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Progress toward norovirus vaccines: considerations for further development and implementation in potential target populations

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Abstract

Human norovirus infection causes significant medical and financial costs in the USA and abroad. Some populations, including young children, the elderly, and the immunocompromised, are at heightened risk of infection with this virus and subsequent complications, while others, such as healthcare workers and food handlers are at increased risk of transmitting it, and some are at risk of both. Human noroviruses are heterogeneous with new strains emerging periodically. In addition to viral diversity, incompletely understood characteristics, such as virus–host cell binding and duration of immunity after infection add to the challenges of creating a norovirus vaccine. Although much progress has been made in recent years, many questions remain to be answered. In this review, we discuss the important areas and relevant literature in considering human norovirus vaccine development and potential targets for implementation.

Keywords

lciviridae; epidemiology; gastroenteritis; norovirus; Norwalk agent; vaccine development

Human norovirus is a major health and financial burden in the USA and abroad. Globally, it causes 12–24% of community or clinic-based cases of acute gastroenteritis (AGE), 11–17% of emergency room or hospital cases and anywhere from 70,000 to 200,000 deaths annually, [1–3]. In the USA alone, it causes 19–21 million cases of AGE [4,5], up to 70,000 hospitalizations across all age groups [6], and nearly 800 deaths annually [7]. Human norovirus infection is costly, with annual estimates of US$493 million for hospitalizations [6] and US$284 million for outpatient and ER visits [8], both in the USA. Costs associated with foodborne norovirus infections are even greater, with just over US$2 billion in combined medical, productivity, and mortality-related costs, as well as 5000 lost quality-adjusted life years (QALYs) [9].

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Human norovirus is implicated as a cause of AGE in both sporadic and outbreak settings. It is the number one cause of community or outpatient cases of AGE among all ages [4,10]. Among US adults, a prospective multi-center study found that norovirus is the number one cause of severe AGE requiring emergency room visits, having been detected in 26% of patients tested [11]. A recent review that included 175 studies from 48 countries that examined the prevalence of human norovirus using RT-PCR among cases of AGE found an overall prevalence of 18%, with higher prevalence in community or outpatient settings versus hospital settings (20–24% vs 17%, respectively) [1]. Prevalence among those complaining of foodborne illnesses is even higher. Studies from the USA and Australia have reported human norovirus prevalence of 42–53% among incidents of foodborne gastroenteritis [12,13]. Human noroviruses are also the most common cause of AGE outbreaks in the USA and abroad [14,15]. In the USA, surveillance data from the National Outbreak Reporting System implicates human norovirus as the leading cause of single-etiology AGE outbreaks, responsible for 68% of identified outbreaks [16].

Given this public health importance, vaccines targeting human norovirus are currently under development and testing [17,18]. In this article, we describe populations at highest risk for human norovirus disease and transmission and key issues and gaps that remain to be addressed for further development and ultimate implementation of a human norovirus vaccine among these potential target populations.

Clinical infection with human norovirus

Earlier studies suggested that as few as 18 virions could cause clinical infection [19], with more recent studies reporting the 50% human infectious dose at 1320 genomic equivalents [20]. Once infected by human norovirus, predominant symptoms include sudden-onset vomiting, abdominal cramping and watery diarrhea. Gastroenteritis due to human norovirus infection has been described as a brief, self-limited infection in many with symptoms resolving normally within 2–3 days, but both symptoms and clinical severity can differ by patient population [21], with elderly and the immunocompromised patients at risk for more severe symptomatology and complications [22,23]. Treatment of symptomatic infection is generally supportive with fluid and electrolyte repletion, although research to identify antiviral treatment strategies is currently underway [24]. The mainstay of current outbreak control efforts is identification and containment of the source, maintenance of strict personal hygiene, and decontamination of environmental surfaces [25]. Vaccines present a new approach to prevention of human norovirus disease.

Transmission of human norovirus

Transmission of human norovirus is predominantly fecal–oral, although infectious vomitus can also spread the disease [21]. Shedding of virus in stool can be copious and has been detected by real time RT-PCR in healthy adults up to 56 days after oral inoculation with norovirus [26]. Specific routes of transmission implicated in outbreaks include direct person-to-person contact, ingestion of contaminated food or water, and contact with contaminated environmental surfaces or fomites [27]. Data from National Outbreak Reporting System indicates that human norovirus outbreaks in the USA occur most commonly via person-to-

person (69%) or food-borne transmission (23%), with foodborne outbreaks occurring mostly in food preparation settings (90%) and non-foodborne outbreaks occurring mostly in long-term care facilities (80%) [15].

Populations with high burden of human norovirus disease

In considering vaccine development and implementation, risk for infection and transmission of human norovirus must be taken into account. Reasons for vaccination need to take into account that some groups have higher risk of infection and clinical complications, while others have higher risk for transmitting infection to other groups, and some can have both (Table 1). A key objective of vaccinating the latter group would be to arrest transmission of virus from high-risk sources to persons in the former group and the general population. The goal of vaccinating the former group would be to directly decrease the morbidity, mortality and associated costs attributed to human norovirus infection.

Young children

Human norovirus infection is common among young children. A literature review examining international data found that the prevalence of human norovirus was 18% among children less than 5 years old with AGE [1]. Emergency department visits and hospitalizations are also seen frequently among this age group. One study found that 12% of such visits were attributed to human norovirus [2], while another found that hospitalization rates were 9.4 hospitalizations/10,000 population, representing the highest rate of any age group [10]. In addition, an estimated 27 norovirus-associated deaths occur per year among US children <5 years old [7]. In settings with national rotavirus vaccine programs, human norovirus has become the leading cause of AGE in children less than 5 years old requiring medical attention [28,29]. In the community setting, an English study found human norovirus to be the most common cause of infectious intestinal disease, and children had the highest incidence. Children less than 5 years old had a higher incidence of human norovirus infection compared with all other age groups, with 21.4 episodes/100 person-years among those less than 5 years old compared with 3.3 episodes/100 person-years among those above 5 years old. Incidence among children 0–1 years old was the highest, at 27/100 person-years [30]. In terms of infectiousness, a prospective cohort study of the natural history of human calicivirus infections (including noroviruses and the closely related sapoviruses) in the community reported that children have longer duration of diarrhea compared with those over 12 years old [31]. This study found that gastroenteritis due to human norovirus was common among all age groups, and diarrhea was a predominant symptom among children. The duration of diarrhea decreased with increasing age, with symptoms lasting 6 days in those less than 1-year-old, 4 days in 1–4 year olds, 4 days in 5–11 year olds, and 3 days in those greater than 12 years. The duration of diarrhea is epidemiologically important, particularly given the low infectious dose of human norovirus and implications for transmission. Also important for transmission is viral shedding in stool. Among children in this same study, RT-PCR detected human norovirus shedding 22 days after infection, with those less than 1-year-old most frequently shedding human norovirus in their stool at this time point. Of note, human norovirus may have been present longer than 22 days after infection, but this was the last time point that the investigators checked fecal specimens. A more recent study among a

Peruvian birth cohort over 2 years measured the median duration of viral excretion and found the length of viral shedding was even longer: 31.5 days among children with diarrheal symptoms and 30 days among asymptomatic children [32]. In addition, data from modeling studies predict the basic reproductive number, or R_0, for human norovirus to be 4 for children less than 4 years old, providing support for young children's role in propagating infection [33]. Children suffering from malnutrition may also serve as reservoirs of human norovirus, as suggested by a murine model of malnutrition showing impaired norovirus control, decreased antibody response and enhanced viral evolution [34]. These data highlight the importance of this age group in spread of infection as well as their substantial burden from human norovirus disease, and this epidemiology points to inclusion of a developed vaccine in young children, which could decrease disease burden both directly and indirectly.

The elderly

Severe human norovirus disease occurs at the extremes of ages and, as with the very young, emergency department visits and hospitalizations are also seen frequently among adults aged ≥65 years. In terms of clinical symptomatology, one study showed that hospitalized patients older than 65 years were more likely to have longer duration of diarrhea and were at higher risk for severe clinical outcomes compared with younger patients [22]. The burden of fatal human norovirus disease is also largely confined to the elderly [7,10,35], with the elderly having a higher case fatality rate than any other age group [36]. Indeed, in the USA, among persons 65 years or older, human norovirus is second only to *Clostridium difficile* as a cause of death from gastroenteritis, with approximately 800 deaths reported annually [7]. A modeling study from England and Wales suggests a similarly prominent role of human norovirus among gastrointestinal infections resulting in death among the elderly [37]. In this analysis, human norovirus was the only significant gastrointestinal pathogen in regression models estimating deaths from infectious intestinal disease other than *Clostridium difficile* in this age group. These authors found that 20% of such deaths, or roughly 80 deaths per year, were associated with human norovirus. Healthcare settings have been shown to bear a large burden of disease related to human norovirus [38], and most outbreak-associated deaths occur in these settings [39,15]. In addition, nursing homes have reported higher rates of all-cause hospitalization and deaths during periods with ongoing human norovirus outbreaks compared with other time periods [40]. Given that the elderly largely populate long-term healthcare facilities, these enclosed settings are ripe for person-to-person transmission and subsequently for adverse outcomes related to human norovirus outbreaks. These are also costlier, as mean hospital charges among the elderly with human norovirus gastroenteritis cost more than twice that of children [6].

Travelers

Human norovirus infections are an important cause of traveler's diarrhea. It is second only to *Escherichia coli*, with diverse human norovirus genotypes having been found in 9–16% of diarrheal stools of travelers to Mexico, Guatemala and India [41–43]. Human norovirus has been implicated as the etiology for outbreaks in other leisure settings, including recreational parks, hotels, camps, cruise ships, tour buses and ski lodges [44–46]. Many of these settings include numerous persons in close proximity or confined spaces, who can transmit infection to each other. Outbreaks in these leisure settings may be caused by a point source, or can be

a marker for increased activity in the general populations. In one large European investigation of 13 ships reporting 43 outbreaks in 2006, the authors were unable to identify a point source for the outbreaks and postulated that the outbreaks may have been related to general increased activity of human norovirus in the population during that period [47]. In addition to these leisure settings, outbreaks have been reported on airplanes while travelers are en route to their destinations [48].

The military

Other populations at risk for infection include those dwelling in group settings, such as the military. Given the close living situations, shared bathrooms in barracks, and close quarters during deployment, human norovirus has been responsible for a variety of AGE outbreaks among those in the military. A systematic review of long-term travelers, including military recruits, found the prevalence of human norovirus infection between 4 and 13% among those with AGE [49], while one cross-sectional study of AGE on a military base during Operation Iraqi Freedom, detected human norovirus in stool of 23% of recruits experiencing AGE [50]. This higher figure compared with that reported by systematic review may be a result of improved diagnostic testing for human norovirus. In these deployed populations, gastroenteritis frequently leads to lost duty time, decreased reserve readiness and hospitalizations, with substantial economic implications [51].

The immunocompromised

Human norovirus is often associated with chronic or recurrent gastroenteritis among the immunocompromised who include those born with congenital immunodeficiency syndromes, those with HIV/AIDS, transplant recipients on immunosuppressive treatment and recipients of cancer chemotherapy. While it is unclear whether these patients pose an increased risk for transmission to immunocompetent persons, they are at high risk for suffering severe complications [23]. In one study, immunocompromised transplant patients with AGE were more commonly infected with human norovirus than any other tested pathogen. Additionally these patients, when compared to those with non-norovirus AGE, had more severe complications, including ICU admission, renal dysfunction, and weight loss [52]. In terms of potential for transmission, infection can be persistent and viral shedding in stool can be prolonged in immunodeficient patients, with some shedding human norovirus in stool for up to 22 months, and even up to 8 years, when biopsy specimens among patients with duodenal villous atrophy were examined [22,53]. Furthermore, evidence from studies in chronically infected immunocompromised patients suggests that they may serve as potential reservoirs for human norovirus, and give rise to novel strains [54,55]. Among immunocompromised children, greater burden of human norovirus may also play a part in their serving as a potential reservoir, as in one study they were found to have higher viral load in stool specimens compared with immunocompetent counterparts with AGE, although this was not statistically significant [56].

Occupational groups at risk for transmitting human norovirus infection

Another important perspective to consider as a potential target for reducing the overall disease burden is those who are at risk for transmitting the infection. The low infectious dose

and hardiness of human norovirus on environmental surfaces allows for rapid spread of infection from just one infected person to potentially many others, and puts healthcare workers and food handlers at risk for transmission of infection [15,57]. Of the estimated 9.4 million episodes of foodborne illness attributable to known agents each year in the USA, the majority, 58%, are caused by human norovirus [5]. Infected food handlers have been implicated as the source of infections in 70% of food-borne norovirus outbreaks in the USA, the majority of whom did not use proper hand hygiene when preparing foods [15]. This analysis also noted that foodborne norovirus infections affect all ages and cause significant burden to the healthcare system, including clinic visits, emergency room visits, hospitalizations, and rarely, deaths. Thus, food handlers may be important targets for breaking the transmission cycle of human norovirus.

Healthcare environments are the most common settings for human norovirus outbreaks, in which direct person-to-person contact is the most common route of transmission [15,58,59]. Data collected over an 8-year period in Australia from more than 1600 human norovirus outbreaks reported to the Victoria Health Department demonstrated that the majority (62% of genogroup (G) I outbreaks and 91% of GII outbreaks) occurred in healthcare settings [60]. Similarly, 5 years of data from 13 European countries reporting human norovirus outbreaks found that 72% of the 6579 outbreaks with a reported setting occurred in a healthcare setting, be it a residential institution or hospital [61]. Findings from a Dutch study indicate that infected healthcare workers play a larger role in transmission during outbreaks, compared with asymptomatic patients, who also shed large amounts of virus in stool [62]. In this study, however, symptomatic patients were found to be more infectious than symptomatic healthcare workers. The authors report that this finding is likely related to enhanced awareness of personal protective hygiene measures among healthcare workers compared with patients. This highlights that healthcare workers can contribute to human norovirus transmission in healthcare settings, and while rigorous hand hygiene and surface decontamination can play a role in controlling outbreaks, preventing healthcare workers from becoming ill may also be an effective approach for interruption of nosocomial transmission.

Human norovirus biology & its implications for vaccine development

Noroviruses are small positive single-stranded 7.5–7.7 kb RNA viruses of the genus *Norovirus*, one of five genera within the *Caliciviridae* family. The first norovirus was discovered in 1972 by Kapikian *et al.* [63] after investigation of an outbreak in Norwalk, Ohio. Based on differences in the major capsid protein (VP1), seven norovirus genogroups have now been classified, of which viruses from GI, GII, and GIV infect humans [64]. GI contains nine genotypes while GII contains 22 [64]. The norovirus genome contains three open reading frames (ORF), with ORF1 encoding six nonstructural proteins, ORF2 encoding VP1, whose P2 subdomain accounts for most of the antigenicity of each virus, and ORF3 encoding the minor capsid VP2 [64,65]. When the VP1 capsid protein is expressed independently of other viral components *in vitro*, it assembles into an empty virus-like particle (VLP) that are structurally and antigenically identical to a native virus particle. These VLPs, which encode no genetic material, induce antibody responses when inoculated into humans and have subsequently become a major vehicle for norovirus vaccine

development [66]. Recently, norovirus was reported to be able to grow *in vitro* in a specific human B-cell line for which the presence of certain enteric bacteria seems important [67]. If replicated in other laboratories, this discovery will facilitate a range of advancements that could accelerate vaccine development efforts.

Noroviruses are genetically diverse; however, the overwhelming majority of human disease is a result of infection with strains of the GII.4 genotype [68,69]. Worldwide, GII.4 is responsible for both outbreaks [69–72] as well as sporadic cases in the community [2,12,73]. An analysis of 3616 human norovirus outbreaks reported to CaliciNet, a USA laboratory-based surveillance system for norovirus outbreaks, demonstrated that the majority of both foodborne and person-to-person outbreaks were caused by GII.4 strains [69]. Furthermore, this study showed long-term care facilities and the elderly were more frequently affected by GII.4 outbreaks. The GII viruses are more persistently shed in the stool, with GII viruses on average lasting 34.5 days compared with 8.5 days for GI virus excretion among a Peruvian birth cohort followed over 2 years [32]. GII viruses are also more often implicated in serious health outcomes. A systematic review of 843 outbreaks from around the world demonstrated that GII.4 strains were more likely associated with higher hospitalization and mortality rates, after controlling for other factors [39].

GII.4 viruses are not static, and undergo frequent genetic shifts. Over the past 20 years, new GII.4 variants have emerged every 2–4 years, generally replacing the predecessor as the predominant cause of outbreaks and endemic disease. These changes arise from mutation and recombination, with shifts in the capsid protein epitopes being a mechanism for immune evasion [74]. Furthermore, it has been postulated that protective herd immunity drives these changes [70,73,75,76] possibly through molecular evolution of the virus, causing antigenic variation resulting subsequently in the emergence of novel epidemic strains [77,78]. By definition, this dynamic suggests that natural immunity to norovirus infection does occur, which is a prerequisite for any vaccine development efforts. However, the level of cross-protection against different genotypes for inclusion of new emergent strains is unclear, suggesting that periodic vaccine reformulation may be required.

Non-GII.4 genotypes are also important in outbreak settings, although to a lesser degree. Various GI and other GII genotypes (including GI.3, GI.6, GI.7, GII.3, GII.6, and GII.12) were more often implicated in foodborne outbreaks in a 5-year study in the USA [69]. These GI.6 outbreaks showed unusual peak activity during summer months, and with less frequently reported clinical severity compared with GII.4 [79]. A study among US military recruits in Turkey in 2009 identified four rare genotypes within GII, two of which had been reported among troops deployed in Iraq, and none from the local Turkish population [80]. Most recently, a novel GII.17 variant has been identified in Jiangsu and Guangdong provinces in China, where it appears to have replaced GII.4_Sydney as the predominant human norovirus [81,82]. Taken together, these findings highlight the importance of vaccine coverage beyond GII.4 strains and suggest candidate vaccines include representative VLPs from at least both genogroups.

Immunity to human norovirus

Intrinsic susceptibility to human norovirus infection

Various host factors are also important for human norovirus infection. Histo-blood group antigens (HBGA), including ABO, secretor, and Lewis types have been shown to play an important role in susceptibility to infection [83–85]. The expression of HBGA in saliva, mucous membranes, and secretions is regulated by the a(1,2)-fucosyltransferase (FUT2) gene. Mutations in this gene may be associated with susceptibility for infection with human norovirus with strong evidence that individuals possessing a certain mutation in FUT2 are resistant to infection with human norovirus [86]. Those who possess a functional FUT2 gene, the so-called secretor-positive individuals, were found to have higher risk of human norovirus AGE in one study [87]. Another study found that both secretor-positive and secretor-negative individuals had infections with human norovirus, although genotypes differed among those with and without the functional gene, with GII.4 infections found among secretor-positive children and non-GII.4 infections more common in secretor-negative children [88]. While HBGA expression may play an important role in the binding of human norovirus and subsequent infection, factors unrelated to these antigens may also be at play as some evidence for human norovirus binding of intestinal epithelial cells without involvement of HBGA has been demonstrated [89]. Additional studies are needed to continue to clarify the role of HBGA, FUT2 gene expression, and other factors contributing to human norovirus interaction and subsequent infection of epithelial cells.

Antibody response to human norovirus infection

Serologic data from natural infection have shown that antibodies may be protective against infection with human norovirus, but these results are mixed in terms of strain-specificity and cross protection. Studies have shown elevated antibody responses after clinical infection among adults and children [90]. Antibody presence increases with age, as seen in one study among hospitalized patients with AGE, where levels of GII.4 IgG and IgA both rose with advancing age, with those aged less than 2 years having the lowest levels of these antibodies and adults having the highest [91]. Children with higher preexisting antibodies who experience subsequent norovirus infections have lower clinical severity; in one study of children from Finland, conducted over the course of 2 years, those with low human norovirus-specific IgG antibody titers had higher likelihood to acquire norovirus infection later on, compared with children with high titers [92]. One study assessed the presence of five genotype-specific antibodies (GII.4 US95/96, GII.4 New Orleans, GII.12, GI.1, GI.3) from acute human norovirus infection in children, as well as the blocking potential of these antibodies [93]. These researchers found that high titers of preexisting GII.4 New Orleans IgG protect against infection from that particular strain, but this antibody was not able to protect children from infection with other GII genotypes in circulation. Results from serological surveys of adults have likewise shown conflicting results with regards to the protective role of pre-existing antibodies to human norovirus. Although data from diverse geographic locations demonstrate high prevalence of human norovirus-specific antibodies [94] among adults and older studies among adults experimentally inoculated with human norovirus did not show consistent protection of preexisting antibodies [95], more recent studies do show protective effect of blocking antibodies [96]. In terms of the strain

specificity of the antibody response, a clear-cut picture has not emerged. Early studies demonstrated mixed cross-reactivity among GI and GII viruses in infected populations [97–99]. Subsequent data from both human and animal models continue to show this mixed response. These results suggest that persistence of high human norovirus antibody titers may be protective among children, although difference in exposure histories may complicate the situation for adults and cross-strain protection remains unclear. Given these issues, when considering vaccinating the elderly, prior exposure histories and age-related immune system decline may pose problems in mounting a robust response against human norovirus.

Mucosal responses, as measured by salivary and fecal antibodies, may also play a role in protection from norovirus infection. A recent placebo controlled study examined 57 patients, inoculated with GI.1 virus, who had salivary and fecal human norovirus-specific IgA measured pre- and post-inoculation [100]. This study found that pre-existing human norovirus-specific IgA salivary levels protected subjects from gastroenteritis, as these IgA levels were greater in infected subjects who did not develop gastroenteritis, compared with those who developed gastroenteritis. While fecal norovirus-specific IgA levels were not observed to confer this same protection in infected subjects, pre-existing fecal human norovirus-specific IgA levels were inversely correlated with peak viral load after infection, which may play a role in duration of viral shedding. Additional studies are needed on the mucosal response in the setting of vaccine development.

Duration of immunity

Duration of protection after human norovirus infection or vaccine administration remains to be determined. Data from an early oral challenge study with a GI.1 norovirus among 12 healthy adult males showed that initial infection caused clinical illness in half of the group; when re-challenged 27–42 months later, the same six subjects became clinically ill with the same agent [101]. A third challenge among four of those who were ill twice 4–8 weeks after the second challenge, however, yielded only one clinically ill subject. The authors concluded that short-(up to 8 weeks) and long-term (up to 34 weeks) immunity exists for this GI.1 virus. A later oral challenge study found up to 6 months protection without evidence for protective effect from initial infection of pre-inoculation serum antibodies [95].

Results from a modeling study designed to estimate the length of immunity conferred from human norovirus infection used a model that tracked six subject types: infection and disease, exposed but not symptomatic, infected with symptoms, infected without symptoms, immune to disease but not infection, and genetically resistant [33]. Models were fitted to gastroenteritis data available from the UK, and included a variety of scenarios of infectiousness and susceptibility of the population. These models predicted protection between 4.1 and 8.7 years, higher than has been shown in the observed oral challenge data. Although the models had good fit with observational data from the UK, the analysis was limited for two reasons. First, it assumed that individuals were immune to disease, rather than to infection and second, it assumed a single strain for human norovirus, with infection from one strain conferring protection against all others. Despite these limitations, if these models prove accurate, this would have a great impact on determination of vaccination schedule, as doses given less frequently may confer adequate protection. Further

observational studies are warranted to determine the duration of immunity, to inform duration of vaccine protection and need for re-formulation and re-administration.

Serologic response to vaccine administration

Similar to reports from natural infection, vaccine studies without oral challenge in animal and healthy adult volunteers have also demonstrated varying levels of antibody responses and cross-strain activity. Several studies have tested different doses of oral and intranasal preparations of GI.1 VLP vaccine and found both elevated IgG and IgA responses. While dose escalation demonstrates increase in serum IgG response [102], this response was found to level off at 250 mg of inoculum. In addition, responses to VLP vaccine were not as high as after norovirus infection [103]. In mice inoculated with two strains of murine norovirus (MNV-1/MNV-3), disparate levels of antibody response were seen, with MNV-3 inducing homotypic and cross-reactive protection, while MNV-1 showed only modest homotypic protection and no heterotypic protection [104]. An early multivalent (GI.1, GII.1, GII.3, GII.4) VLP study showed that, in humans administered GI and GII VLPs, homotypic sera from humans produced the largest serological response against VLPs, whereas heterotypic titers were lower. In addition, GI VLPs elicited a more robust heterotypic response compared with GII infections. This same study also examined mice and found the serological response was higher for homotypic VLPs, but heterotypic responses did occur, with higher within-genogroup cross-reactivity compared with across genogroups [105]. A trivalent (GI.1/GII.4/RV VP6) parenteral norovirus-rotavirus vaccine tested in mice also showed inter-genogroup cross-reactivity and protection to both norovirus and rotavirus, with a sustained serologic response up to 6 months after vaccine administration [106]. A recent Phase I trial of a bivalent adjuvant GI.1/GII.4 intramuscular vaccine given to adults up to 49 years old also showed a robust antibody-secreting cell response [107].

Recently, a Phase I clinical trial tested the serum antibody response to a multivalent (GI.1/GII.4c) norovirus VLP vaccine to determine titers of blockade antibody, indicating cross-strain induced immunity to both the vaccine components, as well to a variety of additional GI and GII VLPs not part of the vaccine formulation [108]. Among 10 adults receiving two doses of the VLP vaccine, researchers found elevated IgG antibody responses to both the GI.1/GII.4 vaccine (10.3 and 6.2 geometric mean fold rise [GMFR], respectively) as well as the non-vaccine VLPs (1.6–7.6 GMFR), peaking at 7 days post-vaccination. Although this showed a promising cross-protective response, as the vaccine elicited a response against various strains, the rise in the non-GII.4 GII VLPs did not increase greater than fourfold, which defined the seroresponse rate in the study. In another recent report, adults inoculated orally with GI.1 VLPs demonstrated an elevated blocking antibody serologic response to both the challenge antigen as well as to one or more heterologous GI antigens. In addition, a smaller number of volunteers developed blocking antibodies to one or more GII antigens, although these responses were less robust than the homotypic response [109]. Of note, while serologic studies indicate protective effect of serum antibodies in children, the vaccine studies cited here focused on healthy adults, highlighting a recurrent lack of available data on children, the elderly and the chronically ill, all of whom might be potential targets of a human norovirus vaccine.

Vaccine trials

Vaccine trials using homologous and heterologous challenge have assessed safety, immunogenicity and efficacy among healthy adult populations. A monovalent intranasal GI.1 VLP vaccine administered at two doses to healthy adults aged 18–50 years old was evaluated [110], and after receiving both doses, 70% of subjects demonstrated a serologic response, which was defined as a fourfold increase in serum antibodies; these were lower after vaccination than after challenge. The vaccine was efficacious against homologous challenge, with 69% of placebo recipients getting virus-associated gastroenteritis compared with 37% of vaccine recipients, yielding a vaccine efficacy of 47%. Vaccine recipients also had reduced relative frequency of GI.1 infection as measured by positive RT-PCR after viral challenge, with 82% of placebo recipients having infection compared with 61% of vaccine recipients. In addition, vaccine recipients had lower mean Vesikari scores, a widely used 20-point scale created to describe clinical severity of gastroenteritis [111], and a delayed onset of illness. The intranasal route of administration caused stuffy nose but otherwise was safe.

It will be crucial to develop a vaccine that offers heterologous protection against not only the GII.4 viruses but also the circulating GI viruses. Therefore, a multivalent vaccine should be considered to offer protection against noroviruses from both genogroups [105,112]. Multivalent vaccine challenge studies, which incorporate a GII.4 component, have also recently been conducted. A Phase I/II challenge study used a bivalent parenteral (intramuscular) GI.1/GII.4 VLP vaccine [113]. The GII.4 component included a consensus sequence based on the sequences of different GII.4 variant strains. Patients were selected with criteria including low pre-vaccine antibody levels to the GII.4 oral challenge strain and also the presence of salivary HBGAs that conferred susceptibility to norovirus infection. One hundred and nine patients were ultimately enrolled, randomized and took the oral challenge of norovirus, which although also a GII.4 virus, differed from the vaccine strains in the amino-acid sequence of the norovirus capsid protein. Serologic responses increased greatly in vaccinated patients compared with placebo, against both GI.1 and GII.4 strains, although the response was greater for GI.1, which had a 100% serologic response compared with 84% for GII.4. Although the authors did find protection against severe clinical symptoms, with reduction on a self-reported severity scale of vomiting and diarrhea as well as a reduction in the modified Vesikari score among vaccine recipients compared with placebo, the vaccine did not significantly protect against infection with human norovirus. Vaccine efficacy in this study was noted to be 13.6% for human norovirus infection, as defined by identification of human norovirus on PCR or antibody rise, and 22% for infection plus clinical illness. They did note that viral load was lower in vaccinated ill subjects compared with placebo, and that viral shedding was shorter, although not statistically significant, among vaccinated subjects. These results indicate protection against severe clinical disease rather than protection from human norovirus infection, as well as general safety of this parenteral formulation as the authors did not report any severe adverse events.

Further development & deployment of norovirus vaccines

Key areas remain to be clarified before informed decisions can be made regarding human norovirus vaccine use (Table 2). One such area is further understanding the epidemiology,

burden and viral genotypic diversity of human norovirus among potential target groups. A limitation of both the GI.1 VLP and GI.1/GII.4 VLP vaccine efficacy studies was the exclusion of the populations that are at highest risk for norovirus complications, as those studies were restricted to healthy adults. How will the elderly or children, or the immunocompromised respond to vaccine? Will prior exposure histories among the elderly necessitate a different vaccine for them, than for pediatric populations? Furthermore, what, if any, is the role of herd immunity in protecting older populations? Can vaccinating children against human norovirus also protect older populations, as noted for rotavirus vaccine? [114]. In terms of targeting strains and how often to vaccinate, although GII.4 strains are the most important for person–person and foodborne outbreaks, will the GII.4 variants targeted by a vaccine protect against future variants? The phenomenon of herd immunity and subsequent emergence of new strains has been discussed, and given the emergence of new GII.4 variant strains roughly every 2 to 4 years, would a new vaccine formulation need to occur as frequently? Should this be targeted by risk group as well? For example, in the military, would a consensus vaccine based on past outbreaks suffice, or would that leave out potential predominant strains in the community to which they will be deployed?

Another limitation, not only of the vaccine trials completed to date but in the literature overall, is the unknown duration of protection conferred by these vaccines; therefore, the question of how often to administer vaccine also remains to be clarified. Although data from modeling exercises show longer conferred protection, data from human studies with oral challenge have not yet demonstrated long-term protection. A short-term effective vaccine may be appropriate for some populations, such as travelers, or pre-deployment for the military, but for the populations at highest risk for complications, notably children, the elderly, and the immunocompromised, this may not be practical.

Another outstanding issue to address is that of cost–effectiveness. The current costs associated with human norovirus infection are burdensome and a vaccine, once developed and incorporated into the vaccine schedule, is likely to be cost-saving for children less than 5 years old. A recent study estimated that, in the USA, a vaccine with efficacy of 50% that provided 12 months of protection, and was administered to those less than 5 years old, would avert 1–2.2 million cases of human norovirus AGE annually, but would incur costs. Savings would start to occur only if the vaccine conferred at least 48 months of protection and cost US$25 [115]. A study from Peru similarly showed that introducing a vaccine that would decrease the incidence of human norovirus diarrhea by 47% among children would avert over 900,000 cases of AGE in high-incidence settings, such as the rural jungle, but overall cost-savings were less certain [116]. Vaccination could decrease clinical and hospital burden, particularly among the children and elderly, and these populations could potentially regain QALYs that were otherwise lost, but additional studies in diverse settings are needed to determine cost–effectiveness of a potential vaccine.

Finally, there are practical issues to consider when incorporating a new vaccine. The challenges of incorporating a new vaccine into the current schedule differ by risk profile. For children, a new human norovirus vaccine could be folded into the schedule that already exists and thus not introduce an extra visit, particularly if testing of a norovirus-rotavirus multivalent vaccine study shows favorable co-administration and lack of interference with

all other current vaccines in use. On the other hand, as there are already 10 vaccines recommended in the childhood schedule, including up to 26 doses administered (but not including the repeat vaccinations recommended for yearly influenza) [117], there may be resistance to inclusion of another vaccine in an already full immunization schedule. In addition, given the recent resurgence of measles in the USA related to willingly unvaccinated children, a special educational campaign may need to be incorporated before suggesting yet another childhood vaccine to parents. For the elderly, uptake of other vaccines (such as zoster) has already proven challenging and introducing a new vaccine may also prove difficult to incorporate [118], although for those who are nursing home-bound, logistics may be more favorable. The military has generally had early adoption of vaccines and if human norovirus vaccine became written into policy, this may be easier to incorporate. Food handlers also remain a challenging group, as high turnover may not lend itself to adequate coverage or cost–effectiveness. Healthcare workers also represent a challenging occupational group, despite being at higher risk of exposure and also higher risk of transmitting infection to others. Looking at examples from influenza vaccine uptake in elderly care units in the UK, most of the healthcare workers declined annual influenza vaccine, citing that they were healthy and did not require vaccine [119]. This indicates that an intensive educational campaign would likely have to precede any vaccination campaign.

Conclusions

Human norovirus is ubiquitous and infection affects most of the population, although some are at higher risk of disease and its complications and others for transmission. Given the differences in these populations, once a human norovirus vaccine is developed, to be maximally effective and cost-effective, the risk profile will need to guide vaccine administration, with special consideration given to populations that are at highest risk of infection and subsequent complications, as well as to those capable of transmitting infection to others. Current vaccines under development have been tested in healthy adult populations with only modest protection from clinical infection and disease demonstrated. Data from other studies have shown potential for cross-protective activity of multivalent vaccines. While results from healthy adult populations may be applicable to military recruits, travelers, food handlers, and healthcare workers, these results cannot readily be extrapolated to children, the elderly, and anyone with a complicated medical history. Further studies will need to clarify vaccine safety and efficacy in these latter populations. Any vaccine developed will have to elicit a high enough titer of antibodies to confer protection, will need to incorporate several strains, and will require followup studies to determine duration of protection and the potential need and timing for re-vaccination.

Expert commentary & five-year view

Human norovirus continues to be a significant clinical and economic burden in the USA and abroad. VLP vaccines are promising interventions and during the next 5 years, further studies will continue to address the duration of immunity, cross-protection and safety among target populations. These target populations can be divided into those that are at risk of disease and those at risk of propagating disease, although some populations are at risk for

both. Further studies will help determine optimal characteristics to protect and prevent disease transmission among both of these groups.

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Key issues

- Human norovirus remains a significant clinical and economic burden globally.
- Several groups should be considered as potential targets for vaccination: children, the elderly, the immunocompromised, travelers, the military, food handlers and healthcare workers.
- Some groups at high risk for infection and clinical complications, some groups are at risk for transmission to others, and some are at risk for both.
- Data from current clinical trials of VLP-based vaccines show some promise with prevention of severe gastroenteritis among healthy subjects but similar data from some target populations, including children, the elderly, and the immunocompromised, are not available.
- Although GII.4 is most often implicated in endemic and outbreak settings, the role of non-GII.4 genotypes, and cross-protection between genotypes, need to be clarified among target populations.
- Observation and modeling studies have given estimates of duration of immunity to human norovirus ranging from 6 months to 8.7 years but further research is warranted to determine this among target groups.
- Refined estimates of cost–effectiveness based on actual vaccine performance are needed.
- Optimal timing and incorporation of a human norovirus vaccine into the current immunization schedule remains to be clarified.

Table 1

High-risk populations susceptible to and at risk for transmitting norovirus and characteristics that justify their prioritization as potential vaccine targets.

Target population	High disease burden	High transmission risk to other groups	Characteristics
Young children	Yes	Yes	• Highest overall norovirus incidence rate and norovirus-associated hospitalization rate • Norovirus is leading cause of pediatric acute gastroenteritis requiring medical attention in countries using rotavirus vaccines • Highest R_0 of any age group, suggesting high transmission potential
Elderly and nursing home residents	Yes	No	• Greatest burden of fatal disease • Nursing homes have higher rates of deaths during norovirus outbreak periods • Higher hospital charges per case compared with children
Travelers	Yes	No	• 9–16% of traveler's diarrhea attributable to norovirus • Numerous leisure settings implicated
Military	Yes	No	• Norovirus common cause of acute gastroenteritis in deployed troops • Lost duty time, decreased reserve readiness as a result of disease
Immunocompromised patients	Yes	Yes	• Suffer severe clinical complications • Persistent viral shedding, up to months or years • Potential reservoir for new strain emergence
Healthcare workers	No	Yes	• Most common setting for norovirus outbreaks • Infected healthcare workers can propagate infection to vulnerable patient populations
Food handlers	No	Yes	• Most foodborne illnesses in US with identified agent are caused by norovirus • Implicated as infectious source in majority of foodborne norovirus outbreaks in USA • Poor compliance with hand hygiene and exclusion while ill

Table 2

Current knowledge and remaining gaps for development of a norovirus vaccine program.

Study (year)		Current knowledge	Remaining gaps	Ref.
Vega *et al.* (2014); Debbink *et al.* (2013); Leshem *et al.* (2013); Fu *et al.* (2015); Lu *et al.* (2015)	Strains to target	GII.4 most often implicated in both outbreaks and endemic disease	Role of non-GII.4 genotypes, particularly in specific target populations	[69,71,79,81,82]
Atmar *et al.* (2011); Bernstein *et al.* (2014)	Vaccine efficacy	Some demonstrated protection against disease and decreased clinical severity in healthy adults	Other age and risk groups; Cross-reactivity and heterologous protection	[110,113]
Parrino *et al.* (1977); Johnson *et al.* (1990); Simmons *et al.* (2013)	Duration of immunity	Observational studies: up to 6 months; mathematical modeling: up to 8.7 years	Further data from observational studies; Improved models to account for strain heterogeneity	[101,95,33]
Lopman *et al.* (2011); Batz *et al.* (2012); Bartsch *et al.* (2012); Mirelman *et al.* (2015)	Cost-effectiveness	Hospitalizations cost US$493 million per year; foodborne infections cost US$ 2 billion; vaccine estimated to be cost saving in certain scenarios	Refined estimates based on actual vaccine performance	[6,9,115,116]
Lu *et al.* (2009); O'Reilly *et al.* (2005)	Incorporation into immunization schedule	Crowded schedule for children; poor coverage with other vaccines in the elderly	Need and timing for booster doses; interference with other vaccines; potential acceptance among target groups	[118,119]