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Status of vaccine research and development for norovirus☆

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ABSTRACT

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.

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

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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.1/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 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 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

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