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.

Existing preventive, diagnostic and treatment measures and their limitations

Noroviruses (NoVs) cause acute debilitating illness characterized by vomiting and diarrhea. The US Centers for Disease Control and Prevention estimates that it is the most common cause of acute gastroenteritis in the United States with 21 million cases each year and an estimated 70,000 hospitalizations and 8,000 deaths (1). NoVs have also emerged as an important cause of gastroenteritis worldwide. These infections can occur in all age groups and commonly result in significant morbidity and mortality, particularly in the very old and very young. A recent systematic review estimated NoV prevalence to be 14 percent and found that rates of NoV are higher in community-based and outpatient health care settings compared to hospital-associated cases. This suggests that NoV causes less severe cases, however the sheer frequency of illness results in a larger burden of severe disease (2). It is estimated that up to 200,000 children die from complications of NoV infection worldwide annually (3). In addition, NoV illnesses and outbreaks exact significant socioeconomic toll on businesses, hospitals, schools, and other closed settings, such as dormitories and military barracks. Current gaps in epidemiology of NoV exist, particularly for lesser-developed countries where advanced molecular diagnostics have been limited. Global regions are not well represented (e.g., Africa, South East Asia), and case definitions have not broadly included the full spectrum of case presentation, including vomiting as the predominant symptom. Thus, NoV incidence is likely underestimated and additional high-quality studies are needed.

*Norovirus* is divided into five genogroups (I-V), with GI, GII and GIV causing human infections. Each genogroup is further subdivided into genotypes based on analysis of the amino acid sequence of its major viral capsid protein VP1. The Norwalk virus, the prototype human NoV is classified as a GI virus. Over 80% of confirmed human NoV infections are associated with genotype GII.4. Serotyping as commonly done for viruses through neutralization assays is impossible as the virus cannot be cultured in vitro and therefore the true biological significance of these classifications is unknown. Pathogenesis is thought to be dependent on binding of the virus to human histoblood group antigens (HBGA) on the epithelium of the small intestine. HBGA are glycans found on the surface gut epithelium (as well as red blood cells, saliva, respiratory epithelia). The expression of these glycans has been shown to affect the susceptibility to infection with certain NoV; namely in human challenge studies where only individuals who have a functional glycosylase enzyme, and consequently express certain HBGAs are susceptible to infection with Norwalk virus. Resistance to infection to other NoV genotypes due to a non-functional glycosylase has also been described.

The inability to culture NoV hampers research on pathogenesis, vaccine development, and diagnostics. Although molecular diagnostics are available, the fact that NoV can be shed at low levels for long periods of time makes disease attribution difficult. A quantitative diagnostic approach will likely yield better sensitivity and specificity in testing. The Global Enteric Multicenter Study (GEMS) used a conventional multiplex real-time polymerase chain reaction (RT-PCR) study for the detection of several enteric RNA viruses, including NoV. GEMS attributed moderate-to-severe diarrheal disease to NoV in only one of its seven sites. However, such a diagnostic approach, which does not differentiate between NoV positivity in acute disease and asymptomatic controls, is likely to have poor sensitivity and specificity and underestimate the role of NoV as a cause of diarrhea, especially in high-transmission settings (4).
finding that NoV rates are higher in community-based studies compared to hospital-based studies, indicates that the disease has a milder presentation. However, the fact that NoV is the leading cause of clinical diarrhea in the United States also suggests that NoV could likely be a cause of severe disease among children in lesser-developed countries. Future epidemiological studies should consider including quantitative diagnostics in their design to more precisely attribute pathogen etiology.

The virus is highly transmissible requiring a very low infectious dose of <10−100 virions, causing an acute illness of fever, nausea, vomiting, cramping, malaise, and diarrhea persisting for 2 to 5 days. The disease is mostly self-limiting although severe outcomes and longer durations of illness are most likely to be reported among the elderly and immuno compromised groups. All age groups are susceptible, as immunity after infection appears both strain specific and limited in duration. Beyond supportive care including oral rehydration, there are no treatments currently available to decrease the severity of NoV-induced illness. In countries where sustained universal rotavirus vaccination has been introduced, NoVs have become the main cause of gastroenteritis in children.

II. Overview of Current Efforts

A. Biological feasibility for vaccine development

Evidence that vaccine development is biologically feasible including from development of naturally acquired immunity, from vaccine development for related pathogens, from animal models or in vitro data

There are currently no licensed vaccines for NoV. Given the high estimated mortality and morbidity attributed to NoV in both developed and lesser-developed countries, the global health value of a NoV vaccine is quite high, similar to other enteric vaccines currently under development, including Shigella, cholera, and enterotoxigenic E. coli. A recent review on NoV vaccine development explored the factors complicating vaccine design (5). These include the lack of appropriate model systems to explore pathogenesis and vaccine target efficacy, unknown duration of protective immunity, antigenic variation among and within genogroups/genotypes, and unknown effects of pre-exposure history. Preclinical development is challenging due to the lack of relevant models (limited to a chimpanzee model which has been halted due to ethical restrictions on the use of non-human primates, and a gnotobiotic pig model) and a cell culture.

Despite the limitations, vaccine feasibility has been convincingly demonstrated with the development of a vaccine candidate based on a recombinant approach using a self-assembling virus-like particle (VLP) that has shown protection in two human challenge efficacy studies. Currently, it is being developed as a bivalent GI.1/GII.4 vaccine administered intramuscularly. NoVs have extensive antigenic and genetic diversity, with more than 25 genotypes recognized among the 3 genogroups containing human viruses. This has best been documented with the GII.4 genotype (dominant strain replacement every two to four years), though GI.1 and GII.2 isolates have also demonstrated stability over the past 30 years. While significant variation is known to occur with the epitopes responsible for seroresponse, there is evidence to suggest that more conserved domain epitopes across groups and strains may serve as a protective antigen in an adjuvanted vaccination regimen. There are also preclinical and clinical data that support broadened activity beyond the vaccine VLP strains. More encouragingly, although there is a lack of correlation of pre-existing serum antibody as measured by ELISA with protection from infection, the presence of serum antibodies that blocks binding of NoV virus-like particles (VLPs) to HBGAs have been associated with a decreased risk of infection and illness following homologous viral challenge. This blocking assay could play a critical role in facilitating further development and optimization of this vaccine. Should a vaccine be licensed and developed based on this bivalent approach, future studies will need to determine whether strain replacement has occurred, which may require formulation modification.

Protective immunity after natural NoV infection may persist between four and eight years based on
modeling of epidemiological data. Early human challenge/re-challenge studies have observed protection from six months to two years. Thus, duration of protection is still unknown and the rapid incubation period from infection to illness onset may challenge the timing of effective memory response activation. The influence on multiple exposures and pre-existing immunity may also complicate the vaccine approach and immune responses.

B. General approaches to vaccine development 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.

The inability to culture NoV has obviously limited any traditional whole-cell vaccine approaches, but recombinant technology, more precisely NoV recombinant virus-like particles (VLPs) produced by the expression and spontaneous self-assembly of the major capsid protein VP1 has played a major role in generating the current body of knowledge and leading approaches in NoV vaccine development. Efficacy trials will be essential in answering the issues raised above including duration of vaccine-induced immunity, implications of antigenic diversity and drift on vaccine-induced protection, and the consequence of pre-existing immune responses.

From a lesser-developed country perspective, there are unique issues to consider for vaccine development and feasibility. Dose number and schedule are important, as well as possibly different circulating strains compared to those found in developed countries. Furthermore, the current vaccine approach for an indication in adults is based on an intramuscular injection of the vaccine with an effective immune response that is thought in part to rely on the boosting of memory from previous natural infection. As this vaccine would likely be targeted to younger children, the effectiveness of the vaccine in a naïve infant may be less, and an alternative mucosal priming-parenteral boost hypothetically may be necessary, but also complicates the development of a vaccine for use in a resource-limited setting.

While more high-quality data is needed, it is clear that NoV has a global distribution that causes significant morbidity and mortality. A recent systematic review on mortality due to diarrhea among children less than five years of age found that NoV was associated with hospitalization in approximately 14 percent of cases, behind rotavirus (38 percent) and enteropathogenic E. coli (15 percent) (6). There is a dearth of data from Africa where the effects of NoV-associated gastroenteritis may be more severe.

Based on available epidemiological data, it appears that NoV has a similar epidemiology to rotavirus in incidence among children less than five years of age, with the highest incidence rates and multiple infections occurring in the first two years of life. A recent birth cohort study in Peru illustrates this epidemiology (see Figure 1 below) (7). Changing dynamics in strain variation and circulation could likely also extend the high incidence beyond two years of life. Thus, a successful vaccination strategy will need to target children at the earliest opportunity in order to have maximum effect.
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.

While recent favorable advances have been made with a demonstration of vaccine efficacy in healthy adult human challenge studies, as well as evidence of a correlate of protection and broadly protective antibodies, translation to low- and middle-income countries will present challenges. More research is needed on virus-host associations and epidemiology to assure adequate coverage of the current bivalent vaccine approach. Further defining a correlate of protection that is also present in a developed-world setting is important and could be supported indirectly by seroepidemiological approaches. Further clinical development is being planned. Favorable results from trials in adults from the developed world (e.g., travelers or other high-risk populations) would provide hope that such a vaccine approach might be effective in low- and middle-income country populations.

Using the example of rotavirus vaccines, there are sufficient field sites, experience, and regulatory pathways to take a NoV vaccine through safety studies and pivotal trials in the developing world. Acute gastroenteritis endpoints are well defined and accepted in this population. The incidence of NoV disease is high enough to support such a trial with reasonable numbers.

Formulation of a NoV vaccine (ideally lyophilized) needs to be considered to achieve acceptability (and low cost) in developing countries. In addition, the Expanded Programme on Immunization vaccine landscape is already quite crowded, and appropriate integration of another vaccine into the schedule would need to be considered.
IV. Status of Vaccine R&D Activities

Summarize status of vaccine design, pre-clinical and clinical trial activity, including platforms, vectors, 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.

Table 1 outlines the NoV vaccine candidates currently under development. The most advanced candidate is a recombinant VLP capsid protein vaccine formulated with Alum and MPL adjuvants. It is given in a two-dose series separated 28 days apart (8). The candidate has completed proof-of-concept in two human challenge studies, where protection, particularly against severe disease, was achieved. It is currently being advanced towards expanded Phase 2 and 3 trials. Takeda Vaccines is sponsoring the development of this vaccine, but it also has significant academic and government involvement including the US Department of Defense. In part, this vaccine is modeled on the success of the currently licensed human papillomavirus VLP vaccines. An alternative VLP candidate is being developed by Arizona State University using the same construct as the Takeda VLP, but it is produced in a plant-based vector (further development and commercialization would require licensure from Takeda) and it has not yet entered clinical development.

A NoV VLP-rotavirus protein combination vaccine candidate is currently in preclinical development, and clinical trials may begin in the near term (9). The vaccine is being co-developed by the University of Tampere (Finland) and UMN Pharma (Japan). The trivalent combination consists of NoV capsid (VP1)-derived VLPs of GI-3 and GII-4 and rotavirus recombinant VP6 (rVP6), a conserved and abundant rotavirus protein. Components are expressed individually in the baculovirus expression system and combined. Preclinical studies in mice demonstrated strong and high avidity NoV and rotavirus type-specific serum IgG responses. Cross-reactivity with heterologous NoV VLPs and rotaviruses were elicited. Blocking antibodies were also described against homologous and heterologous norovirus VLPs, suggesting broad NoV-neutralizing activity of the sera. Mucosal antibodies of mice immunized with the trivalent combination vaccine inhibited rotavirus infection in vitro.

Finally, the University of Cincinnati has conducted preclinical immunological studies on a NoV P particle construct (10). This vaccine candidate is derived from the protruding (P) domain of the NoV VP1 capsid protein. P particles can be easily produced in E. coli expression systems at high yield, and thus could represent a manufacturing advantage through relatively low cost of goods. Recent preclinical research supports heterologous cross-reaction of the intranasal P particle vaccine as well as intestinal and systemic T cell responses using a gnotobiotic pig model. The heterologous protective efficacy of the P particle vaccine was comparable to that of the VLP vaccine in pigs (60 percent) and the homologous protective efficacy in humans (47 percent). Clinical development plans for this vaccine are unknown.

Table 1: Development Status of Current Vaccine Candidates (POC = Proof-of-concept trial)

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<th>Candidate Name/Identifier</th>
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References


