Status of vaccine research and development for norovirus

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\section*{A R T I C L E   I N F O}

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\section*{A B S T R A C T}

The global health community is beginning to gain an understanding of the global burden of norovirus-associated disease, which appears to have significant burden in both developed- and developing-country populations. Of particular importance is the growing recognition of norovirus as a leading cause of gastroenteritis and diarrhea in countries where rotavirus vaccine has been introduced. While not as severe as rotavirus disease, the sheer number of norovirus infections not limited to early childhood makes norovirus a formidable global health problem. This article provides a landscape review of norovirus vaccine development efforts. Multiple vaccine strategies, mostly relying on virus-like particle antigens, are under development and have demonstrated proof of efficacy in human challenge studies. Several are entering phase 2 clinical development. Norovirus vaccine development challenges include, but are not limited to: valency, induction of adequate immune responses in pediatric and elderly populations, and potential for vaccine-strain mismatch. Given current strategies and global health interest, the outlook for a norovirus vaccine is promising. Because a norovirus vaccine is expected to have a dual market in both developed and developing countries, there would likely be scale-up advantages for commercial development and global distribution. Combination with or expression by another enteric pathogen, such as rotavirus, could also enhance uptake of a norovirus vaccine.

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\section*{1. About the disease and pathogen}

Noroviruses (NoVs) cause acute, debilitating gastroenteritis characterized by vomiting and diarrhea. The US Centers for Disease Control and Prevention (CDC) estimate 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 8000 deaths nationwide [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 [2] estimated NoV prevalence to be at 14\% and found that rates of NoV are higher in community-based and outpatient health care settings compared to hospital-associated cases. While this may seem to suggest that NoV causes less severe cases than other causes of diarrheal disease, the sheer frequency of illness results in a larger burden of severe NoV disease overall [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 a significant socioeconomic toll on businesses, hospitals, schools, and other closed settings such as dormitories, military barracks, and cruise ships. However, there are current gaps in the epidemiology of NoV, particularly for lesser-developed countries where advanced molecular diagnostics have been limited. Global regions such as Africa and Southeast Asia are not well represented by data, and case definitions have not broadly included the full spectrum of case presentations, including vomiting as the predominant symptom. Thus, global NoV incidence is likely underestimated and additional high-quality studies are needed.

The Norovirus genus 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. Norwalk virus, the prototype human NoV species, 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 for NoV, as the virus cannot
be cultured in vitro. 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 (HBGAs) on the epithelium of the small intestine. HBGAs are glycans found on the surface gut epithelium (as well as red blood cells, saliva, and 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 [3]. Studies have also described resistance to infection from other NoV genotypes due to a non-functional glycosylase.

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 after infection (average 4 weeks) makes disease attribution difficult. Recent attempts have been made to rigorously define the burden of acute enteric diseases in the developing world. The Global Enteric Multicenter Study (GEMS) used a conventional (non-quantitative) multiplex real-time polymerase chain reaction (RT-PCR) study for the detection of several enteric RNA viruses, including NoV [4]. GEMS attributed moderate-to-severe diarrheal disease to NoV in only one of its seven sites across Africa and Asia. However, the case-control study design utilized in the GEMS study in which control selection may not have eliminated healthy individuals who had prior norovirus in the preceding month (i.e. eligibility excluded only those with diarrhea reported in past 7 days) may have not been able to differentiate between NoV positivity in acute disease and asymptomatic controls and, thus, is likely to have poor sensitivity and specificity and underestimate the role of NoV as a cause of diarrhea, especially in the high-transmission settings [5]. In fact, recent results from the multicenter MAL-ED birth cohort study, which controlled for a longer duration of shedding in controls, found that NoV GII was responsible for the most cases of diarrheal illness among children overall and particularly in countries where rotavirus vaccine had been introduced [6]. Although the MAL-ED observation is consistent with the finding that NoV rates are higher in community-based studies compared to hospital-based studies, indicating 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, a notion furthered by the WHO Food Epidemiology Reference Group (FERG) which has identified norovirus as one of the most important pathogens transmitted by food in the world.

In order to more accurately attribute pathogen etiology at the inpatient, outpatient, and community levels, future epidemiological studies should consider including quantitative diagnostics, frequent sampling, and well-considered control subjects in their design. Furthermore, to attain high-quality global data to influence policy decisions and generate global commitment, a surveillance network similar to that which was established in advance of rotavirus vaccine introduction may be needed.

NoV is also highly transmissible, requiring a very low infectious dose of <10 to 100 virions, causing acute illness of fever, nausea, vomiting, cramping, malaise, and diarrhea persisting for two to five days. The disease is mostly self-limiting, although severe outcomes and longer durations of illness are more likely to be reported among the elderly and immunocompromised groups. Because immunity after infection is limited in duration and appears strain-specific, all age groups are susceptible. Apart from supportive care such as 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.

2. Overview of current efforts

2.1. Biological feasibility for vaccine development

There are currently no licensed vaccines for NoV. While current estimates of under-five mortality rank NoV (71,000) less than rotavirus (197,000) and enteropathogenic E. coli (79,000), NoV is above enterotoxigenic E. coli (42,000) [7]. Additionally, the high estimated morbidity attributed to NoV, which occurs in all age groups in both developed and developing countries, suggests that the global health value of a NoV vaccine may rank equivalently with other enteric vaccines under development when evaluating both disability and mortality measures.

A recent review on NoV vaccine development explored the factors complicating vaccine design [8]. 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 and genotypes, and unknown effects of pre-exposure history. Preclinical development is challenging due to the lack of relevant models—currently limited to a chimpanzee model, which has been halted due to ethical restrictions on the use of nonhuman primates, and a gnotobiotic pig model—and the lack of NoV cell culture.

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.

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 against disease in two human challenge efficacy studies. Currently, it is being developed as a bivalent GI/GII.4 vaccine administered intramuscularly. NoVs have extensive antigenic and genetic diversity, with more than 25 genotypes recognized among the three genogroups containing human viruses. This has best been documented with the GII.4 genotype (dominant strain replacement every two to four years), though G1.1 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 seroresponsiveness, there is evidence to suggest that more conserved domain epitopes across groups and strains may serve as a protective antigen in an adjudicated 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 block 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. If a vaccine based on this bivalent approach is developed and licensed, future studies will need to determine whether the broadly protective immune responses elicited by the vaccine remain effective as strain variation occurs naturally (or in response to wide-spread vaccination). Modification of the formulation may be required if non-vaccine
strains emerge for which the vaccine does not induce functional antibodies. Based on modeling of epidemiological data, protective immunity after natural NoV infection may persist between four and eight years. Early human challenge/rechallenge 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 of multiple exposures and pre-existing immunity may also complicate the vaccine approach and immune responses.

2.2. General approaches to vaccine development for low- and middle-income country markets

From a low- and middle-income-country perspective, there are unique issues to consider for vaccine development and feasibility. Dose number and schedule are important, and circulating strains may be different 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 to rely in part on the boosting of memory from previous natural infection. As this vaccine would likely be targeted to younger children, the effectiveness of such a vaccine in a naïve infant could be less. It will be most interesting to learn about the priming of functional antibody responses from current phase 2 trials in these settings which are currently underway (ClinicalTrials.gov Identifier: NCT02153112). If necessitated, an alternative mucosal-parenteral boost and/or adjuvant may be necessary, but this would complicate the development of a vaccine for use in a resource-limited setting.

From a developed-country perspective, there are a number of target populations and indications for which a norovirus vaccine could be developed and bring substantial public health value. These include not only travelers to lesser-developed countries and military personnel on deployment, but also healthcare workers and food handlers in developed countries who are at higher risk given the frequency and impact of NoV outbreaks in these populations. Elderly populations in group and institutional settings also experience frequent outbreaks and tend to have more severe disease and associated mortality, but may present a challenge to induce effective immune responses.

While more high-quality data on the epidemiology of NoV across ages and geographic areas are 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% of cases, behind rotavirus (38%) and enteropathogenic E. coli (15%) [7]. There is a dearth of data from Africa, where the effects of NoV-associated gastroenteritis may be more severe. Furthermore, because NoV affects all ages, it likely contributes to additional global disease burden beyond mortality. Forthcoming results from the World Health Organization (WHO) Foodborne Epidemiology Reference Group should provide better estimates for relative disease burden across age strata to consider.

In summary, 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 multiple infections and the highest incidence rates occurring in the first two years of life. Changing dynamics in strain variation and circulation could likely also extend the high incidence beyond two years of life. A successful vaccination strategy would therefore need to target children at the earliest opportunity in order to have maximum public health benefit.

3. Technical and regulatory assessment

Favorable advances have been made with a bivalent VLP-based vaccine. Firstly, proof of concept for efficacy has been recently demonstrated in a human challenge model with 50 vaccine and 48 placebo recipients, which observed 52% protection against all severity levels of disease (p = 0.028) and 68% (p = 0.068) and 100% (p = 0.054) protection against moderate-to-severe disease and severe disease only, respectively [9]. Furthermore, evidence for a correlate of protection is emerging based on induction of antibodies which bind to HBGAs [10]. HBGAs are hypothesized to serve as attachment factors for noroviruses, and data from clinical trials show that vaccinated subjects with higher HBGA-blocking antibodies had lower levels of infection and less severe disease. Furthermore, HBGA-blocking antibodies found among placebo recipients at pre-challenge were associated with protection.

An important concern about NoV is that strains change over time, and immune response to natural infection does not provide effective neutralizing antibodies for heterologous strains. However, a recent report describes broadly reacting HBGA-blocking antibodies in the recipients of a candidate bivalent VLP (G1/GII.4) vaccine for diverse strains and genotypes not included in the vaccine, suggesting broadly protective capability of the vaccine [11]. While these studies are encouraging, translation to low- and middle-income countries will present challenges, including adequate immune responses to a variety of circulating strains, substantiation of a correlate of protection that is also present in developed-country settings, and integration of a NoV vaccine into a crowded EPI immunization schedule. Limited data from cohort studies suggest that the timing of a vaccine may be important. In one birth cohort study in Peru it appears that norovirus has a similar epidemiology to rotavirus in incidence under 5 years of age occurring within the first year of life though highest incidence and multiple infections occur in the first two years of life [12]. However, changing dynamics in strain variation and circulation could likely extend the high incidence beyond 2 years of life. Additionally, more research is needed on virus-host associations and epidemiology to ensure adequate strain coverage of the current bivalent vaccine approach. Specifically, there is a need for rigorous studies that can accurately describe NoV prevalence and disease status in developed and developing world settings through the use of more sensitive and specific indicators of genotype-specific NoV exposure from serum.

For an adult traveler vaccine indication, further Phase 2 clinical development is being planned for the bivalent VLP parenteral vaccine being developed by Takeda Vaccines. These types of studies can be challenging due to the lack of predictability of disease outbreaks, the common finding of copathogenicity of diarrheal disease, frequent mismatch of vaccine strains with virus strains encountered, as well as field study challenges in following and collecting specimens from travelers. Despite these challenges, travelers’ diarrheic vaccine studies have successfully been performed in the past. Trials among elderly in nursing homes could also be considered, although lack of predictability of outbreaks and adequate immune responses may present similar challenges. Favorable results from trials in adult travelers or other high-risk populations in developed countries would support the effectiveness of such a vaccine approach in low- and middle-income country populations.

For a developing-country vaccine indication, plans for a multisite, pediatric, age-descending Phase 2 study in Colombia, Panama, and Finland are currently underway (ClinicalTrials.gov Identifier: NCT02153112). After these introductory clinical trials, rotavirus vaccine development pathways have demonstrated that there are sufficient field sites, experience, and regulatory pathways to take a NoV vaccine through large-scale safety studies and pivotal trials in low- and middle-income countries. Although acute gastroenteritis
clinical endpoints for rotavirus infection (e.g., the Vesikari scale) have been defined and accepted for developing-country populations, these types of clinical endpoints may not adequately address outcomes for mild-to-moderate disease, which is more common with NoV compared to rotavirus. However, the incidence of NoV disease (particularly of mild-to-moderate severity) is high enough to support such a trial with reasonable numbers—assuming there is broad enough coverage against circulating and emerging strains. Another important consideration for a NoV vaccine is that, in order to achieve acceptability in developing countries, the vaccine formulation should ideally be non-cold-chain-dependent and low cost. In addition, the Expanded Programme on Immunization (EPI) vaccine schedule is already quite crowded, and appropriate integration of another vaccine into the schedule would need to be navigated with EPI decision-makers. A combination vaccine with parenteral rotavirus vaccine is conceivable, given the similarities in disease indication and early age at which introduction is needed, and could be a more acceptable solution for low- and middle-income countries.

4. Status of vaccine R&D activities

Table 1 outlines the NoV vaccine candidates currently under development. The most advanced candidate is a recombinant VLP capsid protein vaccine, which has been formulated with Aluminum Hydroxide and Monophosphoryl lipid Adjuvants. It is given in a two-dose series separated 28 days apart [13]. The candidate has completed proof-of-concept in two human challenge studies, where protection against disease—particularly, against severe disease—was achieved [9]. Takeda Vaccines is sponsoring the development of this vaccine with significant academic collaboration and involvement with the US Department of Defense. The vaccine is modeled in part on the success of the currently licensed human papillomavirus (HPV) VLP vaccines. An alternative VLP candidate is being developed at Arizona State University using the same construct as the Takeda VLP, but their candidate is produced in a plant-based vector and has not yet entered clinical development.

A NoV VLP-rotavirus protein combination vaccine candidate is also currently in preclinical development, and clinical trials may begin in the near term [14]. The vaccine is being codeveloped 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 then combined. Preclinical studies in mice demonstrated strong and high-avidity NoV and rotavirus type-specific serum IgG responses, and cross-reactivity with heterologous NoV VLPs and rotaviruses was 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. Most recently, the developers have described an adjuvant effect of the rotavirus capsid V6 protein has on the norovirus VLP response which would be advantageous if this obviates the need for addition of an exogenous adjuvant in the target population [15].

The University of Cincinnati has conducted preclinical immunological studies on a NoV P particle construct vaccine candidate [16]. This 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 using a gnotobiotic pig model supports heterologous cross-reaction of the intranasal P particle vaccine as well as intestinal and systemic T cell responses. The heterologous protective efficacy of the P particle vaccine was comparable to that of the VLP vaccine in pigs (60%) and the homologous protective efficacy in humans (47%). Clinical development plans for this vaccine are unknown.

Finally, a novel construct which experimentally combined VLPs of norovirus GII.4 and enterovirus 71 (EV71, cause of hand, foot and mouth disease) was recently reported by developers from the Institut Pasteur of Shanghai and the Chinese Academy of Sciences [17]. In a mouse study they were able to demonstrate functional antibodies to both viruses without evidence of interference. Such a novel combination vaccine may offer additional value to areas of the world where both these diseases are prevalent, however further work is needed to understand valency requirements to adequately cover the six EV71 genogroups that are evolving and geographically unique around the world [18].

5. Likelihood for financing

Currently, Takeda Vaccines is predominantly funding development of its VLP-based candidate, though the US Department of Defense has also provided some support to Ligocyte, which Takeda bought in 2012. While industry funding would likely be able to take a vaccine to the developed-country market, further development in lower- and middle-income country markets would require funding from a range of sources, including vaccine-manufacturing partners in potential target markets, state governments, and global health nonprofit organizations. Furthermore, one should not discount the considerable value opportunity that public and private markets in the emerging economy markets bring to development and introduction of new vaccines [19]. Similar to rotavirus vaccine introduction which has been observed in many emerging economies, a norovirus vaccine may make economic and public health sense to many countries which can afford to introduce such a vaccine.

Gavi, the Vaccine Alliance has indicated an interest in enteric vaccines, including one for NoV, though their strongest preference would be for a combined vaccine, such as the NoV VLP-rotavirus fusion candidate. Alternatively, combination with or expression by another enteric pathogen could also enhance uptake of a NoV vaccine. Because a NoV vaccine is expected to have a dual market—both developed and developing countries—there would likely be scale-up advantages for commercial development and global distribution.

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**Table 1** Development status of current vaccine candidates (POC = proof-of-concept trial).

<table>
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<tr>
<th>Candidate name/identifier</th>
<th>Developer</th>
<th>Pre-clinical</th>
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<th>Phase II</th>
<th>POC</th>
<th>Phase III</th>
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<td>[13]</td>
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<td>[G1-I/GII-4 VLP</td>
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<td>[14]</td>
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<tr>
<td>NoV-EV71/VLP combination</td>
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VLP = virus like particle; POC = proof-of-concept; NoV = norovirus; Refs = references.
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Conflict of interest

Mark Riddle – Takeda Vaccines: advisory board and named investigator on a Cooperative Research and Development Agreement with the US Navy; Richard Walker – No relevant conflicts of interest to disclose.

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References