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Recent advances in human norovirus research and implications for candidate vaccines

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Abstract

Introduction: Noroviruses are a leading cause of acute gastroenteritis worldwide. An estimated 21 million illnesses in the United States and upwards of 684 million illnesses worldwide are attributed to norovirus infection. There are no licensed vaccines to prevent norovirus, but several candidates are in development.

Areas covered: We review recent advances in molecular epidemiology of noroviruses, immunology, and in-vitro cultivation of noroviruses using human intestinal enteroids. We also provide an update on the status of norovirus vaccine candidates.

Expert opinion: Molecular epidemiological studies confirm the tremendous genetic diversity of noroviruses, the continuous emergence of new recombinant strains, and the predominance of GII.4 viruses worldwide. Duration of immunity, extent of cross protection between different genotypes, and differences in strain distribution for young children compared with adults remain key knowledge gaps. Recent discoveries regarding which epitopes are targeted by neutralizing antibodies using the novel in vitro culture of human noroviruses in human intestinal enteroids are enhancing our understanding of mechanisms of protection and providing guidance for vaccine development. A future norovirus vaccine has the potential to substantially reduce the burden of illnesses due to this ubiquitous virus.

Keywords

norovirus; acute gastroenteritis; vaccine development; immunology

1. INTRODUCTION

Globally, noroviruses are the leading cause of acute gastroenteritis (AGE)[1]. Noroviruses were first discovered as a causative agent of AGE in 1972, when they were retrospectively

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identified by immune electron microscopy in patient stool samples from a 1968 AGE outbreak in an elementary school in Norwalk, Ohio [2]. Since then, noroviruses have been identified as an important cause of both outbreaks and sporadic cases of AGE. Humans of all ages are at risk for norovirus infection, although children and the elderly are especially vulnerable to severe disease [3-6].

Norovirus gastroenteritis is defined by a sudden onset of diarrhea and/or vomiting, but may also include nausea, stomach pain, fever, headache, and body aches [7]. These symptoms typically begin 12 to 48 hours after exposure to norovirus and are usually self-limiting, resolving within 1 to 3 days [7]. More severe outcomes are possible and are most often associated with severe dehydration among children, immunocompromised individuals, and the elderly [8]. Noroviruses are primarily spread directly from person to person through fecal-oral transmission or aerosolized vomitus [7]. However, they can also be transmitted through contaminated food, water, and environmental surfaces. As few as 18 to 1,015 viral particles may cause infection, although a more recent study estimated that the infectious dose is more similar to those of other RNA viruses (1,320 to 2,800 particles) [9,10]. Symptomatic individuals with norovirus infection shed high amounts of virus in the feces, with a peak of viral shedding ranging from 0.5–1,640 $\times 10^9$ viral copies per gram of feces at around 2 to 5 days following infection and norovirus can continue to be shed in the feces for weeks after symptoms resolve [11]. Viral particles can also be identified in vomitus of infected persons, although at lower levels than stool [11]. A 2018 meta-analysis estimated that 7% of norovirus infections are asymptomatic, although the asymptomatic prevalence varied in different studies from 0 to 30% [12]. There is evidence of asymptomatic individuals shedding high levels of viral particles similar to symptomatic individuals, although the level of transmission from asymptomatic individuals is likely lower than from symptomatic individuals [13]

The burden of illness associated with norovirus infection is considerable and of public health significance. In the United States, annual estimates of norovirus-associated AGE are as high as 21 million illnesses, with 1.7-2.9 million outpatient visits, 348,000-610,000 emergency department visits, 80,000-145,000 hospitalizations, and 650-1100 deaths [14,15]. Worldwide this burden is even greater, with estimates upwards of 684 million illnesses and over 200,000 deaths every year [16]. Noroviruses are the leading cause of foodborne illness, both in the United States and globally [16]. Evidence from recent birth cohort studies have further highlighted the burden of norovirus infection in young children. For example, one study in Peru found that 36% of infants experienced more than five norovirus infections within their first two years of life [17].

This high overall disease burden results in substantial economic costs. Annually, norovirus illnesses result in approximately $60 billion in total societal costs worldwide, including $4.2 billion in direct health care costs [18]. One recent publication estimated the norovirus associated-DALYs (disability adjusted life years, an estimate of the years of healthy life lost from a given disease) in England and Wales to be between 1159 and 4283, or 0.3-1.2 years of healthy life lost per thousand cases of norovirus [19]. These estimates are consistent with prior estimates from Australia, the United States, and the Netherlands and highlight the impact of norovirus infection on both the individual and population level [20-22]. Globally,

the norovirus-associated DALYs (95% uncertainty intervals) from 2010 were estimated as 15.1 million (11.6 —19.5 million), the highest DALYs among 22 foodborne bacterial, protozoal, and viral diseases [16].

No vaccines are currently licensed to prevent norovirus disease. However, several vaccines are in preclinical and clinical trials, and these could have substantial impact in future prevention efforts. The World Health Organization (WHO) highlighted noroviruses as a priority for vaccine development in their 2016 Product Development for Vaccines Advisory Committee [23]. Current vaccine efforts are limited to recombinant approaches due to the lack of a vaccine-approved cell line that is necessary to pursue inactivated or live-attenuated vaccines. In this paper, we review recent advances in molecular epidemiology, immunology, and in-vitro cultivation of noroviruses, all as they relate to vaccine development, and provide an update on the status of norovirus vaccines in development.

2. MOLECULAR EPIDEMIOLOGY AND STRAIN DIVERSITY

2.1 Molecular epidemiology

Noroviruses are classified within the *Caliciviridae* family, and have a 7.5 kb linear, positive sense, single-stranded RNA genome that is enclosed in a non-enveloped icosahedral capsid [24]. The genome is organized into three open reading frames (ORFs). ORF1 encodes a polyprotein that is co- and post-translationally cleaved into six non-structural viral proteins, including the RNA-dependent RNA polymerase (RdRp). ORF2 encodes VP1, the major structural capsid protein, which is composed of a shell (S) and two protruding (P) regions (P1 and P2). ORF3 encodes VP2, a minor structural capsid protein. Based on sequence differences of the VP1 protein, noroviruses are classified into at least ten genogroups (GI-GX), with most infections in humans caused by GI and GII viruses [25]. Noroviruses can be further classified into genotypes and P (polymerase)-types based on amino acid diversity of VP1 and nucleotide diversity of the RdRp region, respectively. Currently, there are at least 49 genotypes and 60 P-types [25]. Genogroup II genotype 4 (GII.4) viruses are further divided into epidemiologically important variants that carry the name of the location for the first strain from which the complete capsid sequence was submitted to GenBank (e.g., GII.4 Sydney).

Lateral gene transfer or gene conversion (recombination) and point mutations (genetic drift) are key mechanisms in the evolution and diversity of noroviruses. Increasing evidence of recombination along the ORF1-ORF2 junction indicates that recombination may be more significant for viral pathogenesis and fitness than previously recognized and ORF1-ORF2 recombination is increasingly recognized as the most important mechanism of norovirus evolution [26-28]. Hence, dual typing of noroviruses is increasingly used and dual types are listed with the genotype listed first, followed by the P-type in brackets (e.g. GII.3[P12]) [25].

2.2 Strain diversity

A major challenge in norovirus vaccine development is the large number of genetically and antigenically diverse strains against which a vaccine needs to protect. Since the mid-1990s, GII.4 strains have been the predominant genotype circulating globally and have

been responsible for roughly 50-70% of outbreaks in the past several decades [26,29]. Since 2002, GII.4 variants have emerged every 2 to 4 years, with notable pandemic GII.4 variants-Farmington Hills 2002, Hunter 2004, Den Haag 2006b, New Orleans 2009, and Sydney 2012. This pattern of GII.4 variant emergence and epochal evolution has occurred for several years, with emergence of phenotypically distinct variants, several of which have been associated with an increasing number of outbreaks.

While GII.4 variants continue to dominate globally, non-GII.4 strains also play an important role in norovirus disease burden and transmission. In Asia, a novel recombinant GII.17 strain (GII.17[P17]) emerged and caused a majority of cases during the 2014–2015 season [30-33] and in 2016–2017 a novel recombinant GII.2[P16] virus emerged in several Asian countries and outnumbered GII.4 viruses in several provinces in China [34-36]. Interestingly, a higher proportion of GII.2[P16] was found in young adults at a higher viral load than other genotypes [37,38]. However, this change seemed to be confined to Asia as GII.2[P16] viruses did not cause more than 20% of the outbreaks on other continents [26,29,39], perhaps explained by most reported norovirus outbreaks in China occurring in schools while in North America, Europe and Australia most reported outbreaks are from healthcare facilities, including long-term care institutions for the elderly [40-43].

In recent years, many countries have reported the emergence and spread of RdRp/capsid recombinant noroviruses. In the United States, a new recombinant GII.4 Sydney emerged in 2015 (GII.4 Sydney[P16]) and replaced the 2012 variant (GII.4 Sydney[P31], formerly GII.Pe-GII.4 Sydney) as the primary cause of outbreaks [26]. In Alberta, Canada, GII.4 Sydney[P16] was predominant in 2015-2016 and 2017-2018 [44], GII.2[P16] was predominant in 2016-2017 [44], and GII.12[P16] emerged in 2018-2019 and caused 10% of outbreaks and 17% of sporadic cases [45]. Among recent surveillance reports of sporadic norovirus cases in New Zealand, Australia, Italy, Hong Kong, Brazil, and Bangladesh, GII.2[P16] and GII.4 Sydney[P16] viruses were commonly reported strains [38,46-49]. In Shanghai, China, surveillance data of sporadic norovirus cases from 2012 to 2017 found 12 likely recombinant strains [50]. There is evidence that polymerase recombinants can emerge with or without unique amino acid changes in their capsid genes [26,51]. Interestingly, the P16 polymerase has been associated with both GII.4 Sydney and the recently emerged GII.2 viruses, suggesting its importance in viral fitness compared to other P-types [26,27,48]. However, in the U.S. no increased risk of hospitalization or death were reported among outbreaks caused by P16 polymerase harboring strains, suggesting that this polymerase may enhance viral fitness but not virulence [27].

Collectively, these findings provide evidence that new norovirus strains continue to emerge and that recombinants with novel RdRp genes may confer evolutionary advantages. These data show that recombination and acquisition of new capsid or polymerase genes is a prominent driver of strain diversity and may happen more frequently than previously recognized. With no broadly-reactive neutralization epitope currently recognized, changes in circulating strains could pose challenges for vaccine development, and therefore global norovirus strain surveillance is important. Furthermore, it is unclear if differences in strain distribution exist between young children and adults, differences which may impact vaccine development and implementation of future vaccines.

3. IMMUNOLOGY

Many gaps remain in the understanding of natural immunity to human noroviruses, complicating predicting the effect of vaccine-induced immunity. Mathematical modeling suggests that duration of naturally-induced immunity from infection may last up to 10 years [58]. It is critical to understand the duration of immunity, both homotypic and heterotypic, to inform the vaccine schedule necessary for sustained protection.

3.1 Birth cohort studies

Since infants have no prior exposure to norovirus infection, longitudinal birth cohorts provide a valuable opportunity to prospectively describe the natural history of norovirus infections and subsequent immunologic responses. Data from such studies can be used to assess the effects of prior human norovirus infection on the risks of subsequent infection and disease caused by homotypic and heterotypic strains. Birth cohorts in low- and middle-income countries (LMICs) with a high level of endemic norovirus also represent important sources of information regarding sequential infection, patterns of humoral response, and the impact of health status on immune response. A review of ten community-based birth cohort studies from LMICs that assessed symptomatic infection as well as asymptomatic infection by collecting and testing frequent, routine stool specimens found that approximately 70% of children under the age of two had at least one diarrheal episode associated with norovirus infection, and at least 90% experienced at least one norovirus infection (symptomatic or asymptomatic) [59]. A study in Peru found that 36% of infants had more than five norovirus infections within the first 2 years of life, with a high level of homotypic antibody protection against infection with both GII.4 and GII.6 [17]. In addition, heterotypic protection against GI.3 infection was also noted after prior infection with a GII.4 strain. A surprising finding from this study was the increased risk of infection for some genotypes after prior heterotypic infections, although additional studies are needed to confirm these data [17]. Overall, data from birth cohort studies complement human challenge studies in healthy adults to provide burgeoning evidence of homotypic immunity and some level of heterotypic immunity after repeat infections [59].

3.2 Innate Immunity

Understanding of the innate immune system’s role in responding to norovirus infection is evolving, with new evidence shedding light on potential cellular-level mechanisms of protection [52]. Specifically, recent findings show that the innate immune response is triggered to restrict virus replication through mechanisms such as IFN-induced transcriptional responses and production of pro- and anti-inflammatory cytokines [60]. However, it remains unclear to what extent T-cell mediated immunity is influenced by strain diversity. Additionally, it is unclear how influential cellular immunity will be in vaccine effectiveness. The innate immune response does not fully activate until at least 2 days post-infection, so it is likely that antibodies provide the primary mode of protection from infection [61-63]. However, this may differ by immune status and T-cells may be more important for control in elderly populations.

3.3 Neutralizing antibodies

There have been several recent advances in the identification of conserved norovirus B and T cell epitopes [52]. Neutralizing epitopes on the norovirus capsid are primarily located on the P2 domain; however, several conserved epitopes in often-inaccessible regions of the viral capsid have been identified, suggesting that conformational changes may also contribute to neutralization of the virus [52,53]. Several monoclonal antibodies directed to epitopes on the P domain of VP1 have recently been described that showed both histo-blood group antigen (HBGA) blockade and neutralization activity [64]. In a study from Australia and New Zealand, all three recombinant GII.4 Sydney 2012 strains had significant positive selection at residue 373 of epitope A, an epitope that is hypothesized to be an a key component of antigenic change and related to loss of blockade antibody binding [46,54-56]. Notably, a neutralizing antibody was recently reported that mapped to a conserved epitope present on multiple GII.4 strains and was able to neutralize a pandemic strain of norovirus in an human intestinal enteroid cell culture system [53]. This epitope was located separately from the epitope to which HBGAs bind, suggesting mechanisms beyond direct interference with ligand binding. Epitope mapping and genomic analyses have also highlighted the relationship between mutations at specific antigenic sites and emergence of new variants among GII.4, which could be beneficial in the development of cross-protective vaccines [57].

3.4 Correlates of protection

Vaccine development has been limited by the absence of a primary, confirmed correlate of protection against norovirus infection [65]. Several candidates have been explored to determine whether there is correlation with protection against infection or disease, however no one candidate has indicated absolute correlation. These current candidates include serum HGBA-blocking antibodies, serum hemagglutination inhibition antibodies, salivary, serum, and fecal immunoglobulin (Ig) A, and virus-specific IgG memory B-cells, although the primary focus has been on serum HGBA-blocking antibodies [65]. Two challenges studies previously demonstrated an association between titers of HBGA-blocking antibodies and AGE symptoms or infection [66,67]. Also, a case study of chronic norovirus disease in an immunocompromised host identified strain-specific antibody blockage titers correlated with symptom improvement[68]. Furthering our understanding of correlates of protection, and ideally identification of the best predictor of protection, against norovirus disease has implications for future vaccine study endpoints, choice of adjuvants and route of immunization [65].

4. IN-VITRO CULTURES OF HUMAN NOROVIRUS

Until recently, the inability to culture noroviruses has significantly limited *in vitro* experiments [69,70]. Use of murine noroviruses (MNV) and other cultivable viruses in the *Caliciviridae* family has helped in unraveling many aspects of basic norovirus virology. However, the clinical symptoms and natural infection route for MNV differ from human noroviruses. Only recently have major advancements been made in cultivation of human noroviruses *in vitro* through both a human-derived continuous B-cell line, BJAB, and a stem-cell derived human intestinal enteroid (HIE) culture system.

4.1 In-vitro culture of human norovirus using B cells

In 2014, Jones et al demonstrated that human noroviruses could be cultivated in a human-derived continuous B-cell line, BJAB [71]. The replication of human noroviruses in this culture was enhanced by presence of enteric bacteria or the addition of histo-blood group antigen carbohydrates [72]. A limited number of human norovirus strains have been successfully cultivated using this culture system [69,72,73]. However, this system is primarily limited by the low level of viral replication yielded from this cultivation and transferring this method to other laboratories has been challenging [72].

4.2 In-vitro culture of human norovirus using human intestinal enteroids

The establishment of a stem-cell derived human intestinal enteroid (HIE) culture system for human noroviruses in 2016 represented a major breakthrough in norovirus research [74]. The HIE culture system recapitulates the human intestinal epithelium, the initial cells that noroviruses interact with to enter into the human host gut epithelium [60,75]. HIEs have become a tool for the evaluation and better understanding of biological mechanisms, viral pathogenesis, cellular pathways, and the immunology of noroviruses. Using HIE, it is possible to measure neutralizing antibodies after natural infection or vaccination. Although multiple norovirus genotypes have been successfully cultivated in HIE cultures, efforts with GII.4 viruses seem to be most successful whereas attempts with GI viruses have been largely unsuccessful [75].

This novel HIE model has been used to study the innate immune response to norovirus infection [60], measure the impact of disinfectants such as chlorine and alcohols on viral inactivation [76], and characterize neutralizing antibodies including the characterization of the first human monoclonal antibodies that neutralize pandemic GII.4 viruses [75]. However, there are several challenges to the system. The medium used for HIE culture is expensive. Some strains require bile in the medium [75], and the success rate of replication is also strain-dependent with a higher success rate for fecal samples collected from children compared to adults [76]. Despite these limitations, the HIE culture system has been instrumental in furthering our understanding of the virology and immunology of noroviruses for the design and evaluation of vaccine candidates.

5. VACCINES IN HUMAN CLINICAL TRIALS

Several norovirus vaccines are in the pre-clinical phase of vaccine development, two vaccines are currently in clinical trials, and two vaccines were recently approved for clinical trial testing (Figure 1). Sections 5.1–5.4 will detail the four vaccines in or recently approved for human clinical trials, while in Section 6 preclinical vaccine candidates will be discussed.

5.1 Takeda Vaccine (Bivalent GI.1/GII.4 vaccine)

The vaccine furthest along in development is a bivalent GI.1/GII.4 vaccine being developed by Takeda Pharmaceutical Company Limited. This vaccine was first developed as a virus-like particle (VLP) vaccine to be administered intranasally, with a GI.1 genotype and monophosphoryl lipid A and chitosan as adjuvants [77]. VLPs consist of 90 dimers of the norovirus VP1 capsid protein that when expressed in eukaryotic cells, spontaneously

self-assemble into non-infectious virus-like particles that are antigenically similar to live virus [78]. Findings from a human clinical trial challenge study showed that this intranasal monovalent vaccine was well tolerated and reduced norovirus-associated AGE after challenge with a homologous virus [77].

Takeda subsequently reformulated this formulation into a bivalent GI.1/GII.4 VLP vaccine with the GII.4 component derived from an amino acid consensus of GII.4 Houston, Yerseke, and Den Haag variant strains [79]. A human clinical trial challenge study in 2012–2013 found that this vaccine was immunogenic, well tolerated, and decreased severity, but not incidence, of norovirus-associated illness [80]. Recently, data from the first field efficacy study using this bivalent vaccine were reported. The participants in this study included 4,712 healthy adult trainees at the US Navy Recruit Training Command at Great Lakes, IL who were randomized to one intramuscular injection of the bivalent vaccine or a saline placebo and monitored for occurrence of AGE for 45 days. The primary endpoint of protection against homotypic infection was not able to be evaluated because of the limited number of homotypic norovirus infections (6 moderate/severe cases due to GI.1 or GII.4 strains), however vaccine efficacy for moderate/severe norovirus-associated AGE due to any strain was 61.8% (95.01% CI, 20.8 to 81.6) [81].

While this study was limited in its conclusions, due to the short follow-up of participants and limited statistical power, these findings are encouraging and further supported by analyses of the antibody response post-vaccination. Investigators later analyzed samples from three participants from the Takeda Phase 2b trial to look at the serological repertoire before and after vaccination [53] and found that vaccine-elicited antibodies were able to induce broad blockade and neutralization of GII.4, but that the serological response was dominated by pre-existing immunity and vaccine boosting of antibodies initiated by norovirus exposure prior to vaccination [53]. These data highlight that vaccination efficacy might differ by the exposure history of the target population. For example, adults with a much broader history of prior exposure may illicit stronger immune responses than norovirus-naïve infants.

5.2 Vaxart Oral Vaccine (Monovalent GI.1 oral vaccine)

The biotechnology company Vaxart is developing a recombinant vaccine for norovirus prevention that would be administered as an oral tablet in adults or by liquid dosing for children [82]. These formulations have the advantage that they are stable at ambient temperature and do not need a ‘cold chain’ to maintain product quality [83]. This vaccine was originally designed as a monovalent vaccine using a non-replicating adenovirus 5 vector to express the VP1 gene from the GI.1 norovirus strain and double-stranded RNA as adjuvant [82]. This same non-replicating adenovirus 5 vector platform was developed for an oral influenza vaccine, which has had successful phase I human studies [84-86]. Safety and immunogenicity from a phase 1 clinical trial for this monovalent norovirus vaccine demonstrated it was safe, well-tolerated, and generated systemic, mucosal, and memory IgA/IgG antibodies [82].

Recently, findings were presented from a phase 1b study in which the oral vaccine platform was used to express GI.1 or GII.4 VP1 with monovalent or bivalent dosing. Eighty participants were randomized to one of four groups: 1) GII.4 vaccine, 2) GI.1 vaccine,

3) bivalent GII.4 and GI.1 vaccine, or 4) placebo tablets. There was no indication of immunological interference upon co-administration of the bivalent vaccine and a similar adaptive immune response was induced post-vaccination as is produced after natural infection, demonstrating robust immunogenicity [87]. The GI.1, GII.4, and bivalent GII.4/GI.1 vaccines had no indication of serious adverse events and met all primary endpoints for safety [88]. Vaxart reportedly plans to conduct a Phase 2 bivalent study beginning in 2020 to confirm dosage of their bivalent norovirus vaccine candidate [88]. However, there remain unanswered questions regarding the boosting of antibodies for pediatric populations and whether this vaccine will protect high risk groups.

5.3 Chinese Academy of Sciences (Tetravalentvaccine)

In May 2019, the Institut Pasteur of Shanghai (IPS) under the Chinese Academy of Sciences received a clinical research permit from the Chinese National Medical Products Administration to conduct a clinical trial in humans for a tetravalent vaccine that had been under pre-clinical development for four years [89]. The formulation of this tetravalent vaccine has not yet been published.

5.4 National Serum & Vaccine Institute, China (Bivalent GI.1/GII.4)

The National Serum & Vaccine Institute in China is currently enrolling healthy people aged 6 months to 59 years in a phase 1 clinical trial to evaluate the safety and immunogenicity of a bivalent vaccine (https://clinicaltrials.gov/ct2/show/NCT04188691). This bivalent vaccine expresses GI.1 and GII.4 VLPs and is administered intramuscularly.

6. VACCINES IN PRE-CLINICAL TRIALS

There are currently at least four vaccines in pre-clinical trials (Figure 1). These include 1) a trivalent VP6 vaccine developed by the University of Tampere, Daiichi-Sankyo Company, Limited & UMN Pharma Inc., Japan, 2) a plant-based GII.4 VLP norovirus vaccine developed by Arizona State University, 3) a trivalent GII.4 norovirus, hepatitis E, astrovirus P particle vaccine developed by the Cincinnati Children's Hospital Medical Center, the University of Cincinnati, and Virginia Polytechnic Institute and State University, and 4) a lactic acid bacteria (LAB)-based VP1 norovirus vaccine from the Ohio State University. A recent review summarized findings from these preclinical trials [90] and since that review there have only been a few published updates for the Daiichi-Sankyo trivalent VP6 vaccine and the Ohio State University LAB-based VP1 vaccine, which we will discuss in this review.

6.1 Trivalent VP6 vaccine (University of Tampere, Daiichi-Sankyo Company, Limited & UMN Pharma Inc., Japan)

The trivalent VP6 vaccine consists of GII.4 and GI.3 VLPs and the rotavirus VP6 capsid protein, however new research has assessed the effect of adding CVB1 VLPs to protect against coxsackievirus B (CVB) serotypes CVB1-6 [91]. CVB is an enterovirus which cause a range of illnesses from mild respiratory symptoms to more severe illness, such aseptic meningitis and myocarditis, with infants being an especially vulnerable population [91]. The inclusion of CVB1 VLP caused no interference in the norovirus and rotavirus-specific antibody responses, supporting the addition of this antigen to the candidate vaccine

[91]. These findings also supported prior evidence that the rotavirus VP6 protein acts as an efficient adjuvant, improving immune response [91-94]. A bivalent vaccine with GI.4 and GII.4-2006a VLP derived from a plant *Nicotiana benthamiana* expression system has been tested on mice with intradermal immunization, demonstrating induction of antibodies against vaccine-derived and heterologous norovirus genotypes when co-administering with rotavirus VP6 [94].

6.2 Lactic acid bacteria (LAB)-based VP1 norovirus vaccine (The Ohio State University)

The Ohio State University recently developed a new lactic acid bacteria (LAB)-based norovirus vaccine candidate. The LAB was used as a vector to express the major capsid VP1 gene of a GII.4 norovirus strain [95]. This vaccine candidate was tested for immunogenicity by orally inoculating gnotobiotic piglets, showing induced norovirus-specific immune responses. A challenge study further showed vaccination was able to prevent norovirus infection of pig intestines [95].

7.0 CONCLUSION

In conclusion, while there are currently no licensed vaccines to prevent norovirus infection, several vaccines are in clinical trials which, if lead to licensed products, will have the potential to have a tremendous impact on reducing norovirus morbidity. Although noroviruses are extremely diverse, the two-vaccines in clinical trials include GII.4 which continues to cause at least half of all norovirus illnesses worldwide [26,29]. Birth cohort studies may help elucidate duration and extent of homotypic and heterotypic immunity, but it is still unknown whether vaccines currently in clinical trials will provide cross-protection against non-GII.4 noroviruses. Vaccine effectiveness will likely depend on formulation of the vaccines and the level of cross-protection with other genotypes as an ideal vaccine would induce immunity to a wide variety of frequently circulating genotypes and protect against future emerging strains. Continuous monitoring of circulating norovirus strains will help inform possible future reformulations. Additionally, the impact of recombination on vaccine effectiveness warrants future investigation. For example, it is unknown if a vaccine based on GII.4 VLPs derived from GII.4 Sydney[P31] would be as effective against the recently emerged recombinant GII.4 Sydney[P16] strains. Future studies are needed to determine potentially cross-protective antibody binding sites as well as antibody responses in key vaccine target populations. The HIE culture system [74,76] can provide invaluable information for refining the design and vaccination strategies to provide broad, long-term protection against norovirus.

8.0 Expert Opinion

The described advances in molecular epidemiology of noroviruses, immunology, and *in-vitro* cultivation are all key research areas that inform vaccine development. The progress in norovirus vaccine development over the past decade is promising. A future vaccine that is effective at preventing norovirus infection has the potential to have a dramatic impact on reducing the burden of norovirus. Even partial reduction in the estimated global burden of 684 million illnesses annually and $60 billion total societal costs worldwide has potential for substantial public health impact. Notably, pediatric vaccination could potentially prevent

up to 85% of pediatric norovirus cases and subsequent dramatic reductions in societal costs, depending on the efficacy, duration of immunity, and cost of vaccination [96,97].

In-vitro cultivation of noroviruses using HIE is a ground-breaking discovery and a crucial tool for not only better understanding the virology and immunology of noroviruses, but for analyzing the neutralizing potential of vaccine-induced antibodies. Given the proclivity of noroviruses to adapt and escape immunity, it is vital for vaccines to protect against a wide breadth of strains both to promote cross-protection as well as to minimize the possibility of promoting new strain emergence through selective pressure. However, many questions remain regarding the duration of natural and vaccine-induced immunity and the extent of cross-protection against different genotypes and genogroups. The spread and circulation of non-GII.4 noroviruses may complicate current vaccine strategies, as pipeline vaccine candidates include GII.4 components as a primary factor to elicit immune response. It is unclear yet whether these vaccine candidates will provide cross-protection against different variants and genotypes, although preliminary data from the Takeda bivalent GI.1/GII.4 VLP vaccine trial indicated cross-protection of the vaccine against heterotypic strain [81].

As there are several gaps in our current understanding of norovirus immunology and molecular epidemiology as they relate to vaccine development and potential effectiveness, continued research is needed to better understand these key issues and promote efficient and successful vaccine development. As clinical and pre-clinical vaccine trials progress, the next five years look to be an exciting time in the field of norovirus prevention research.

DECLARATION OF INTEREST

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

DISCLAIMER:

The findings and conclusion in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Article highlights

- Noroviruses cause an estimated 684 million illnesses of acute gastroenteritis worldwide resulting in approximately $60 billion in societal costs.
- There are no vaccines currently licensed to prevent norovirus infection or disease, although several candidate vaccines are in clinical and pre-clinical trials.
- Increased surveillance efforts have helped to identify and characterize several novel genotypes and updated norovirus classification now recognizes 5 genogroups infecting humans with the majority of infections associated with GI and GII viruses.
- GII.4 strains have been the predominant strains over the past several decades, but in recent years some geographic regions have seen the emergence and predominance of non-GII.4 strains.
- The routine use of dual typing of the capsid and polymerase regions of norovirus strains has helped identify and highlight the role of recombination in the evolution of noroviruses.
- The identification of conserved B and T cell antibody binding sites helps inform vaccine development, particularly characterization of broadly neutralizing epitopes against different strains.
- The duration of natural or vaccine-induced immunity remains unclear, as does the extent of cross protection against different genogroups or genotypes. Birth cohort studies may help elucidate some of these knowledge gaps.
- Human intestinal enteroids can now be used to culture noroviruses and are a valuable tool for evaluating the immunology of noroviruses and for measuring neutralizing antibodies after vaccination.

	Preclinical	Phase 1	Phase 1b	Phase 2	Phase 2b
Virus-Like Particle (VLP)	**Daiichi-Sankyo Company, Limited & UMN Pharma Inc., Japan** • GI.3, GII.4, rotavirus VP6 • Intramuscular injection • Trials in mice	**Chinese Academy of Sciences** • Tetravalent vaccine • Trials in healthy children and healthy adults		**Takeda Pharmaceutical Company Limited** • GI.1, GII.4 • Intramuscular injection • Trials in children 6 weeks through 8 years of age, adults, the elderly >60 years, and military recruits	
	Arizona State University • GII.4 • Intranasal • Trials in mice	**National Serum & Vaccine Institute, China** • GI.1, GII.4 • Intramuscular injection • Trials in healthy children and healthy adults			
Recombinant Adenovirus			**Vaxart, Inc.** • GI.1, GII.4 • Oral Pill • Trials in healthy adults		
P Domain	**Cincinnati Children's Hospital Medical Center & University of Cincinnati** • GII.4, Hepatitis E, Astrovirus • Intranasal • Trials in mice				
Lactic acid bacteria	**The Ohio State University** • GII.4 • Oral • Trials in pigs				

Figure 1.

Vaccine candidates in development, by type and pre-clinical or clinical phase. Adapted with permission from [90].