to be highly effective in the prevention of cervical human papilloma virus infections and can be synthesized in large quantities, thus providing sufficient antigen for large vaccine volumes. The proof of concept that these vaccines provide protection was obtained from an adult challenge study using intranasal GI.1 VLP, developed by university investigators and sponsored by LigoCyte Pharmaceuticals.[111] Additional studies of this vaccine candidate, which includes the adjuvant monophosphoryl lipid A (MPL, GlaxoSmithKline), demonstrated that 2 doses elicit a significant B cell response.[114,116] LigoCyte Pharmaceuticals advanced further with a bivalent vaccine including GI.1 and GII.4 VLPs, the latter was constructed based on a consensus of 3 different GII.4 strains.[117] The highest homologous and heterologous antibody titers to the bivalent vaccine were elicited following immunization of animals via the intramuscular route.

Further development of this intramuscular vaccine is being carried on by Takeda Vaccines. Two adult phase I studies on safety and immunogenicity showed that the vaccine was, in general, well tolerated with only mild to moderate pain and tenderness and mild headache being slightly more common in vaccine compared to placebo recipients. The majority of subjects seroconverted after the first dose of a 2-dose regimen and maintained antibodies (measured by a norovirus pan antibody assay) for 393 d[118] The serum antibody response to the intramuscular vaccine was high and peaked at day 7 after the first dose of a 50/50 μg formulation, with no evidence of boosting when a second dose was administered 28 d later, suggesting previous priming by natural exposure to related noroviruses. A recently concluded challenge study, including 18 to 50 y old healthy adults receiving 2 doses of the vaccine and challenged with a GII.4 strain,[119] has provided promising results. Immunogenicity and safety results can be summarized as follows: HBGA blocking antibody titers increased following vaccination and not placebo, and geometric mean titers for GII.4 were higher following vaccination than after live virus challenge in placebo subjects, indicating that intramuscular injection provides a robust response. Non-attributable serious adverse events (SAEs) were observed throughout the 30-day post-challenge observation period. During the inpatient phase of the study, severe norovirus illness occurred in 0/50 vaccinees vs. 4/48 (8%) placebo recipients (100% reduction, P=0 .054), moderate or severe illness occurred in 6% versus 19% (68% reduction, P= 0.068) and illness, whether mild, moderate or severe, occurred in 18% vs. 38% (52% reduction, P=0 .042). In addition, the severity of illness was significantly reduced in vaccinated subjects who became symptomatic compared to symptomatic placebo recipients (P=0 .023). There were also indications of impact on viral shedding: less vaccine compared to placebo recipients shed virus, at lower amounts and for shorter durations, which would be a very important feature of the vaccine for infection control. Fast track phase IIb/III trials in both adults and children are expected within the next few years.

Vaccine candidates in preclinical development

P particle-based vaccines

Researchers from the Cincinnati Children's Hospital Medical Center are developing these P-particle vaccine candidates. The main capsid protein VP1 has an S (for shell) domain and P (for protruding) domain, which plays an important role in the binding of the virus to HBGAs.[120] The P particle is an octahedral nanoparticle that is being considered as candidate for norovirus vaccine, as well as a delivery system for other antigens, such as rotavirus, influenza and hepatitis E.[17] This candidate induced both humoral and cellular immune responses in a mouse model. Production of P particles in *E. coli* expression systems, in contrast to VLPs that use eukaryotic expression systems, may be an advantage for massive antigen production.[112]

Vaccine candidates in preclinical development

Trivalent vaccine including
norovirus GII.4 and GI.3 VLPs and rotavirus rVP6

This vaccine has been developed by researchers from the Vaccine Research Center at the University of Tampere in Finland and supported by UMN Pharma Inc.., Japan. The intent is to protect against norovirus infection using VLPs and rotavirus infection by using a recombinant VP6 particle. Results from a recent study in BALB/c mice indicate that the vaccine produced a significant immune response.[115] Broadly reactive anti-norovirus IgG antibodies against different viruses within the genogroups were detected, mucosal antibodies capable of inhibiting rotavirus infectivity were induced, and cell mediated immunity for both viruses was also detected. Immunity was sustained for 6 months, and interference between the vaccine components was not observed. Studies in humans are expected to follow.

Multivalent alphavirus replicon particles (VRPs)

This system has been developed by researchers from University of North Carolina, USA.[121] It is based on equine encephalitis virus plasmids with insertion of *Norovirus* capsid clones. The plasmid is used as a delivery system to introduce the capsid genes into cells that produce norovirus VLPs. More recently, a "null VRP," which lacks a transgene and does not include norovirus insertions, has been used as an adjuvant in conjunction with VLPs, demonstrating an increase in both systemic and mucosal immune responses in a BALB/c mouse model.[113]

Vibrio cholerae

Pathogen and disease overview

Vibrio cholerae is the causative agent of cholera disease, an illness characterized by massive aqueous diarrhea that can rapidly lead to death due to dehydration and electrolyte imbalance.[122] Since 1817, there have been 7 cholera pandemics, the last of which started in 1961; presently cholera affects an estimated 3-5 million people per year, causing about 120,000 deaths.[122] According to the WHO, 129,064 cholera cases and 2,102 deaths were reported in 2013,[123] reflecting a notable decrease from the previous year (245,393 cases and 3,034 deaths) however, illness rates are assumed to be underestimates.

V. cholerae is a Gram-negative curve-shaped bacterium first isolated in 1884 by Robert Koch.[122] It belongs to the Vibrionaceae family, which primarily includes water living bacteria along with at least 2 other species that can cause illness in humans: *Vibrio parahaemolyticus* and *Vibrio vulnificus*.[122] Reactivity of antibodies against lipopolysaccharide O antigen has led to the identification of about 200 different serogroups of *V. cholerae*, of which strains belonging to O1 and O139 serogroups have been associated with human epidemics. In fact, the 7 cholera pandemics that have occurred since 1817 were all caused by O1 strains; as a result, they are the most well studied to date. Two main biotypes of *V. cholerae* O1, Classical and El Tor, can be distinguished according to a set of phenotypic features, including the capacity to agglutinate the red blood cells of chicken and sheep, susceptibility to polimixin B, susceptibility to lytic bacteriophages and the pattern of growth displayed in Voger Proskauer medium.[124] The first 6 cholera pandemics have been attributed to the Classical biotype, whereas the 7th was attributable to El Tor. Furthermore, 3 different serotypes of *V. cholerae* O1 (Ogawa, Inaba and Hikojima) have been established within biotypes based on their reactivity to antibodies against other regions within the lipopolysaccharide.[125]

The main virulence factor of *V. cholerae* is the cholera toxin (CT), a secreted holotoxin consisting of one catalytic subunit (subunit A, CT-A) and 5 repetitions of the receptor-binding subunit (subunit B, CT-B).[126] CT binds to GM1-monoganglioside molecules present on the apical side of epithelial cells in the small bowel, inducing a signaling cascade that results in an excessive release of electrolytes and water toward the lumen.[126] Both CT subunits are encoded within a mobile genetic element, the lisogenic CTXϕ bacteriophage, which can be inserted in different sites on the *V. cholerae* genome.[122]

Control of cholera epidemics in developing countries is directly related to improvements in hygiene and the availability of non-contaminated drinking water. As neither of these improvements will be fully accomplished in the short term, the WHO has supported the use of oral vaccination as a strategy to reduce the impact of cholera in low-income countries, in parallel to progresses in water, sanitation and hygiene interventions. Although an ideal vaccine has not yet been developed, vaccines licensed for distribution in some countries are available. In parallel, there is work in-progress related to the development of new vaccine candidates and improvements to current vaccines.

Three orally administered formulations to prevent cholera are currently licensed, *Dukoral*®, *Shanchol*® and *mORCVAX*®. *Dukoral*® was licensed in 1991 and has been distributed in 65 countries around the globe, whereas *Shanchol*® was first licensed in 2009 being only distributed in India.[127] Now, after being prequalified by the WHO in 2001 and 2011, respectively, *Dukoral*® and *Shanchol*® can be distributed globally.[123] The "oral cholera vaccine stockpile" is a globally available reserve, which attempts to store and provide cholera vaccines to be used when and where required, especially in outbreaks and humanitarian crises scenarios, along with other actions to control and prevent the spread of the disease.[127] This stockpile was created in 2011 and includes these 2 formulations.

mORCVAX®, another killed whole cell vaccine, is identical to *Shanchol*® but is manufactured by another company and is currently only licensed for distribution in Vietnam.[21] Additionally, a formulation that was licensed in some countries as *Orochol*®/*Mutacol*® halted production in 2004. Recently, results of a phase I trial of a newly manufactured formulation, starting from master stock of CVD103-HgR, have been reported and require further evaluation in order to be licensed in the USA.[128]

Current vaccines licensed worldwide

Dukoral*® *(Crucell, Switzerland) is a whole cell formulation that contains a mix of heat and formalin-killed *Vibrio cholerae* O1 from Classical and El Tor biotypes, Inaba and Ogawa serotypes, as well as the purified recombinant CT-B.[129] *Dukoral*® is licensed for administration to individuals starting at 2 y of age,

and is distributed in packages containing 2 doses with bicarbonate buffer.[147] The safety and immunogenicity of *Dukoral*® was evaluated in volunteers in the USA, Sweden and Peru, a country in which cholera emerged in 1991.[129] The 2 doses are to be administered 2 weeks apart. No major post-vaccination side effects have been observed and vibrocidal antibodies, in addition to anti-CT IgG and IgA, were induced.

An evaluation of the ability to confer protection was conducted in rural areas of Bangladesh.[130] At the time, the purified CT-B included in the vaccine was not the recombinant form used in the current formulation. Three doses of this vaccine formulation were administered in 6-week intervals to 21,141 individuals, while 21,220 received *Escherichia coli* K12 as a placebo. A significantly lower number of cholera cases occurred in the vaccinated group, indicating that the formulation was efficacious. Protection was high during the first 6 months (85%, 95% CI: 62-94%), and considered complete (100%, 95% CI lower boundary (LB): 80%) in children 2-5 y of age.[131] Protection was also clearly evident after the first (62%, 95% CI LB: 50%) and second years (58%, 95% CI LB: 44%) and declined after the third year (18%, 95% CI LB: -14%). No protection was observed during the fourth year. A further evaluation was carried out in Pampa de San Juan de Miraflores, Peru. This study included about 35,000 individuals, both children and adults.[132] Three doses of the formulation containing the recombinant CT-B were administered. The first 2 doses were given 2 weeks apart, prior to cholera season, and the third was administered about 11 months later, prior to the start of the subsequent year's cholera season. No protection was observed following the administration of the first 2 doses (-4% protection, 95% CI: -43-87%), whereas 61% protection (95% CI: 28-79%) was observed after the third dose.

WHO has supported vaccination campaigns with *Dukoral*® and currently recommends its use, particularly to contain outbreaks in high-risk areas and for travelers visiting endemic regions.[123] The suggested administration is 2 doses for adults and children over 6 y of age and 3 doses for children under 6 y of age, with a minimum interval of 1 week between each dose and a maximum interval of 6 weeks.

Shanchol® (Shantha Biotechnics, India)

This formulation includes whole killed *V. cholerae* O1 strains from classical (Inaba and Ogawa serotypes) and El Tor (Inaba) biotypes. It differs from *Dukoral*® in that it contains an additional killed *V. cholerae* O139 strain, therefore making it a bivalent vaccine, and also in that it lacks the recombinant CT-B.[133] *Shanchol*® is licensed for administration in children 1 y of age and older (compared to 2 y of age and older for *Dukoral*®).[21] Safety and immunogenicity of *Shanchol*® was evaluated in a double-blind, placebo-controlled trial, including 101 vaccinated individuals (50 adults and 51 children 1-17 y of age) and 100 placebos (50 adults and 50 children) in India. Two doses were administered 14 d apart, and after 28 d vaccination-related side effects did not differ between vaccinated groups and placebos receiving *Escherichia coli* K12. Vibrocidal antibodies against *V. cholerae* O1 and O139 were detected in the serum samples of vaccinated groups at significantly higher levels than the groups that received placebo, although the response to O139 was lower than that observed against O1.[133]

Efficacy of *Shanchol*® was evaluated in a placebo-controlled trial carried out in Kolkata, India in about 67,000 individuals (31,932 vaccinees and 34,968 placebos), including adults and children >1 y of age. Overall, after 3 y of follow up, the vaccine was shown to confer 66% protection (95% CI LB: 53%) after administration of 2 doses.[134] In children 1-4 y old, an age range particularly affected by cholera, efficacy after 3 y was 43% (95% CI LB: 7%), and after 2 y was higher (83%, 95% CI LB: 43%).

Shanchol® is recommended to be administered in 2 doses 2 weeks apart.[123] Recently, administration of *Shanchol*® following this scheme proved to be effective (86.6% efficacy, 95% CI: 56.7-95.8%) in a cholera outbreak scenario in Guinea.[135] These results support the use of *Shanchol*® and the generation of the oral cholera vaccine stockpile.

Current vaccines with restricted license

mORCVAX® (VABIOTECH, Vietnam)

As mentioned above, this formulation is identical to *Shanchol*®, and the same dosage is recommended, but it is manufactured by a different company and it has conducted separate evaluation trials in Vietnam.[127] The current mORCVAX® contains 5 different *V. cholerae* strains: 1 *V. cholerae* serogroup O1 Inaba El Tor, 1 serogroup O1 Inaba classical, 2 serogroup O1 Ogawa classical and 1 serogroup O139.[136] Safety and immunogenicity of the current formulation was evaluated in a 143 adults in Vietnam, with 74 receiving 2 doses of the vaccine 2 weeks apart and 69 receiving killed *E. coli* K12 as placebo. No adverse effects were evident in either group while vibrocidal antibodies were significantly induced after vaccination, even when response against *V. cholerae* O139 was scarce compared to that stimulated against *V. cholerae* O1.[136] Efficacy has been only evaluated for a similar previous formulation (ORC-Vax), which contained a different *V. cholerae* serogroup O1 Inaba strain and only 1 serogroup O1 Ogawa strain. The study was carried out in an outbreak scenario in Hanoi, Vietnam, including 54 matched cholera cases and controls.[137] Vaccination was found to be significantly higher in controls (16/54) than in cases (8/54), with an efficacy of 54% (95% CI: -31-84%). By taking into account other factors that were significantly associated with cholera cases in a univariate analysis (such as eating dog meat or raw vegetables and not drinking boiled or bottled water most of the time) efficacy was raised to 76% (95% CI: 4-94%).[137]

CVD103-HgR

This formulation was designed at the Center for Vaccine Development, University of Maryland, Baltimore (Maryland, USA) and is based on an attenuated *V. cholerae* O1 classical Inaba strain (*CVD103-HgR*). In contrast to the 2 previously mentioned licensed vaccines, this formulation included live non-toxigenic bacteria, after knocking out both the CT-A encoding gene and the hemolysin A (*hlyA*) gene.[128] The last mentioned mutation was achieved by insertion of a cassette conferring resistance to mercury, which allowed the identification of the strain by growth

in culture media containing this heavy metal. The formulation was licensed as *Orochol*® in Switzerland, New Zealand, Australia and other countries, and as *Mutacol*® in Canada.[128] Results of safety and immunogenicity assessments of CVD103-HgR in various geographical regions for both adults and children have been published in several reports.[21] Despite evident protection against cholera in volunteer challenges, protection was not significant after massive evaluation in the endemic region of North Jakarta, Indonesia (14% protection, 95% CI LB: -24%).[138] The low number of cholera cases at the time of evaluation may have influenced these results (50 cases occurred in placebo recipients during the 4-year follow-up period). One hypothesis to explain the low number of cholera cases in the placebo group is a potential herd protective effect, as suggested after reanalysis of data obtained during the evaluation of *Dukoral*® in Dhaka, Bangladesh.[139] Thus, efficacy of the vaccine may be higher than estimated. Nevertheless, production of the vaccine by Crucell (Netherlands) was halted in 2004. In 2009, the PaxVax Corporation (USA) acquired the right to restart production of CVD103-HgR, and a new evaluation was performed in order to assess safety and immunogenicity.[128] In this study, 66 volunteers were vaccinated with a single oral dose of approximately 4.4 $x10^{10}$ colony forming units (CFU), a higher dose than that administered in evaluations performed at earlier stages. Symptoms were significantly less frequent in vaccinated individuals compared to the placebo group. Vibrocidal antibodies were significantly induced in 89% of cases, peaking at 10-14 d after vaccination. Production of anti-CT serum IgG was induced in 59% of vaccinated individuals with the titer peaking at day 28 post-vaccination. Further human trials aimed at obtaining a cholera vaccine licensed in the USA for administration predominantly to travelers visiting endemic zones are in progress.

Vaccines in early stages of clinical development

Four orally administered vaccine formulations, all based on live attenuated *V. cholerae* strains, have been shown to be safe and immunogenic in humans, and/or have demonstrated the capacity to confer protection to small groups of volunteers.

Peru-15 (CholeraGarde®)

This formulation was developed from a *V. cholerae* O1 El Tor Inaba strain. Attenuation was obtained by deleting genes encoding for CT-A, RTX toxin and recombinase A (RecA) (making the strain unable to recombine homologous genetic material using this mechanism).[21] Safety and immunogenicity was first evaluated in 2 groups of 12 inpatient and 50 outpatient adult volunteers in the USA.[140] Administration of 10^8 and 10^9 CFU caused no major symptoms compared to the groups who received bicarbonate buffer mixed with milk as placebo (vaccine vehicle) and induced significant production of vibrocidal antibodies and anti-toxin IgG. A second evaluation in 59 volunteers showed similar results after administration of 10^8 and 10^9 lyophilized bacteria.[141] The ability of Peru 15 to confer protection against a challenge with a pathogenic strain (*V. cholerae* O1 El Tor Inaba 10^5 CFU) was evaluated in a subgroup of 36 subjects. Seven out of 12 of the individuals who received placebo suffered diarrhea (5 cholera cases), compared to none of 24 vaccinated subjects.[141]

Two other studies performed in Dhaka, Bangladesh, proved the safety and immunogenicity of Peru-15. The first included 70 adults who received buffer as placebo, or a single dose of 2 x 10^8 CFU.[142] Vibrocidal and anti-LPS antibody responses were significantly induced; however, anti-CT response was modest. Only 7% and 27% of vaccinated subjects developed anti-CT IgA or IgG antibody secreting cells (ASC), respectively, whereas 20% developed anti-CT IgA in serum. Anti-CT IgG levels in serum were low and anti-CT IgM levels were not specified. A later study included a total of 240 Bangladeshi children between 9 months and 5 y of age.[143] Doses of 2 x 10^7 CFU and 2 x 10^8 CFU of Peru-15 were given to different groups, including a placebo group receiving buffer. Neither fever nor diarrhea was reported in any of the vaccinated groups, and mild symptoms were similar in vaccine and placebo recipients. Vibrocidal antibodies were induced after administration of both doses, and specific anti-LPS IgA and IgG antibodies increased significantly after vaccination. In contrast, as noted in previous studies of adult Bangladeshi volunteers, anti-CT IgG response after vaccination in children was low. These results contrast with those obtained after vaccination of volunteers in the USA, in which Peru-15 stimulated significant production of anti-toxin antibodies. Nevertheless, overall safety results and success in inducing production of vibrocidal and anti-LPS antibodies, in addition to preliminary protection results, indicate that Peru-15 could be a successful vaccine candidate.

***V. cholerae* 638**

This formulation developed by the Finlay Institute (Havana, Cuba) was originated from the *V. cholerae* strain C7258 (El Tor, Ogawa), first isolated during an outbreak in Peru in 1991. *V. cholerae* 638 was obtained after deletion of genes encoding both subunits of CT (CT-A and CT-B) as well as genes encoding the accessory cholera enterotoxin (Ace) and the zonula occludens toxin (Zot).[144] Furthermore, the gene encoding for the hemaglutinin protease (HA/P) was inactivated by insertion of the reporter *celA*, which encodes the endoglucanase A of *Clostridium thermocellum*. This property allows for rapid identification of the strain in carboxymethylcellulose indicator agar stained with Congo Red. Morphology, biochemical properties, growth rates and colonization capacity of the bacteria were not affected by these mutations.[144] Safety and immunogenicity of *V. cholerae* 638 were preliminarily evaluated in 56 adult volunteers in Havana, Cuba (42 vaccinees and 14 placebos) who received single doses ranging from 4 x 10^7 to 2 x 10^9 CFU living bacteria, followed by another study including 36 adult volunteers (24 vaccinees and 12 placebos) who received a single dose of $2x10^9$ CFU living bacteria. Similar results were obtained in both studies. None of the vaccinees developed diarrhea, and *V. cholerae* 638 induced vibrocidal antibodies against *V. cholerae* classical Ogawa and anti-LPS IgG and IgA.[145]

The capacity of *V. cholerae* 638 to confer short-term protection was first reported in 2005 in a group of 24 Cuban adult volunteers who received the vaccine compared to a matched group of 21 placebos receiving only bicarbonate buffer.[146] After vaccination, individuals were challenged either with a mutant nontoxigenic *V. cholerae* strain, a parent of *V. cholerae* 638 or a wild-

type *V. cholerae* O1 El Tor Ogawa strain. Two of 13 vaccinated subjects who received the non-toxigenic strain developed diarrhea, compared to 5/9 who received placebo. None of 12 vaccinated subjects challenged with the virulent strain developed diarrhea, compared to 7/9 placebo recipients. In 2010, another similar study was published involving a group of 21 Cuban volunteers who received bicarbonate buffer or *V. cholerae* 638 and who were challenged with a virulent strain 28 d later.[147] Seven out of 9 individuals who received placebo, but none of the 12 vaccinated subjects, developed diarrhea.

CVD112

This vaccine candidate was developed at the Center for Vaccine Development at the University of Maryland, Baltimore (Maryland, USA) by deleting genes encoding for CTA, the toxins Ace and Zot, as well as the core-encoded pilus (Cep) in a *V. cholerae* O139 strain.[148] CVD112 administered in a dose containing 10^6 CFU did not cause adverse effects in adult volunteers, while a higher dose of 10^8 CFU was associated with increased side effects. A challenge with the virulent wild-type *V. cholerae* O139 AII837 strain was carried out at day 28 post-vaccination in 8 vaccinees (4 vaccinated with the lower dose and 4 with the higher) and 15 unvaccinated controls. Only 1 vaccinee, who received the lower dose, developed diarrhea versus 12 of the unvaccinated controls. No further studies have been published following this initial report in 1995.[148]

VA1.3 (Vaccine attempt 1.3)

Based on a non-toxigenic *V. cholerae* O1 El Tor, this formulation lacks the CTX prophage but carries the gene encoding for CT-B and is resistant to ampicillin.[149] VA1.3 was designed in Kolkata, India, where it was evaluated in 304 volunteers, in order to assess its safety and immunogenicity. In this study, 186 individuals received a single dose containing $5x10^9$ CFU of VA1.3 and the remaining received bicarbonate buffer as a placebo. Only two vaccinated subjects developed mild diarrhea not requiring oral rehydration; none of the placebo recipients developed symptoms. Vibrocidal antibodies were significantly induced 15 d post-vaccination, and anti-CT antibodies determined in a subset of volunteers also increased.[149] A new version of this formulation, named **VA1.4**, was produced with financial support from the Indian government. This new strain lacks the gene conferring resistance to ampicillin in an attempt to avoid eventual lateral transfer of this gene to vaccinated subjects' resident bacteria. Results of a phase I trial evaluating VA1.4 were recently published.[150] No significant adverse effects were observed after administration of 2 doses of the formulation (14 d apart) to 44 adult volunteers in India, compared to 43 placebos. Seroconversion and a rise in anti-vibrocidal antibody titers were evident and significant after the first dose. Anti-CT response in the form of neutralizing antibodies was weak compared to that induced by VA1.3. Future trials should evaluate if administration of a second dose and/or anti-CT neutralizing response are required for VA1.4 to be an effective vaccine.

Vaccine candidates in preclinical development

Other vaccine formulations based on both *V. cholerae* O1 and O139 strains have been designed and have been shown to be safe, immunogenic, as well as to have the capacity to confer protection in animal models.

IEM 108

This candidate is based on a *V. cholerae* O1 El Tor, Ogawa strain, lacking the CTX prophage, in which genes encoding for CT-B and RstR were introduced.[151] RstR is a transcriptional repressor protein involved in immunity against CTX phage, which if expressed would avoid potential reversion to a toxigenic phenotype by lateral transfer of a new phage from another El Tor strain.[151] Evaluation of IEM108 was performed in rabbits by administration of a single dose of 10^9 CFU. This dosage proved to be safe and to stimulate vibrocidal and anti-CT antibodies, peaking at 14 and 21 d post-vaccination, respectively. Furthermore, vaccination with IEM prevented fluid accumulation in rabbit ligated loops after challenge with virulent *V. cholerae* El Tor and classical strains, in addition to challenge with purified CT.[151] These results suggest that IEM 108 could potentially confer protection against both biotypes.

VCUSM2

This is a metabolic auxotroph, unable to grow in the absence of aminolevulenic acid (ALA), derived from an O139 Bengal strain, which was responsible for cholera cases in Bangladesh and India.[152] This candidate aims to preserve the antigenic repertoire of the virulent strain while reducing its toxigenic effect by means other than directly knocking out toxin or other virulence genes. There have been reports that an ALA-auxotroph *V. cholerae* O1 El Tor mutant strain had reduced capacity to colonize the bowel, while preserving its immunogenic potential.[152] In order to obtain a similar phenotype in an O139 Bengal strain, gene *hemA* encoding the enzyme glutamyl tRNA reductase was inactivated. The resulting VCUSM2 strain elicited production of vibrocidal, anti-LPS and anti-CT antibodies in rabbits after 2 doses containing 10^{10} CFU of live bacteria were administered 2 weeks apart.[152] Additionally, it conferred protection against challenge with the wild-type Bengal O139 strain in the ligated ileal loops and the RITARD model (removable intestinal tie-adult rabbit diarrhea).

TLP01

Developed in Havana, Cuba, this is a live bacteria formulation designed from the virulent *V. cholerae* O139 CRC266 strain, obtained after deletion of the CTX prophage (knock-out of the HA/P gene by insertion of the *celA* reporter gene) and deletion of *mshA*, which encodes the major structural subunit of the mannose-sensitive haemagglutinin (MSH) pilus.[153] The logic behind this mutation is based on the fact that the MSH pilus is the receptor for transduction of the VGJϕ phage, which can eventually carry and transfer the CTX phage genome into a toxin co-regulated pilus (TCP) negative strain. Therefore, this mutation may prevent reversion to a toxigenic phenotype. Administration of TLP01 in a single dose of 10^9 CFU stimulated production of antivibrocidal antibodies as well as anti-LPS IgG, IgA and IgM

in rabbits. A similar anti-LPS response was observed in serum after administration of 3 x 10^{10} CFU to rats.[153]

Conclusions

In this first of this 2 part series, aimed at reviewing the full spectrum of vaccine development against viral and bacterial pathogens causing acute gastroenteritis, we have focused on rotavirus, norovirus and *Vibrio cholerae*. Licensed vaccines have proven, in the case of rotavirus, to be highly efficacious and to have a significant public health impact. The main challenges for rotavirus vaccines will be to improve effectiveness in resource-deprived regions, to further reduce the low risk of inducing IS, and most importantly, to increase vaccine usage in the world's poorest regions. Several new multi- or monovalent vaccines may achieve licensure in the following years. Vaccines for norovirus are advancing at a fast pace and are using a radically different strategy than rotavirus; currently the most advanced candidates are based on IM inoculation of VLPs. A major challenge to this strategy, if successful in phase III trials, will be its implementation in already crowded childhood vaccination schedules, an issue that is less problematic for potential adult vaccination strategies. An IM vaccine including both norovirus and rotavirus with high efficacy rates against different virus groups/types would be an attractive vaccine over the currently available vaccine options, especially countries that are resource-deprived or with an absence of IS risk. Vaccines for cholera have been around for several decades, providing protection ranging from 60 to 70% for up to 5 y The main challenges are to increase vaccine use in endemic areas, accepting that these protective efficacy rates are an important factor in reducing cholera morbidity and mortality, and advancing past the current standard practice of recommending vaccination only for travelers to endemic areas or for outbreak control.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Walker CL, Aryee MJ, Boschi-Pinto C, Black RE. Estimating diarrhea mortality among young children in low and middle income countries. PLoS One 2012; 7:e29151; PMID:22235266; http://dx.doi.org/10.1371/journal.pone.0029151
2. Walker CLF, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA, O'Brien KL, Campbell H, Black RE. Global burden of childhood pneumonia and diarrhoea. Lancet 2013; 381:1405-16; PMID:23582727; http://dx.doi.org/10.1016/S0140-6736(13)60222-6
3. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, Rudan I, Campbell H, Cibulskis R, Li M., et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 2012; 379:2151-61; PMID:22579125; http://dx.doi.org/10.1016/S0140-6736(12)60560-1
4. O'Ryan GM, Ashkenazi-Hoffnung L, O'Ryan-Soriano MA, Ashkenazi S. Management of acute infectious diarrhea for children living in resource-limited settings. Expert Rev Anti Infect Ther 2014; 12:621-32; PMID:24661314; http://dx.doi.org/10.1586/14787210.2014.901168
5. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF., et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the global enteric multicenter study, GEMS): a prospective, case-control study. Lancet 2013; 382:209-22; PMID:23680352; http://dx.doi.org/10.1016/S0140-6736(13)60844-2
6. Moyo SJ, Gro N, Matee MI, Kitundu J, Myrmel H, Mylvaganam H, Maselle SY, Langeland N. Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in dar es salaam, tanzania. BMC pediatr 2011; 11:19; PMID:21345186; http://dx.doi.org/10.1186/1471-2431-11-19
7. Razafindratsimandresy R, Heraud JM, Ramarokoto CE, Rabemanantsoa S, Randremanana R, Andriamamonjy NS, Richard V, Reynes JM. Rotavirus genotypes in children in the community with diarrhea in Madagascar. J Med Virol 2013; 85:1652-6; PMID:23797859; http://dx.doi.org/10.1002/jmv.23631
8. Opintan JA, Newman MJ, Ayeh-Kumi PF, Affrim R, Gepi-Attee R, Sevilleja JE, Roche JK, Nataro JP, Warren CA, Guerrant RL. Pediatric diarrhea in southern ghana: etiology and association with intestinal inflammation and malnutrition. Am J Trop Med Hyg 2010; 83:936-43; PMID:20889896; http://dx.doi.org/10.4269/ajtmh.2010.09-0792
9. Ciarlet M, Schodel F. Development of a rotavirus vaccine: clinical safety, immunogenicity, and efficacy of the pentavalent rotavirus vaccine, rotaTeq. Vaccine 2009; 27:6:G72-81; PMID:20006144; http://dx.doi.org/10.1016/j.vaccine.2009.09.107
10. Giaquinto C, Dominiak-Felden G, Van Damme P, Myint TT, Maldonado YA, Spoulou V, Mast TC, Staat MA. Summary of effectiveness and impact of rotavirus vaccination with the oral pentavalent rotavirus vaccine: a systematic review of the experience in industrialized countries. Hum Vaccin 2011; 7:734-48; PMID:21734466; http://dx.doi.org/10.4161/hv.7.7.15511
11. O'Ryan M. Rotarix TM. (RIX4414): an oral human rotavirus vaccine. Expert Rev Vaccines 2007; 6:11-9; PMID:17280473; http://dx.doi.org/10.1586/14760584.6.1.11
12. O'Ryan M, Linhares AC. Update on rotarix (TM): an oral human rotavirus vaccine. Expert Rev Vaccines 2009; 8:1627-41; PMID:19943758; http://dx.doi.org/10.1586/erv.09.136
13. O'Ryan M, Lucero Y, Linhares AC. Rotarix (R): vaccine performance 6 years postlicensure. Expert Rev Vaccines 2011; 10:1645-59; PMID:22085167; http://dx.doi.org/10.1586/erv.11.152
14. Soares-Weiser K, Maclehose H, Bergman H, Ben-Aharon I, Nagpal S, Goldberg E, Pitan F, Cunliffe N. Vaccines for preventing rotavirus diarrhoea: vaccines in use. Cochrane Database Syst Rev 2012; 11:CD008521; PMID:23152260; http://dx.doi.org/10.1002/14651858.CD008521.pub3
15. Yen C, Tate JE, Hyde TB, Cortese MM, Lopman BA, Jiang B, Glass RI, Parashar UD. Rotavirus vaccines: current status and future considerations. Hum Vaccin Immunother 2014; 10:1436-48; PMID:24755452; http://dx.doi.org/10.4161/hv.28857
16. Debbink K, Lindesmith LC, Baric RS. The state of norovirus vaccines. Clin Infect Dis 2014; 58:1746-52; PMID:24585561; http://dx.doi.org/10.1093/cid/ciu120
17. Tan M, Jiang X. Vaccine against norovirus. Hum Vaccin Immunother 2014; 10:1449-56; PMID:24718366; http://dx.doi.org/10.4161/hv.28626
18. Kim YJ, Yeo SG, Park JH, Ko HJ. *Shigella* vaccine development: prospective animal models and current status. Curr Pharm Biotechnol 2014; 14:903-12; PMID:24372251; http://dx.doi.org/10.2174/1389201014666131226123900
19. McLennan CA, Martin LB, Micoli F. Vaccines against invasive *salmonella* disease: current status and future directions. Hum Vaccin Immunother 2014; 10:1478-93; PMID:24804797; http://dx.doi.org/10.4161/hv.29054
20. Ahmed T, Bhuiyan TR, Zaman K, Sinclair D, Qadri F. Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea. Cochrane Database Syst Rev 2013; 7:CD009029; PMID:23828581; http://dx.doi.org/10.1002/14651858.CD009029.pub2
21. Pastor M, Pedraz JL, Esquisabel A. The state-of-the-art of approved and under-development cholera vaccines. Vaccine 2013; 31:4069-78; PMID:23845813; http://dx.doi.org/10.1016/j.vaccine.2013.06.096
22. Snedeker KG, Campbell M, Sargeant JM. A systematic review of vaccinations to reduce the shedding of *Escherichia coli* O157 in the faeces of domestic ruminants. Zoonoses Public Health 2012; 59:126-38; PMID:21824378; http://dx.doi.org/10.1111/j.1863-2378.2011.01426.x
23. Varela NP, Dick P, Wilson J. Assessing the existing information on the efficacy of bovine vaccination against *Escherichia coli* O157:H7- a systematic review and meta-analysis. Zoonoses Public Health 2013; 60:253-68; PMID:22856462; http://dx.doi.org/10.1111/j.1863-2378.2012.01523.x
24. de Zoete MR, van Putten JPM, Wagenaar JA. Vaccination of chickens against *Campylobacter*. Vaccine 2007; 25:5548-57; PMID:17224215; http://dx.doi.org/10.1016/j.vaccine.2006.12.002

25. Mead JR. Prospects for immunotherapy and vaccines against *Cryptosporidium*. Hum Vaccin Immunother 2014; 10:1505-13; PMID:24638018; http://dx.doi.org/10.4161/hv.28485
26. Gladstone BP, Ramani S, Mukhopadhya I, Muliyil J, Sarkar R, Rehman AM, Jaffar S, Gomara MI, Gray JJ, Brown DW., et al. Protective effect of natural rotavirus infection in an Indian birth cohort. N Eng J Med 2011; 365:337-46; PMID:21793745; http://dx.doi.org/10.1056/NEJMoa1006261
27. Velazquez FR, Matson DO, Calva JJ, Guerrero L, Morrow AL, Carter-Campbell S, Glass RI, Estes MK, Pickering LK, Ruiz-Palacios GM. Rotavirus infections in infants as protection against subsequent infections. N Eng J Med 1996; 335:1022-8; PMID:8793926; http://dx.doi.org/10.1056/NEJM199610033351404
28. Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, Birmingham M, Glass RI. Global mortality associated with rotavirus disease among children in 2004. J Infect Dis 2009; 200 1:S9-S15; PMID:19817620; http://dx.doi.org/10.1086/605025
29. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis 2012; 12:136-41; PMID:22030330; http://dx.doi.org/10.1016/S1473-3099(11)70253-5
30. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003; 9:565-72; PMID:12737740; http://dx.doi.org/10.3201/eid0905.020562
31. Estes MK, Desselberger U. Rotaviruses: cause of vaccine-preventable disease yet many fundamental questions remain to be explored. Curr Opin Virol 2012; 2:369-72; PMID:22762866; http://dx.doi.org/10.1016/j.coviro.2012.06.002
32. Greenberg HB, Estes MK. Rotaviruses: from pathogenesis to vaccination. Gastroenterology 2009; 136:1939-51; PMID:19457420; http://dx.doi.org/10.1053/j.gastro.2009.02.076
33. O'Ryan M. The ever-changing landscape of rotavirus serotypes. Pediatr Infect Dis J 2009; 28:S60-S2; PMID:19252426; http://dx.doi.org/10.1097/INF.0b013e3181967c29
34. O'Ryan ML, Hermosilla G, Osorio G. Rotavirus vaccines for the developing world. Curr Opin Infect Dis 2009; 22:483-9; PMID:19623063; http://dx.doi.org/10.1097/QCO.0b013e32833040a9
35. O'Ryan ML, Matson DO, Estes MK, Bartlett AV, Pickering LK. Molecular epidemiology of rotavirus in children attending day care centers in Houston. J Infect Dis 1990; 162:810-6; PMID:2169496; http://dx.doi.org/10.1093/infdis/162.4.810
36. Bernstein DI, Glass RI, Rodgers G, Davidson BL, Sack DA. Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in US children. US rotavirus vaccine efficacy group. JAMA 1995; 273:1191-6; PMID:7707626; http://dx.doi.org/10.1001/jama.1995.03520390051032
37. Joensuu J, Koskenniemi E, Pang XL, Vesikari T. Randomised placebo-controlled trial of rhesus-human reassortant rotavirus vaccine for prevention of severe rotavirus gastroenteritis. Lancet 1997; 350:1205-9; PMID:9652561; http://dx.doi.org/10.1016/S0140-6736(97)05118-0
38. Perez-Schael I, Guntinas MJ, Perez M, Pagone V, Rojas AM, Gonzalez R, Cunto W, Hoshino Y, Kapikian AZ. Efficacy of the rhesus rotavirus-based quadrivalent vaccine in infants and young children in Venezuela. N Eng J Med 1997; 337:1181-7; PMID:9337376; http://dx.doi.org/10.1056/NEJM199710233371701
39. Rennels MB, Glass RI, Dennehy PH, Bernstein DI, Pichichero ME, Zito ET, Mack ME, Davidson BL, Kapikian AZ. Safety and efficacy of high-dose rhesus-human reassortant rotavirus vaccines-report of the national multicenter trial. united states rotavirus vaccine efficacy group. Pediatrics 1996; 97:7-13; PMID:8545227
40. Santosham M, Moulton LH, Reid R, Croll J, Weatherholt R, Ward R, Forro J, Zito E, Mack M, Brenneman G., et al. Efficacy and safety of high-dose rhesus-human reassortant rotavirus vaccine in native american populations. J Pediatr 1997; 131:632-8; PMID:9386673; http://dx.doi.org/10.1016/S0022-3476(97)70076-3
41. Murphy TV, Gargiullo PM, Massoudi MS, Nelson DB, Jumaan AO, Okoro CA, Zanardi LR, Setia S, Fair E, LeBaron CW., et al. Rotavirus Intussusception Investigation Team. Intussusception among infants given an oral rotavirus vaccine. N Eng J Med 2001; 344:564-72; PMID:11207352; http://dx.doi.org/10.1056/NEJM200102223440804
42. Armah GE, Kapikian AZ, Vesikari T, Cunliffe N, Jacobson RM, Burlington DB, Ruiz LP, Jr. Efficacy, immunogenicity, and safety of two doses of a tetravalent rotavirus vaccine RRV-TV in Ghana with the first dose administered during the neonatal period. J Infect Dis 2013; 208:423-31; PMID:23599316; http://dx.doi.org/10.1093/infdis/jit174
43. Block SL, Vesikari T, Goveia MG, Rivers SB, Adeyi BA, Dallas MJ, Bauder J, Boslego JW, Heaton PM, Pentavalent Rotavirus Vaccine Dose Confirmation Efficacy Study G. Efficacy, immunogenicity, and safety of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine at the end of shelf life. Pediatrics 2007; 119:11-8; PMID:17200266; http://dx.doi.org/10.1542/peds.2006-2058
44. Clark HF, Bernstein DI, Dennehy PH, Offit P, Pichichero M, Treanor J, Ward RL, Krah DL, Shaw A, Dallas MJ., et al.Safety, efficacy, and immunogenicity of a live, quadrivalent human-bovine reassortant rotavirus vaccine in healthy infants. J Pediatr 2004; 144:184-90; PMID:14760258; http://dx.doi.org/10.1016/j.jpeds.2003.10.054
45. Vesikari T, Clark HF, Offit PA, Dallas MJ, DiStefano DJ, Goveia MG, Ward RL, Schodel F, Karvonen A, Drummond JE., et al.Effects of the potency and composition of the multivalent human-bovine (WC3) reassortant rotavirus vaccine on efficacy, safety and immunogenicity in healthy infants. Vaccine 2006; 24:4821-9; PMID:16621194; http://dx.doi.org/10.1016/j.vaccine.2006.03.025
46. Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, Dallas MJ, Heyse JF, Goveia MG, Black SB., et al.Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N Eng J Med 2006; 354:23-33; PMID:16394299; http://dx.doi.org/10.1056/NEJMoa052664
47. Tate JEPM, Cortese MM, Lopman BA, Gentsch JR, Fleming J, Steele AD, Parashar UD. Remaining issues and challenges for rotavirus vaccine in preventing global childhood diarrheal morbidity and mortality. Expert Rev Vaccines 2012; 11:211-20; PMID:22309669; http://dx.doi.org/10.1586/erv.11.184
48. Patel M, Pedreira C, De Oliveira LH, Umana J, Tate J, Lopman B, Sanchez E, Reyes M, Mercado J, Gonzalez A., et al.Duration of protection of pentavalent rotavirus vaccination in Nicaragua. Pediatrics 2012; 130:e365-72; PMID:22753550; http://dx.doi.org/10.1542/peds.2011-3478
49. Yih WK, Lieu TA, Kulldorff M, Martin D, McMahill-Walraven CN, Platt R, Selvam N, Selvan M, Lee GM, Nguyen M. Intussusception risk after rotavirus vaccination in US infants. N Eng J Med 2014; 370:503-12; PMID:24422676; http://dx.doi.org/10.1056/NEJMoa1303164
50. Cunliffe NA, Witte D, Ngwira BM, Todd S, Bostock NJ, Turner AM, Chimpeni P, Victor JC, Steele AD, Bouckenooghe A., et al.Efficacy of human rotavirus vaccine against severe gastroenteritis in malawian children in the first two years of life: a randomized, double-blind, placebo controlled trial. Vaccine 2012; 30 1:A36-43; PMID:22520135; http://dx.doi.org/10.1016/j.vaccine.2011.09.120
51. Kawamura N, Tokoeda Y, Oshima M, Okahata H, Tsutsumi H, Van Doorn LJ, Muto H, Smolenov I, Suryakiran PV, Han HH. Efficacy, safety and immunogenicity of RIX4414 in Japanese infants during the first two years of life. Vaccine 2011; 29:6335-41; PMID:21640780; http://dx.doi.org/10.1016/j.vaccine.2011.05.017
52. Li RC, Huang T, Li YP, Luo D, Tao J, Fu B, Si G, Nong Y, Mo ZJ, Liao XY., et al. Human rotavirus vaccine (RIX4414) efficacy in the first two years of life: a randomized, placebo-controlled trial in China. Hum Vaccin Immunother 2014; 10:11-8; PMID:24013441; http://dx.doi.org/10.4161/hv.26319
53. Madhi SA, Kirsten M, Louw C, Bos P, Aspinall S, Bouckenooghe A, Neuzil KM, Steele AD. Efficacy and immunogenicity of two or three dose rotavirus-vaccine regimen in South African children over two consecutive rotavirus-seasons: a randomized, double-blind, placebo-controlled trial. Vaccine 2012; 30 1:A44-51; PMID:22520136; http://dx.doi.org/10.1016/j.vaccine.2011.08.080
54. Vesikari T, Karvonen A, Prymula R, Schuster V, Tejedor JC, Cohen R, Meurice F, Han HH, Damaso S, Bouckenooghe A. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in european infants: randomised, double-blind controlled study. Lancet 2007; 370:1757-63; PMID:18037080; http://dx.doi.org/10.1016/S0140-6736(07)61744-9
55. Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, Abate H, Breuer T, Clemens SC, Cheuvart B, Espinoza F, Gillard P, Innis BL., et al.Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. New Engl J Med 2006; 354:11-22; PMID:16394298; http://dx.doi.org/10.1056/NEJMoa052434
56. Cortese MM, Immergluck LC, Held M, Jain S, Chan T, Grizas AP, Khizer S, Barrett C, Quaye O, Mijatovic-Rustempasic S., et al.Effectiveness of monovalent and pentavalent rotavirus vaccine. Pediatrics 2013; 132:e25-33; PMID:23776114; http://dx.doi.org/10.1542/peds.2012-3804
57. Fernandes EG, Sato HK, Leshem E, Flannery B, Konstantyner TC, Veras MA, Patel MM. Impact of rotavirus vaccination on diarrhea-related hospitalizations in Sao Paulo state, Brazil. Vaccine 2014; 32:3402-8; PMID:24736002; http://dx.doi.org/10.1016/j.vaccine.2014.04.015
58. Steele AD, Neuzil KM, Cunliffe NA, Madhi SA, Bos P, Ngwira B, Witte D, Todd S, Louw C, Kirsten M., et al.Human rotavirus vaccine rotarix provides protection against diverse circulating rotavirus strains in African infants: a randomized controlled trial. BMC Infect Dis 2012; 12:213; PMID:22974466; http://dx.doi.org/10.1186/1471-2334-12-213
59. Patel MM, Lopez-Collada VR, Bulhoes MM, De Oliveira LH, Bautista Marquez A, Flannery B, Esparza-Aguilar M, Montenegro Renoiner EI, Luna-Cruz ME, Sato HK., et al.Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. N Eng J Med 2011; 364:2283-92; PMID:21675888; http://dx.doi.org/10.1056/NEJMoa1012952
60. Noel GMP, Merrot T. Intussusception risk after rotavirus vaccination in US infants. N Engl J Med 2014; 370:1766; PMID:24785219; http://dx.doi.org/10.1056/NEJMc1402790
61. Fu C, He Q, Xu J, Xie H, Ding P, Hu W, Dong Z, Liu X, Wang M. Effectiveness of the lanzhou lamb rotavirus vaccine against gastroenteritis among children. Vaccine 2012; 31:154-8; PMID:23127516; http://dx.doi.org/10.1016/j.vaccine.2012.10.078

62. Dang DA, Nguyen VT, Vu DT, Nguyen TH, Nguyen DM, Yuhuan W, Baoming J, Nguyen DH, Le TL, Rotavin MVTG. A dose-escalation safety and immunogenicity study of a new live attenuated human rotavirus vaccine (rotavin-M1) inVietnamese children. Vaccine 2012; 30 1:A114-21; PMID:22520120; http://dx.doi.org/10.1016/j.vaccine.2011.07.118
63. Danchin M, Kirkwood CD, Lee KJ, Bishop RF, Watts E, Justice FA, Clifford V, Cowley D, Buttery JP, Bines JE. Phase I trial of RV3-BB rotavirus vaccine: a human neonatal rotavirus vaccine. Vaccine 2013; 31:2610-6; PMID:23597719; http://dx.doi.org/10.1016/j.vaccine.2013.04.008
64. Glass RI, Bhan MK, Ray P, Bahl R, Parashar UD, Greenberg H, Rao CD, Bhandari N, Maldonado Y, Ward RL., et al.Development of candidate rotavirus vaccines derived from neonatal strains in India. J Infect Dis 2005; 192 1:S30-5; PMID:16088802; http://dx.doi.org/10.1086/431498
65. Bhandari N, Sharma P, Taneja S, Kumar T, Rongsen-Chandola T, Appaiahgari MB, Mishra A, Singh S, Vrati S. Rotavirus Vaccine Development G. A dose-escalation safety and immunogenicity study of live attenuated oral rotavirus vaccine 116E in infants: a randomized, double-blind, placebo-controlled trial. J Infect Dis 2009; 200:421-9; PMID:19545211; http://dx.doi.org/10.1086/600104
66. Luna EJ, Frazatti-Gallina NM, Timenetsky MC, Cardoso MR, Veras MA, Miraglia JL, Escobar AM, Grisi SJ, Raw I, Precioso AR. A phase I clinical trial of a new 5-valent rotavirus vaccine. Vaccine 2013; 31:1100-5; PMID:23261048; http://dx.doi.org/10.1016/j.vaccine.2012.12.020
67. Lappalainen S, Tamminen K, Vesikari T, Blazevic V. Comparative immunogenicity in mice of rotavirus VP6 tubular structures and virus-like particles. Hum Vaccin Immunother 2013; 9:1991-2001; PMID:23777748; http://dx.doi.org/10.4161/hv.25249
68. Wang L, Huang P, Fang H, Xia M, Zhong W, McNeal MM, Jiang X, Tan M. Polyvalent complexes for vaccine development. Biomaterials 2013; 34:4480-92; PMID:23498893; http://dx.doi.org/10.1016/j.biomaterials.2013.02.041
69. Moon S, Wang Y, Edens C, Gentsch JR, Prausnitz MR, Jiang B. Dose sparing and enhanced immunogenicity of inactivated rotavirus vaccine administered by skin vaccination using a microneedle patch. Vaccine 2013; 31:3396-402; PMID:23174199; http://dx.doi.org/10.1016/j.vaccine.2012.11.027
70. Jiang B, Wang Y, Saluzzo JF, Bargeron K, Frachette MJ, Glass RI. Immunogenicity of a thermally inactivated rotavirus vaccine in mice. Hum Vaccin 2008; 4:143-7; PMID:18382129; http://dx.doi.org/10.4161/hv.4.2.5263
71. Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, Chanock RM. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J Virol 1972; 10:1075-81; PMID:4117963
72. Dolin R. Norwalk agent-like particles associated with gastroenteritis in human beings. J Am Veterinary Med Association 1978; 173:615-9; PMID:100481
73. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. N Eng J Med 2009; 361:1776-85; PMID:19864676; http://dx.doi.org/10.1056/NEJMra0804575
74. Green KY, Ando T, Balayan MS, Berke T, Clarke IN, Estes MK, Matson DO, Nakata S, Neill JD, Studdert MJ, et al.Taxonomy of the caliciviruses. J Infect Dis 2000; 181 2:S322-30; PMID:10804145; http://dx.doi.org/10.1086/315591
75. Jung K, Wang Q, Kim Y, Scheuer K, Zhang Z, Shen Q, Chang KO, Saif LJ. The effects of simvastatin or interferon-α on infectivity of human norovirus using a gnotobiotic pig model for the study of antivirals. PLoS One 2012; 7:e41619; PMID:22911825; http://dx.doi.org/10.1371/journal.pone.0041619
76. Taube S, Kolawole AO, Hohne M, Wilkinson JE, Handley SA, Perry JW, Thackray LB, Akkina R, Wobus CE. A mouse model for human norovirus. mBio 2013; 4:e00450-13; PMID:23860770; http://dx.doi.org/10.1128/mBio.00450-13
77. Cukor G, Blacklow NR, Echeverria P, Bedigian MK, Puruggan H, Basaca-Sevilla V. Comparative study of the acquisition of antibody to norwalk virus in pediatric populations. Infect Immun 1980; 29:822-3; PMID:7216438
78. Greenberg HB, Valdesuso J, Kapikian AZ, Chanock RM, Wyatt RG, Szmuness W, Larrick J, Kaplan J, Gilman RH, Sack DA. Prevalence of antibody to the norwalk virus in various countries. Infect Immun 1979; 26:270-3; PMID:227798
79. Greenberg HB, Valdesuso J, Yolken RH, Gangarosa E, Gary W, Wyatt RG, Konno T, Suzuki H, Chanock RM, Kapikian AZ. Role of norwalk virus in outbreaks of nonbacterial gastroenteritis. J Infect Dis 1979; 139:564-8; PMID:220341; http://dx.doi.org/10.1093/infdis/139.5.564
80. O'Ryan ML, Mamani N, Gaggero A, Avendano LF, Prieto S, Pena A, Jiang X, Matson DO. Human caliciviruses are a significant pathogen of acute sporadic diarrhea in children of Santiago, Chile. J Infect Dis 2000; 182:1519-22; PMID:11023476; http://dx.doi.org/10.1086/315874
81. O'Ryan ML, Vial PA, Mamani N, Jiang X, Estes MK, Ferrecio C, Lakkis H, Matson DO. Seroprevalence of norwalk virus and mexico virus in chilean individuals: assessment of independent risk factors for antibody acquisition. Clin Infect Dis 1998; 27:789-95; PMID:9798035; http://dx.doi.org/10.1086/514949
82. Jiang X, Wang M, Graham DY, Estes MK. Expression, self-assembly, and antigenicity of the norwalk virus capsid protein. J Virol 1992; 66:6527-32; PMID:1328679
83. Xi JN, Graham DY, Wang KN, Estes MK. Norwalk virus genome cloning and characterization. Science 1990; 250:1580-3; PMID:2177224; http://dx.doi.org/10.1126/science.2177224
84. Bucardo F, Reyes Y, Svensson L, Nordgren J. Predominance of norovirus and sapovirus in nicaragua after implementation of universal rotavirus vaccination. PLoS One 2014; 9:e98201; PMID:24849288; http://dx.doi.org/10.1371/journal.pone.0098201
85. Kobayashi S, Fujiwara N, Yasui Y, Yamashita T, Hiramatsu R, Minagawa H. A foodborne outbreak of sapovirus linked to catered box lunches in japan. Arch Virol 2012; 157:1995-7; PMID:22752792; http://dx.doi.org/10.1007/s00705-012-1394-8
86. Tam CC, O'Brien SJ, Tompkins DS, Bolton FJ, Berry L, Dodds J, Choudhury D, Halstead F, Iturriza-Gomara M, Mather K., et al.Changes in causes of acute gastroenteritis in the united kingdom over 15 years: microbiologic findings from 2 prospective, population-based studies of infectious intestinal disease. Clin Infect Dis 2012; 54:1275-86; PMID:22412058; http://dx.doi.org/10.1093/cid/cis028
87. Ramani S, Atmar RL, Estes MK. Epidemiology of human noroviruses and updates on vaccine development. Curr Opin Gastroenterol 2014; 30:25-33; PMID:24232370http://dx.doi.org/10.1097/MOG.0000000000000022
88. Desai R, Hembree CD, Handel A, Matthews JE, Dickey BW, McDonald S, Hall AJ, Parashar UD, Leon JS, Lopman B. Severe outcomes are associated with genogroup 2 genotype 4 norovirus outbreaks: a systematic literature review. Clin Infect Dis 2012; 55:189-93; PMID:22491335; http://dx.doi.org/10.1093/cid/cis372
89. Patel MM, Hall AJ, Vinje J, Parashar UD. Noroviruses: a comprehensive review. J Clin Virol 2009; 44:1-8; PMID:19084472; http://dx.doi.org/10.1016/j.jcv.2008.10.009
90. Vidal R, Solari V, Mamani N, Jiang X, Vollaire J, Roessler P, Prado V, Matson DO, O'Ryan ML. Caliciviruses and foodborne gastroenteritis, Chile. Emerg Infect Dis 2005; 11:1134-7; PMID:16022799; http://dx.doi.org/10.3201/eid1107.041062
91. Monica B, Ramani S, Banerjee I, Primrose B, Iturriza-Gomara M, Gallimore CI, Brown DW, M F, Moses PD, Gray JJ, Kang G. Human caliciviruses in symptomatic and asymptomatic infections in children in Vellore, South India. J Med Virol 2007; 79:544-51; PMID:17385696; http://dx.doi.org/10.1002/jmv.20862
92. O'Ryan ML, Pena A, Vergara R, Diaz J, Mamani N, Cortes H, Lucero Y, Vidal R, Osorio G, Santolaya ME, Hermosilla G, Prado VJ. Prospective characterization of norovirus compared with rotavirus acute diarrhea episodes in chilean children. Pediatr Infect Dis J 2010; 29:855-9; PMID:20581736; http://dx.doi.org/10.1097/INF.0b013e3181e8b346
93. Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinje J, Parashar UD. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis 2008; 14:1224-31; PMID:18680645; http://dx.doi.org/10.3201/eid1408.071114
94. Hemming M, Rasanen S, Huhti L, Paloniemi M, Salminen M, Vesikari T. Major reduction of rotavirus, but not norovirus, gastroenteritis in children seen in hospital after the introduction of RotaTeq vaccine into the national immunization programme in Finland. Eur J Pediatr 2013; 172:739-46; PMID:23361964; http://dx.doi.org/10.1007/s00431-013-1945-3
95. Payne DC, Vinje J, Szilagyi PG, Edwards KM, Staat MA, Weinberg GA, Hall CB, Chappell J, Bernstein DI, Curns AT., et al.Norovirus and medically attended gastroenteritis in US children. N Eng J Med 2013; 368:1121-30; PMID:23514289; http://dx.doi.org/10.1056/NEJMsa1206589
96. Hall AJ, Rosenthal M, Gregoricus N, Greene SA, Ferguson J, Henao OL, Vinje J, Lopman BA, Parashar UD, Widdowson MA. Incidence of acute gastroenteritis and role of norovirus, georgia, USA, 2004-2005. Emerg Infect Dis 2011; 17:1381-8; PMID:21801613; http://dx.doi.org/10.3201/eid1708.101533
97. Trivedi TK, Desai R, Hall AJ, Patel M, Parashar UD, Lopman BA. Clinical characteristics of norovirus-associated deaths: a systematic literature review. Am J Infect Control 2013; 41:654-7; PMID:23266383; http://dx.doi.org/10.1016/j.ajic.2012.08.002
98. Bok K, Green KY. Norovirus gastroenteritis in immunocompromised patients. N Eng J Med 2012; 367:2126-32; PMID:23190223; http://dx.doi.org/10.1056/NEJMra1207742
99. Simon A, Schildgen O, Maria Eis-Hubinger A, Hasan C, Bode U, Buderus S, Engelhart S, Fleischhack G. Norovirus outbreak in a pediatric oncology unit. Scand J Gastroenterol 2006; 41:693-9; PMID:16716968; http://dx.doi.org/10.1080/00365520500421694
100. Hall AJ, Lopman BA, Payne DC, Patel MM, Gastanaduy PA, Vinje J, Parashar UD. Norovirus disease in the United States. Emerg Infect Dis 2013; 19:1198-205; PMID:23876403; http://dx.doi.org/10.3201/eid1908.130465
101. Vidal R, Roessler P, Solari V, Vollaire J, Jiang X, Matson DO, Mamani N, Prado V, O'Ryan ML. Novel recombinant norovirus causing outbreaks of gastroenteritis in Santiago, Chile. J Clin Microbiol 2006; 44:2271-5; PMID:16757638; http://dx.doi.org/10.1128/JCM.01890-05
102. Kroneman A, Vega E, Vennema H, Vinje J, White PA, Hansman G, Green K, Martella V, Katayama K, Koopmans M. Proposal for a unified norovirus nomenclature and genotyping. Arch Virol 2013; 158:2059-68; PMID:23615870; http://dx.doi.org/10.1007/s00705-013-1708-5
103. Siebenga JJ, Vennema H, Zheng DP, Vinje J, Lee BE, Pang XL, Ho ECM, Lim W, Choudekar A, Broor S., et al.Norovirus illness is a global problem: emergence

and spread of norovirus GII.4 variants, 2001-2007. J Infect Dis 2009; 200:802-12; PMID:19627248; http://dx.doi.org/10.1086/605127
104. Parrino TA, Schreiber DS, Trier JS, Kapikian AZ, Blacklow NR. Clinical immunity in acute gastroenteritis caused by norwalk agent. N Eng J Med 1977; 297:86-9; PMID:405590; http://dx.doi.org/10.1056/NEJM197707142970204
105. LoBue AD, Lindesmith L, Yount B, Harrington PR, Thompson JM, Johnston RE, Moe CL, Baric RS. Multivalent norovirus vaccines induce strong mucosal and systemic blocking antibodies against multiple strains. Vaccine 2006; 24:5220-34; PMID:16650512; http://dx.doi.org/10.1016/j.vaccine.2006.03.080
106. Teunis PF, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J, Calderon RL. Norwalk virus: how infectious is it? J Med Virol 2008; 80:1468-76; PMID:18551613; http://dx.doi.org/10.1002/jmv.21237
107. Simmons K, Gambhir M, Leon J, Lopman B. Duration of immunity to norovirus gastroenteritis. Emerg Infect Dis 2013; 19:1260-7; PMID:23876612; http://dx.doi.org/10.3201/eid1908.130472
108. O'Ryan ML, Lucero Y, Prado V, Santolaya ME, Rabello M, Solis Y, Berrios D, O'Ryan-Soriano MA, Cortes H, Mamani N. Symptomatic and asymptomatic rotavirus and norovirus infections during infancy in a Chilean birth cohort. Pediatr Infect Dis J 2009; 28:879-84; PMID:19672213; http://dx.doi.org/10.1097/INF.0b013e3181a4bb60
109. Czako R, Atmar RL, Opekun AR, Gilger MA, Graham DY, Estes MK. Serum hemagglutination inhibition activity correlates with protection from gastroenteritis in persons infected with norwalk virus. Clin Vaccine Immunol 2012; 19:284-7; PMID:22190401; http://dx.doi.org/10.1128/CVI.05592-11
110. Reeck A, Kavanagh O, Estes MK, Opekun AR, Gilger MA, Graham DY, Atmar RL. Serological correlate of protection against norovirus-induced gastroenteritis. J Infect Dis 2010; 202:1212-8; PMID:20815703; http://dx.doi.org/10.1086/656364
111. Atmar RL, Bernstein DI, Harro CD, Al-Ibrahim MS, Chen WH, Ferreira J, Estes MK, Graham DY, Opekun AR, Richardson C, et al. Norovirus vaccine against experimental human norwalk virus illness. N Eng J Med 2011; 365:2178-87; PMID:22150036; http://dx.doi.org/10.1056/NEJMoa1101245
112. Fang H, Tan M, Xia M, Wang L, Jiang X. Norovirus P particle efficiently elicits innate, humoral and cellular immunity. PLoS One 2013; 8:e63269; PMID:23638188; http://dx.doi.org/10.1371/journal.pone.0063269
113. LoBue AD, Thompson JM, Lindesmith L, Johnston RE, Baric RS. Alphavirus-adjuvanted norovirus-like particle vaccines: heterologous, humoral, and mucosal immune responses protect against murine norovirus challenge. J Virol 2009; 83:3212-27; PMID:19176631; http://dx.doi.org/10.1128/JVI.01650-08
114. Ramirez K, Wahid R, Richardson C, Bargatze RF, El-Kamary SS, Sztein MB, Pasetti MF. Intranasal vaccination with an adjuvanted norwalk virus-like particle vaccine elicits antigen-specific B memory responses in human adult volunteers. Clin Immunol 2012; 144:98-108; PMID:22710446; http://dx.doi.org/10.1016/j.clim.2012.05.006
115. Tamminen K, Lappalainen S, Huhti L, Vesikari T, Blazevic V. Trivalent combination vaccine induces broad heterologous immune responses to norovirus and rotavirus in mice. PLoS One 2013; 8:e70409; PMID:23922988; http://dx.doi.org/10.1371/journal.pone.0070409
116. El-Kamary SS, Pasetti MF, Mendelman PM, Frey SE, Bernstein DI, Treanor JJ, Ferreira J, Chen WH, Sublett R, Richardson C, et al.Adjuvanted intranasal norwalk virus-like particle vaccine elicits antibodies and antibody-secreting cells that express homing receptors for mucosal and peripheral lymphoid tissues. J Infect Dis 2010; 202:1649-58; PMID:20979455; http://dx.doi.org/10.1086/657087
117. Parra GI, Bok K, Taylor R, Haynes JR, Sosnovtsev SV, Richardson C, Green KY. Immunogenicity and specificity of norovirus consensus GII.4 virus-like particles in monovalent and bivalent vaccine formulations. Vaccine 2012; 30:3580-6; PMID:22469864; http://dx.doi.org/10.1016/j.vaccine.2012.03.050
118. Treanor JJ, Atmar RL, Frey SE, Gormley R, Chen WH, Ferreira J, Goodwin R, Borkowski A, Clemens R, Mendelman PM. A novel intramuscular bivalent norovirus VLP vaccine candidate - reactogenicity, safety and immunogenicity in a phase I trial in healthy adults. J Infect Dis 2014; 210:1763-71; PMID:24951828; http://dx.doi.org/10.1093/infdis/jiu337
119. Treanor JJ, Atmar RL, Frey SE, Gormley R, Chen WH, Ferreira J, Goodwin R, Borkowski A, Clemens R, Mendelman PM. A novel intramuscular bivalent norovirus virus-like particle vaccine candidate-reactogenicity, safety, and immunogenicity in a phase 1 trial in healthy adults. J Infect Dis 2014; 210:1763-71; PMID:24951828; http://dx.doi.org/10.1093/infdis/jiu337
120. Tan M, Huang P, Xia M, Fang PA, Zhong W, McNeal M, Wei C, Jiang W, Jiang X. Norovirus P particle, a novel platform for vaccine development and antibody production. J Virol 2011; 85:753-64; PMID:21068235; http://dx.doi.org/10.1128/JVI.01835-10
121. Baric RS, Yount B, Lindesmith L, Harrington PR, Greene SR, Tseng FC, Davis N, Johnston RE, Klapper DG, Moe CL. Expression and self-assembly of norwalk virus capsid protein from venezuelan equine encephalitis virus replicons. J Virol 2002; 76:3023-30; PMID:11861868; http://dx.doi.org/10.1128/JVI.76.6.3023-3030.2002
122. Harris JB, LaRocque RC, Qadri F, Ryan ET, Calderwood SB. Cholera. Lancet 2012; 379:2466-76; PMID:22748592;http://dx.doi.org/10.1016/S0140-6736(12)60436-X
123. World Health Organization. Cholera, 2013. Wkly Epidemiol Rec 2014; 89:345-56; PMID:25136711
124. Safa A, Nair GB, Kong RYC. Evolution of new variants of vibrio cholerae O1. Trends Microbiol 2010; 18:46-54; PMID:19942436; http://dx.doi.org/10.1016/j.tim.2009.10.003
125. Wang J, Villeneuve S, Zhang J, Lei P, Miller CE, Lafaye P, Nato F, Szu SC, Karpas A, Bystricky S., et al.On the antigenic determinants of the lipopolysaccharides of vibrio cholerae O1, serotypes ogawa and inaba. J Biol Chem 1998; 273:2777-83; PMID:9446585; http://dx.doi.org/10.1074/jbc.273.5.2777
126. Sears CL, Kaper JB. Enteric bacterial toxins: mechanisms of action and linkage to intestinal secretion. Microbiol Rev 1996; 60:167-215; PMID:8852900
127. World Health Organization. Oral cholera vaccine stockpile for cholera emergency response. [Internet] 09/16/2013. Available from: http://www.who.int/cholera/vaccines/Briefing_OCV_stockpile.pdf?ua=1
128. Chen WH, Greenberg RN, Pasetti MF, Livio S, Lock M, Gurwith M, Levine MM. Safety and immunogenicity of single-dose live oral cholera vaccine strain CVD 103-HgR, prepared from new master and working cell banks. Clin Vaccine Immnunol 2014; 21:66-73; PMID:24173028; http://dx.doi.org/10.1128/CVI.00601-13
129. Begue RE, Castellares G, Ruiz R, Hayashi KE, Sanchez JL, Gotuzzo E, Oberst RB, Taylor DN, Svennerholm AM. Community-based assessment of safety and immunogenicity of the whole cell plus recombinant B subunit (WC/rBS) oral cholera vaccine in Peru. Vaccine 1995; 13:691-4; PMID:7668039; http://dx.doi.org/10.1016/0264-410X(94)00056-S
130. van Loon FP, Clemens JD, Chakraborty J, Rao MR, Kay BA, Sack DA, Yunus M, Ali M, Svennerholm AM, Holmgren J. Field trial of inactivated oral cholera vaccines in Bangladesh: results from 5 years of follow-up. Vaccine 1996; 14:162-6; PMID:8852414; http://dx.doi.org/10.1016/0264-410X(95)00122-H
131. Clemens JD, Sack DA, Harris JR, Chakraborty J, Khan MR, Stanton BF, Kay BA, Khan MU, Yunus M, Atkinson W, et al. Field trial of oral cholera vaccines in Bangladesh. Lancet 1986; 2:124-7; PMID:2873397; http://dx.doi.org/10.1016/S0140-6736(86)91944-6
132. Taylor DN, Cardenas V, Sanchez JL, Begue RE, Gilman R, Bautista C, Perez J, Puga R, Gaillour A, Meza R, et al. Two-year study of the protective efficacy of the oral whole cell plus recombinant B subunit cholera vaccine in Peru. J Infect Dis 2000; 181:1667-73; PMID:10823767; http://dx.doi.org/10.1086/315462
133. Mahalanabis DLA, Sur D, Deen J, Manna B, Kanungo S, von Seidlein L, Carbis R, Han SH, Shin SH, Attridge S, et al. A randomized, placebo-controlled trial of the bivalent killed, whole-cell, oral cholera vaccine in adults and children in a cholera endemic area in Kolkata, India. PLoS One 2008; 3::2323; PMID:18523643; http://dx.doi.org/10.1371/journal.pone.0002323
134. Sur D, Kanungo S, Sah B, Manna B, Ali M, Paisley AM, Niyogi SK, Park JK, Sarkar B, Puri MK., et al. Efficacy of a low-cost, inactivated whole-cell oral cholera vaccine: results from 3 years of follow-up of a randomized, controlled trial. PLoS Negl Trop Dis 2011; 5:e1289; PMID:22028938; http://dx.doi.org/10.1371/journal.pntd.0001289
135. Luquero FJGL, Ciglenecki I, Sakoba K, Traore B, Heile M, Diallo AA, Itama C, Page AL, Quilici ML, Mengel MA., et al. Use of *Vibrio cholerae* vaccine in an outbreak in Guinea. N Engl J Med 2014; 370:2111-20; PMID:24869721; http://dx.doi.org/10.1056/NEJMoa1312680
136. Anh DDCdG, Lopez AL, Thiem VD, Long PT, Son NH, Deen J, von Seidlein L, Carbis R, Han SH, Shin SH., et al.Safety and immunogenicity of a reformulated vietnamese bivalent killed, whole-cell, oral cholera vaccine in adults. Vaccine 2007; 25:1149-55; PMID:17055622; http://dx.doi.org/10.1016/j.vaccine.2006.09.049
137. Anh DDLA, Thiem VD, Grahek SL, Duong TN, Park JK, Kwon HJ, Favorov M, Hien NT, Clemens JD. Use of oral cholera vaccines in an outbreak in Vietnam: a case control study. PLoS Negl Trop Dis 2011; 25:e1006; PMID:21283616; http://dx.doi.org/10.1371/journal.pntd.0001006
138. Richie EE, Punjabi NH, Sidharta YY, Peetosutan KK, Sukandar MM, Wasserman SS, Lesmana MM, Wangsasaputra FF, Pandam SS, Levine MM., et al.Efficacy trial of single-dose live oral cholera vaccine CVD 103-HgR in North Jakarta, Indonesia, a cholera-endemic area. Vaccine 2000; 18:2399-410; PMID:10738097; http://dx.doi.org/10.1016/S0264-410X(00)00006-2
139. Ali M, Emch M, von Seidlein L, Yunus M, Sack DA, Rao M, Holmgren J, Clemens JD. Herd immunity conferred by killed oral cholera vaccines in Bangladesh: a reanalysis. Lancet 2005; 366:44-9; PMID:15993232; http://dx.doi.org/10.1016/S0140-6736(05)66550-6
140. Sack DA, Sack RB, Shimko J, Gomes G, O'Sullivan D, Metcalfe K, Spriggs D. Evaluation of peru-15, a new live oral vaccine for cholera, in volunteers. J Infect Dis 1997; 176:201-5; PMID:9207368; http://dx.doi.org/10.1086/514025
141. Cohen MB, Giannella RA, Bean J, Taylor DN, Parker S, Hoeper A, Wowk S, Hawkins J, Kochi SK, Schiff G., et al.Randomized, controlled human challenge study of the safety, immunogenicity, and protective efficacy of a single dose of peru-15, a live attenuated oral cholera vaccine. Infect Immun 2002; 70:1965-70; PMID:11895960; http://dx.doi.org/10.1128/IAI.70.4.1965-1970.2002
142. Qadri F, Chowdhury MI, Faruque SM, Salam MA, Ahmed T, Begum YA, Saha A, Alam MS, Zaman K,

Seidlein LV., et al.Randomized, controlled study of the safety and immunogenicity of peru-15, a live attenuated oral vaccine candidate for cholera, in adult volunteers in Bangladesh. J Infect Dis 2005; 192:573-9; PMID:16028125; http://dx.doi.org/10.1086/432074

143. Qadri F, Chowdhury MI, Faruque SM, Salam MA, Ahmed T, Begum YA, Saha A, Al Tarique A, Seidlein LV, Park E., et al.Peru-15, a live attenuated oral cholera vaccine, is safe and immunogenic in Bangladeshi toddlers and infants. Vaccine 2007; 25:231-8; PMID:16996172; http://dx.doi.org/10.1016/j.vaccine.2006.08.031
144. Talavera A, Ano G, Pino Y, Castano J, Uribarri E, Riveron L, Gil S, Fernandez S, Cedre B, Valmaseda T., et al. Formulation in tablets of a cholera whole cells inactivated vaccine candidate. Vaccine 2006; 24:3381-7; PMID:16460846; http://dx.doi.org/10.1016/j.vaccine.2005.12.064
145. Valera R, Garcia HM, Jidy MD, Mirabal M, Armesto MI, Fando R, Garcia L, Fernandez R, Ano G, Cedre B., et al. Randomized, double-blind, placebo-controlled trial to evaluate the safety and immunogenicity of live oral cholera vaccine 638 in Cuban adults. Vaccine 2009; 27:6564-9; PMID:19720365; http://dx.doi.org/10.1016/j.vaccine.2009.08.042
146. Garcia L, Jidy MD, Garcia H, Rodriguez BL, Fernandez R, Ano G, Cedre B, Valmaseda T, Suzarte E, Ramirez M., et al. The vaccine candidate *Vibrio cholerae* 638 is protective against cholera in healthy volunteers. Infect Immun 2005; 73:3018-24; PMID:15845509; http://dx.doi.org/10.1128/IAI.73.5.3018-3024.2005
147. Diaz Jidy M, Perez Rodriguez A, Fernandez Llanes R, Bravo Farinas L, Garcia Sanchez H, Valera Fernandez R, Garcia Imia L, Fando Calzada R, Menendez Hernandez J. Challenge clinical trial for evaluation of a vaccine candidate strain against cholera. Rev Cubana Med Trop 2010; 62:194-9; PMID:23437548
148. Tacket CO, Losonsky G, Nataro JP, Comstock L, Michalski J, Edelman R, Kaper JB, Levine MM. Initial clinical studies of CVD 112 *Vibrio cholerae* O139 live oral vaccine: safety and efficacy against experimental challenge. J Infect Dis 1995; 172:883-6; PMID:7658089; http://dx.doi.org/10.1093/infdis/172.3.883
149. Mahalanabis D, Ramamurthy T, Nair GB, Ghosh A, Shaikh S, Sen B, Thungapathra M, Ghosh RK, Pazhani GP, Nandy RK., et al. Randomized placebo controlled human volunteer trial of a live oral cholera vaccine VA1.3 for safety and immune response. Vaccine 2009; 27:4850-6; PMID:19523608; http://dx.doi.org/10.1016/j.vaccine.2009.05.065
150. Kanungo SSB, Ramamurthy T, Sur D, Manna B, Pazhani GP, Chowdhury G, Jhunjhunwala P, Nandy RK, Koley H, Bhattacharya MK., et al.Safety and immunogenicity of a live oral recombinant cholera vaccine VA1.4: a randomized, placebo controlled trial in healthy adults in a cholera endemic area in Kolkata, India. PLoS One 2014; 9:e99381; PMID:24983989; http://dx.doi.org/10.1371/journal.pone.0099381
151. Liang W, Wang S, Yu F, Zhang L, Qi G, Liu Y, Gao S, Kan B. Construction and evaluation of a safe, live, oral *Vibrio cholerae* vaccine candidate, IEM108. Infect Immun 2003; 71:5498-504; PMID:14500467; http://dx.doi.org/10.1128/IAI.71.10.5498-5504.2003
152. Ravichandran M, Ali SA, Rashid NHA, Kurunathan S, Yean CY, Ting LC, Bakar ASA, Lalitha P, Zainuddin ZF. Construction and evaluation of a O139 *Vibrio cholerae* vaccine candidate based on a *hemA* gene mutation. Vaccine 2006; 24:3750-61; PMID:16102875; http://dx.doi.org/10.1016/j.vaccine.2005.07.016
153. Ledon T, Ferran B, Perez C, Suzarte E, Vichi J, Marrero K, Oliva R, Fando R. TLP01, an mshA mutant of *Vibrio cholerae* O139 as vaccine candidate against cholera. Microbes Infect 2012; 14:968-78; PMID:22546527; http://dx.doi.org/10.1016/j.micinf.2012.04.004