I. About the Disease and Pathogen

Basic information on pathogen, including transmission, estimated global disease burden for those at risk, for morbidity and for mortality, including uncertainties/data gaps, geographical distribution, economic burden if available, age groups affected and target groups for vaccination.

Children, regardless of geographic location or economic status, are almost universally infected with rotavirus before their third birthday. However those living in low-income countries are far more likely to be infected earlier and are more likely to develop severe disease and die, due to various factors including limited access to urgent care and higher prevalence of pre-existing conditions such as co-infections and malnutrition in these settings. Child mortality estimates consistently find diarrheal diseases among the leading causes of deaths, a great majority of them occurring in the world’s poorest countries. Using multi-cause proportionate mortality models and analysis of vital registration data, the Child Health Epidemiology Reference Group (CHERG) of WHO and UNICEF estimated that up to 10% of deaths in children under 5 years old (excluding the neonatal period) or 0.75 million (0.538-1.031) was due to diarrheal diseases in 2010 [1]. Recent systematic reviews, based on published studies of diarrhea hospitalization and diarrhea attributable to rotavirus, estimated rotavirus-specific mortality of 453,000 deaths (for 2008) to 197,000 deaths (for 2011) in children younger than 5 years [2,3]. This corresponds to 37% (2008) and 28% (2011) of deaths attributable to diarrhea. In one study, five countries accounted for more than half of all deaths attributable to rotavirus infection: Democratic Republic of the Congo, Ethiopia, India, Nigeria, and Pakistan; India alone accounted for 22% of deaths (98,621 deaths) [2].

Rotaviruses belong to the virus family Reoviridae and are small (70-100-nm) viruses composed of three protein layers covering their segmented double-stranded RNA. The gene segments encode for 11 viral proteins including structural (VP1-VP7) and non-structural proteins (NS1-NS6). VP7 and VP4 proteins are the antigenic determinants of rotavirus serotypes, and have been critical for rotavirus vaccine development as they are targets of neutralizing antibodies during infection. The variations in genetic sequences of VP7, the glycoprotein or G protein, and VP4, the protease-cleaved or P protein classify rotavirus strains according to G-types and P-types. The segmented RNA allows genes encoding the G and P proteins to segregate independently, creating multiple G/P combinations. Genetic diversity and antigenic variation also results from sequential point mutations (antigenic drift) and genetic reassortment (antigenic shift) where gene segments reassort during mixed infections with more than one rotavirus strain and produce reassortant strains.

Current data indicates fluctuations in rotavirus G/P-type that vary also by geography and season, as well as the emergence of unusual types, such as G5, G6, G8, G9, G12, and P[6] for reasons that are not well-understood. This makes accurately predicting serotype circulation and understanding the clinical implications of the serotype changes a challenge. Although surveillance studies have found that a small number of rotavirus strains bearing VP7 G serotypes G1 to G4 and G9 and VP4 P genotypes P1B[4], P2A[6], and P1A[8] were predominant worldwide, they also indicated that the distribution of various G/P combinations varied drastically between continents. All this has implications for the development of next generation rotavirus vaccines targeting geographically relevant, serotype-specific as well as heterotypic protection [4].

Rotavirus disease is characterized by acute onset of watery diarrhea, fever and vomiting. The majority of cases are mild and self-limiting, but a small fraction of cases develop severe dehydration with electrolyte losses in the stools and metabolic acidosis. Treatment consists of rehydration, but high rotavirus mortality rates still prevail in low-income countries in spite of the significant impact of oral rehydration therapy
(ORT) and the general improvement in sanitary conditions. These severe manifestations are more common among children between 3 months and 2 years of age, than in younger infants or older children and adults. The virus infects and replicates in intestinal epithelial cells, damaging their function and causing a malabsorption diarrhea that is the hallmark symptom of rotavirus infections. Although the exact causal mechanisms of diarrhea is not fully understood, studies in animals have suggested a role for a non-structural proteins NS4, an enterotoxin found to cause excessive chloride secretion in an age and dose-dependent manner in animals, as well as the possible role for the virus in causing diarrhea through the stimulation of the enteric nervous system.

Studies of natural infection in neonates and young children have found that acquired immunity does occur; in particular against recurrent disease more than recurrent infections. These studies observed that protection acquired following the first natural infection was greatest particularly against severe outcomes upon future reinfections [5, 6]. Future reinfections can and do occur, but each subsequent infection confers progressively greater protection. Maternal rotavirus antibodies pass transplacentally, and as a result, most neonates, although susceptible to infection, will have asymptomatic or mild disease.

The fact that infection with rotavirus is universal so early in childhood indicates that the virus is not primarily transmitted via fecally contaminated water or food, distinguishing it from other major causes of viral, bacterial or parasitic causes of gastroenteritis. Although the exact mode of transmission is unclear, it is most likely that transmission occurs through person-to-person contact or aerosolized respiratory droplets, as suggested by the seasonality seen in temperate climates (similar to influenza, RSV and measles). Given the major contribution of rotavirus to the global disease burden caused by diarrheal diseases and the fact that improvements in economic development are unlikely to greatly impact disease incidence, vaccination is the primary strategy of control.

II. Overview of Current Efforts

A. Vaccines currently available and their limitations

Include perceived limitations with available vaccines for low and middle-income country markets (LMIC). These could include safety, effectiveness, serotype/strain coverage, supply, affordability, financing, and number of WHO prequalified vaccines, WHO policy recommendations for available vaccines, perceived lack of priority from endemic country authorities.

Human Rotavirus vaccine development began shortly after the human virus was identified in Melbourne, Australia in 1973 as the most common cause of infant diarrhea [7]. The earliest vaccine candidates followed what has been called the “Jennerian” approach, applying Edward Jenner’s concept of using animal strains, which were observed to be naturally attenuated, to induce protection against human strains. Three nonhuman rotavirus vaccines, two bovine rotavirus strains, RIT 4237 (P6[1]G6) and WC3 (P7[5]G6), and a simian (rhesus) rotavirus vaccine (RRV) strain MMU1006 (P5B[3]G3) were investigated [8] during this early stage.

The first, the RIT4237 bovine rotavirus strain vaccine, manufactured by SmithKline-RIT, was found to be safe and highly effective (80%) in preventing severe diarrhea in Finnish children, but no significant efficacy was seen in clinical trials that followed in African and Latin America which led eventually to halt development. The two subsequent vaccine candidates, the bovine WC3 (P7[5]G6), and the rhesus (RRV) strain MMU1006 were developed by researchers at Children’s Hospital in Philadelphia (WC3) and Merck Pharmaceuticals and US NIH and Instituto de Biomedicina, Universidad Central de Venezuela and later Wyeth Pharmaceuticals (RRV) [9]. Although none made it to registration, these two candidates were essentially precursors for the first licensed rotavirus vaccines – Rotashield (RRV) in 1998 and RotaTeq (WC3) in 2006. A monovalent animal strain vaccine was licensed in China, which introduced a monovalent lamb strain rotavirus vaccine, LLR in 2000 and 2001. Developed and produced by the
Lanzhou Institute of Biological products, the live-attenuated oral vaccine was licensed to be given to children aged 2 to 36 months, with yearly boosters, and it has been estimated that between 2001 and 2008, approximately 10 million doses were administered in China. The vaccine did not undergo controlled phase III testing, but efficacy was derived from a case-control study of 838 children aged 2 months to 5 years who were hospitalized with rotavirus infections with community-matched controls which found 73% (95% CI: 61e82%) efficacy for one dose of LLR against hospitalized rotavirus diarrhea [10].

To circumvent the inconsistent efficacy of the early monovalent vaccines, development efforts shifted to taking advantage of the ability of rotaviruses to reassort to produce novel reassortant vaccines that contained immunologically important proteins from human rotaviruses (VP7) and other genes from the animal strain to maintain the attenuated aspect, the so-called “modified Jennerian” approach. Furthermore, with a better understanding of the existence and distribution of rotavirus G serotypes, the second-generation vaccines were formulated to attempt to broaden immunity across serotypes by including several human G serotypes. The first licensed rotavirus vaccine, RotaShield or RRV-TV(Wyeth) was a quadrivalent vaccine based on the attenuated phenotype of the rhesus rotavirus, which represented the four most common circulating human G serotypes: G1, G2, G3 and G4. Various efficacy trial found a high level of protection (80%–100%) in preventing severe diarrheal disease [11]. A year after its introduction, the vaccine was withdrawn when it was reported that the first dose of RRV-TV was associated with a substantial increased relative risk (at least 25-fold) of intussusception within the first 10 days after administration [12]. Although the underlying mechanism is unknown, it has been suggested to be specific to the rhesus rotavirus vaccine strain as other wild-type rotaviruses and live-attenuated vaccines have not found a similar association.

Following this, subsequent development focused on human-bovine reassortant strains or attenuated human strains. Using the previously tested bovine rotavirus strain (WC3) as a backbone, researchers created a pentavalent vaccine that contained 5 separate viruses that expressed either human G1, 2, 3, or 4 VP7s, and a human P(8) VP4 on the bovine WC3 backbone which was developed and licensed after extensive clinical testing, by Merck Pharmaceuticals as RotaTeq® [13]. Alternatively, the observation that the first natural rotavirus infection, either symptomatic or asymptomatic, provides protective immunity against subsequent severe disease, irrespective of serotype, was the underlying logic behind the approach of developing a live attenuated human strain vaccine. Using a wild-type human rotavirus isolate P1A[8]G1 strain which represented the most common human rotavirus VP7 and VP4 antigens and attenuated by multiple tissue culture passages, this was developed by GlaxoSmithKline Biologicals, as Rotarix®, which was licensed following successful trials showing safety, immunogenicity, and efficacy [14].

Both Rotarix and RotaTeq vaccines have since demonstrated the efficacy in developing countries, leading to the WHO recommendation for their widespread introduction [15]. However, recalling the results of the monovalent animal strain vaccines, neither vaccine was as immunogenic or efficacious in infants in developing countries compared to those living in industrialized countries. The efficacy trial of two and three doses of Rotarix® conducted in infants in South Africa and Malawi—countries with a high under-five years childhood mortality rate and high diarrheal disease burden showed that severe rotavirus gastroenteritis (SRVGE) was detected among 4.9 percent of the infants who had received the placebo and 1.9 percent of the infants who had received Rotarix® [16]. This corresponded to an aggregated efficacy of Rotarix® in preventing SRVGE of 61.2 percent (95 percent CI: 44.0 to 73.2 percent). Similarly, in trials of the RotaTeq® vaccine in Asia (Bangladesh and Vietnam) and Africa (Ghana, Kenya, and Mali) the efficacy against SRVGE during the first year of follow up was 64.2 percent in Africa (95 percent CI: 40 to 79 percent) and 51.0 percent in Asia (95 percent CI: 13 to 73 percent) [17]. These results confirm that live oral rotavirus vaccines have diminished protective capacity in developing country populations as compared to industrialized nations or middle-income countries where the efficacy of these vaccines
against SRVGE is greater than 90%. Nonetheless, the introduction of rotavirus vaccination in developing countries should lead to a significant impact on mortality.

B. General approaches to vaccine development for this disease for low and middle income country markets

What are the scientific approaches and indications and target/age/geographic groups being pursued? What public health needs will these vaccines meet if successfully developed? Where there are several different possible indications/target groups, how much consensus is there as to prioritization between these for vaccine development in LMIC.

Although orally administered live attenuated rotavirus strains remain the primary approach to vaccination, and the currently licensed oral live, attenuated rotavirus vaccines offer the benefit of significantly impacting current childhood mortality in LMICs, several limitations have led to the pursuit of improved next generation rotavirus vaccines. This includes diminished efficacy in populations with the highest burden, for reasons which remain largely unconfirmed, likely multifactorial and probably shared with the known decreased ‘takes’ for other oral vaccines such as the oral polio vaccine. The following reasons have been postulated: interference by other viruses or bacteria colonizing the intestine; maternally derived antibody that neutralizes the virus (from breast-milk or trans-placentally acquired); immaturity of the local immune system; tropical enteropathy, etc.; however, direct studies to investigate these factors are complicated by the inability to sample the mucosal compartment in young infants.

Another factor driving the development of alternative vaccines is cost and supply. The current cost of the licensed vaccines remains beyond the means of high burden populations, combined with the fact that current production levels cannot cover all children. The three countries with the highest burden are also known to depend on local manufacturing for their vaccine supply.

Several studies suggest that maternally derived antibody diminish vaccine takes, and therefore typical regimens begin at the second month of age. At least two doses of vaccine (depending on the vaccine), and most likely three are necessary to maximize vaccine immunity. Concomitant administration with OPV facilitates the introduction of rotavirus into EPI programs, but several studies had to be carried out to demonstrate that rotavirus vaccine do not diminish OPV seroresponses. Therefore, the pursuit of next-generation rotavirus vaccines also relate to improving immunogenicity in terms of greater cross-serotype protection, longer duration of protection, and less susceptibility to being compromised by factors such as maternal antibodies allowing for immunization from birth.

Although the evidence indicates that the risk of intussusception with currently licensed vaccines is low, about 1 to 2 per 100,000 infants vaccinated, the concerns over the risk of intussusception remains present and has affected policy recommendations regarding age of first dose. Risk-benefit considerations between countries, which have different disease burden as well as childhood vaccination coverage, and vaccination delay rates will obviously differ. For many high burden countries, vaccination delay is common and the age threshold for the first dose – no later than 15 weeks old was argued to be too restrictive and caused a missed opportunity to prevent rotavirus mortality. WHO SAGE in 2013 reiterated the thresholds for the first dose(<15 weeks) and last dose (<32 weeks) but stated that the age restriction could be overlooked in countries where disease burden is high and delays in vaccinations and deaths from rotavirus are common. Therefore ideally, newer vaccines are seeking to have a greater safety profile where age restrictions would no longer be a major issue. Finally, further downstream there also efforts to develop vaccines that are in formulations more suited for administration and storage in LMICs.

III. Technical and Regulatory Assessment

Highlight perceived positive/negative aspects in clinical/regulatory pathways e.g. well established product development and regulatory pathway to licensure, accepted immune correlates and/or functional
assays, accepted surrogate efficacy endpoints, existence of well accepted animal or challenge models, agreed trial designs and endpoints. Possibilities to develop case for correlates/surrogates should be included.

The mechanisms of protection and virulence of rotavirus remain incompletely understood, despite decades of research and the testing of numerous vaccine candidates. Although studies have shown that rotavirus infection results in serum and intestinal antibody responses, determining the exact role of serum antibodies in mediating protection has been a source of some debate. The role of humoral immunity has also been demonstrated through passive transfer studies in both humans and animals. Passive immunization of infants with orally administered rotavirus-specific antibodies showed amelioration of disease and passive transfer of serum antibodies in monkeys can provide protection against infection. Neutralizing antibodies against VP7 and VP4 antigens clearly play a role in protection after natural rotavirus infection, and studies have also demonstrated that the first infection with rotavirus elicits a predominantly homotypic, serum-neutralizing antibody response to the virus, and subsequent infections elicit a broader, heterotypic response [18]. Evidence from clinical testing of RotaShield, Rotarix® and RotaTeq® indicate that these vaccines induce immunity that goes beyond just serotype-specific immunity as type-specific neutralization response rates after vaccination were much lower than the protection rates observed in clinical trials, and in addition, effectiveness was seen against non-vaccine serotypes as well although which serotypes and the degree of protection varied.

A thorough review of data from a variety of human studies, ranging from challenge trials in adults and natural history studies in young children to clinical trials suggests that serum antibodies, either IgA, IgG or neutralizing antibodies, can act as correlate markers of protection against rotavirus disease [19]. The role of cell-mediated immunity in protection in humans is not known, although results from animal studies do support the role of cytotoxic T lymphocytes in disease protection in animal models. However, despite this, no correlate of vaccine-induced protection has emerged from the development of currently licensed rotavirus vaccines. Currently anti-rotavirus serum IgA induction by the vaccine is the primary assay applied to evaluate vaccine immunogenicity, and the lack of correlation with efficacy implies that each new vaccine candidate will require demonstration of efficacy against clinical endpoints, meaning large field efficacy trials. Though the preferred scientific way to conduct such trials is through placebo-control trials, the availability of the two currently licensed vaccines (Rotarix and RotaTeq) and potentially new licensed vaccines in the population in many countries, limits the ethical justification of conducting placebo control studies. The early experience with RotaShield and intussusception means that substantial post-marketing surveillance activities need to be put into place in every country where rotavirus vaccines are introduced to identified this condition and its possible association with vaccination.

IV. Status of vaccine R&D activities

Summarize status of vaccine design, pre-clinical and clinical trial activity, including platforms, vectors, and adjuvants. Note academic, government, biotech and industry entities engaged. Summarize antigenic targets (if subunit approaches). Section on major advances in last 3-5 years, including key opportunities highlighted by recent science developments in the area.

To overcome the major issue of cost and supply, several groups around the world have developed or are developing live attenuated rotavirus vaccines, using a similar approach to the human-bovine reassortant multivalent vaccine, or alternatively the selection of human neonatal strains. Neonatal strains have been previously explored as vaccine candidates as naturally history studies found that neonates were mostly asymptomatic when infected (indicating a natural attenuation of the infecting strains) and that the neonates subsequently had reduced frequency and severity of rotavirus diarrhea. Two strains are being developed, and one recently reached licensure in India. Bharat Biotech International Limited (BBIL) in India, with assistance from PATH, has developed and licensed in India a monovalent rotavirus vaccine based on a human neonatal G9P(10) rotavirus strain 116-E. In collaboration with Bharat Biotech, several
academic investigators and the Indian Department of Biotechnology, the 116-E rotavirus vaccine was tested in a large efficacy trial with 6,800 infants demonstrating a 56% level of protection against severe rotavirus gastroenteritis [20]. The vaccine has now been licensed as RotaVac® in India and the Indian Government has announced the stepped-wise future introduction of rotavirus vaccines in the Universal Immunization Program (UIP).

Another human neonatal P[6]G3 strain, RV3, originally developed by the group that originally described the human rotavirus, Bishop and colleagues in Australia, was evaluated as an oral vaccine in 3-month-old infants and was found to be safe and well tolerated, but had suboptimal immunogenicity. Reformulated with higher titers, developed in collaboration by Murdoch Children’s Research Institute in Australia and Biofarma, Indonesia, the RV3-BB G3P[6] has apparently been reported safe and immunogenic in Phase 1 and 2 trials and is now undergoing phase 2b trial with evaluation of neonatal dose. Another human strain rotavirus vaccine, Rotavin-IM G1P[8] has also been reported to be developed and licensed in Vietnam.

Others are developing multi-valent reassortant rotavirus vaccine based on bovine rotavirus strains. The Serum Institute of India has developed a pentavalent reassortant rotavirus vaccine based on a bovine rotavirus strains originally constructed by the US NIAID, the BRV-Hu (pentavalent G1-4 with G9), and is currently engaged with PATH in a phase 3 efficacy trial in multiple regions of India [21]. Others are developing a tetravalent G1, 2, 3, 4 vaccine using a series of bovine reassortants that use a UK bovine strain as a backbone that has been licensed by the National Institutes of Health to several companies; the ChengDu Institute of Biological Products (CDIBP) in China; and Instituto Butantan in Brazil, and Shanta Biotech in India. Earlier efficacy trials with this vaccine in Finland showed it to be nonreactogenic, highly immunogenic, and highly efficacious.

As an alternative to the orally administered live vaccine strategy, various academic and public health organizations, including PATH, are developing non-replicating rotavirus vaccines (NRRV) for parenteral administration to overcome the hurdles resulting in diminished efficacy of live rotavirus vaccines in the high burden populations. The technologies span the range from fully intact, inactivated rotavirus particles to subunits rotavirus proteins. Three types of candidates have received the most attention: triple- and double-layered virus-like particles (VLPs); inactivated rotavirus particles; and recombinant subunit proteins. PATH is developing a VP8 subunit expressed in E coli as a chimeric protein vaccine in which the VP8 subunit of the VP4 outer capsid protein of rotavirus, which contains most of the neutralizing epitopes in VP4 is fused to the tetanus toxin P2 epitope (to enhance immunogenicity). This P2-VP8 vaccine when tested in a phase 1 trial in adults demonstrated to be safe and well tolerated and elicited significant neutralizing antibody responses [22]. Descending age studies are being conducted to demonstrate immunogenicity in infants, which, if successful, will lead to the conduct of efficacy trials.
Table 1: Development Status of Current Vaccine Candidates (POC = Proof of concept trial)

<table>
<thead>
<tr>
<th>Candidate Name/Identifier</th>
<th>Preclinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>POC</th>
<th>Phase III</th>
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<tbody>
<tr>
<td>Live approaches</td>
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<tr>
<td>RotaVac monovalent rotavirus vaccine developed by Bharat, India</td>
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<td>X</td>
<td>(efficacy demonstrated; additional studies ongoing)</td>
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<tr>
<td>RV3_BB developed by Murdoch Children’s Research Institute in Australia and Biofarma, Indonesia</td>
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<tr>
<td>BRV Pentavalent Rotavirus Vaccine developed by Serum Institute of India (SII)</td>
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<td>(efficacy trial ongoing)</td>
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<td>Tetravalent G1, 2, 3, 4</td>
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<td>• ChengDu Institute of Biological Products (CDIBP) in China</td>
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<td>• Instituto Butantan in Brazil,</td>
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<tr>
<td>• Shanta Biotech in India</td>
<td>X</td>
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<tr>
<td>Rotavin-IM G1P[8], Vietnam</td>
<td>X</td>
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<td>(reported as licensed)</td>
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<tr>
<td>Non-replicating rotavirus vaccines (NRRV)</td>
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<td>P2-VP8 recombinant subunit rotavirus vaccine developed by PATH</td>
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<td>(phase 1 study in adults completed; studies in toddler and infants ongoing)</td>
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</tbody>
</table>

References


