HHS Public Access

Author manuscript

Expert Rev Vaccines. Author manuscript; available in PMC 2019 September 01.

Published in final edited form as:

Expert Rev Vaccines. 2018 September ; 17(9): 773–784. doi:10.1080/14760584.2018.1510327.

Progress on norovirus vaccine research: public health considerations and future directions

Claire P. Mattison[a,b], **Cristina V. Cardemil**[b], and **Aron J. Hall**[b]

[a]Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA

[b]Division of Viral Diseases, Viral Gastroenteritis Branch, Centers for Disease Control and Prevention, Atlanta, USA

Abstract

Introduction: Noroviruses are the leading cause of foodborne illness worldwide, account for approximately one-fifth of acute gastroenteritis (AGE) cases globally, and cause a substantial economic burden. Candidate norovirus vaccines are in development, but there is currently no licensed vaccine.

Areas covered: Noroviruses cause approximately 684 million cases and 212,000 deaths per year across all age groups, though burden estimates vary by study and region. Challenges to vaccine research include substantial and rapidly evolving genetic diversity, short-term and homotypic immunity to infection, and the absence of a single, well-established correlate of protection. Nonetheless, several norovirus vaccine candidates are currently in development, utilizing virus-like particles (VLPs), P particles, and recombinant adenoviruses. Of these, a bivalent GI.1/GII.4 VLP-based intramuscular vaccine (Phase IIb) and GI.1 oral vaccine (Phase I) are in clinical trials.

Expert Commentary: A norovirus vaccine should target high-risk populations, including the young and the elderly, and protect them against the most common circulating norovirus strains. A norovirus vaccine would be a powerful tool in the prevention and control of norovirus while lessening the burden of AGE worldwide. However, more robust burden and cost estimates are needed to justify investments in and guide norovirus vaccine development.

Keywords

Norovirus; acute gastroenteritis; burden of disease; virus-like particles; vaccine development

CONTACT Claire P. Mattison, lkz5@cdc.gov, Oak Ridge Institute for Science and Education, 1299 Bethel Valley Rd, Oak Ridge, TN 37830.

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. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Names of specific vendors, manufacturers, or products are for identification only and do not imply endorsement by the Centers for Disease Control and Prevention or the US Department of Health and Human Services.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

1. Introduction

Since the first identification of norovirus in stool samples from an acute gastroenteritis (AGE) outbreak among children and staff at an elementary school in Norwalk, Ohio in 1968 [1], the clinical characteristics of norovirus illness have been well described and include nausea, abdominal pain, vomiting, and diarrhea. On average, each person in the United States will experience five episodes of norovirus gastroenteritis in his or her lifetime [2]. Most norovirus illnesses are mild to moderate, but severe illness, including death, can occur in vulnerable populations such as the elderly, the immunocompromised, and children under 5 years of age [3].

The genus *Norovirus* includes a genetically and antigenically diverse group of viruses within the family *Caliciviridae* (i.e. caliciviruses) and consist of at least seven genogroups, three of which – GI, GII, and GIV – infect humans [4]. Genotype GII.4 causes the majority of norovirus outbreaks worldwide, and until 2012 new GII.4 variants emerged every 2–4 years [5,6]. The norovirus genome has three open reading frames (ORFs) of which ORF2 and ORF3 encode the major capsid protein (VP1) that determines the antigenicity of the virus, as well as the minor capsid protein (VP2). ORF1 encodes a large polyprotein that is cleaved by the viral protease in mature non-structural proteins, including the RNA-dependent RNA polymerase [7]. To date, all norovirus vaccine candidates contain noninfectious recombinant VP1 proteins, either as virus-like particles (VLP), as P-particles, or as recombinant adenoviruses.

The burden of disease for norovirus is substantial, affecting all age groups. In the United States, sporadic and outbreak-related cases of norovirus cause approximately 20 million illnesses annually, and worldwide, an estimated 684 million illnesses and 212,000 deaths occur every year due to norovirus [2,8]. With the dramatic decline of rotavirus gastroenteritis after the introduction of rotavirus vaccines in the mid-2000s, norovirus has become the leading cause of severe pediatric AGE in countries that have introduced the rotavirus vaccine [9–12]. However, global, regional, and national estimates of the prevalence and incidence of norovirus have varied by year, setting, and population. This has made it challenging to precisely quantify the economic and social impacts of the disease and thus to justify large investments in norovirus vaccine development.

In June 2016, the World Health Organization's (WHO) Product Development for Vaccines Advisory Committee identified norovirus as a priority disease for vaccine development [13]. While several candidate vaccines have been developed, including those in Phase I and Phase II trials and many others in preclinical stages [14, 15], there is currently no licensed norovirus vaccine. In this review, we summarize the latest estimates of norovirus disease burden, describe norovirus vaccines in development, both preclinical and clinical, and identify challenges facing a norovirus vaccine.

2. Norovirus burden estimates

Norovirus illnesses are ubiquitous and costly. Currently, the only global norovirus illness estimates are by the WHO Foodborne Disease Burden Epidemiology Reference Group

(FERG). They estimated that norovirus caused 684 million (95% uncertainty interval [UI] 491–1,100 million) illnesses in 2010 and was the leading cause of foodborne illness worldwide [8]. Low- and middle-income countries (LMICs) account for 82% of norovirus illnesses and 97% of norovirus deaths worldwide [16]. In the United States, it is estimated that norovirus causes an average of 570–800 deaths, 1.7–1.9 million outpatient visits, and 19–21 million illnesses every year [2]. Globally, norovirus accounts for an estimated $4.2 billion in direct health-care costs and an additional $56.2 billion in lost productivity annually [16]. Norovirus disease, in children under 5 years of age, costs society $39.8 billion, nearly twice the cost for all other age groups combined [16].

Sections 2.1–2.4 will discuss the various burden estimates that exist for norovirus worldwide, as well as potential reasons for the variability between them. These burden estimates generally include both sporadic illnesses and those occurring as part of recognized outbreaks.

2.1. Deaths due to norovirus globally

Global estimates of deaths due to enteric pathogens including norovirus have been published by WHO FERG, the WHO Child Health Epidemiology Reference Group (CHERG), and the Global Burden of Disease (GBD) studies in 2013 & 2015; the 2013 GBD study data were updated and re-analyzed to produce new estimates in 2015 (Table 1) [8,17–19]. Estimates of the number of deaths due to norovirus in all age groups from the GBD 2015 and WHO/FERG 2015 studies ranged from 29,700 (95% UI 4,800–67,600) to 212,489 (95% UI 160,595–278,420), respectively [8, 18]. Norovirus deaths in children under 5 years of age ranged from 8,992 (95% confidence interval [CI] 4,251–19,347) to 71,000 (uncertainty range [UR] 39,000–113,000) [8, 18, 19]. The variability in these estimates is likely due to different methodologies used and the data available. In Table 1, the estimated number of norovirus deaths in those <5 years of age from the WHO Foodborne Disease Burden Epidemiology Reference Group (FERG) study [8] refers to foodborne norovirus deaths only. The 2013 and 2015 GBD analyses used similar methods; however, using updated data, including the results of the reanalysis of the global enteric multicenter study (GEMS), GBD 2015 estimated more than 10 times the number of deaths due to norovirus as compared to the previous 2013 estimate (Table 1) [17, 18]. This wide variability between estimates creates uncertainty around the true number of norovirus deaths globally.

2.2. Systematic reviews on the global and regional burden of norovirus

Globally, norovirus is responsible for approximately one-fifth of AGE cases. A systematic review in 2014 found that 18% (95% CI 17–20%) of AGE cases worldwide were attributable to norovirus, with lower percentages attributable in high-mortality developing countries (14%, 95% CI 11–16%) than in low-mortality developing countries (19%, 95% CI 16–22%) or developed countries (20%, 95% CI 17–22%) [21]. A separate systematic review in 2016 estimated a similar norovirus prevalence among AGE cases in upper-middle–, lower-middle–, and low-income countries (17%, 95% CI 15–18%) [22]. Neither of these meta-analyses found significant differences in norovirus prevalence by the age group.

Region-specific systematic reviews have also provided estimates of norovirus burden. Studies in African countries have focused on children less than 18 years of age and reported a median norovirus prevalence of 11 −14% among AGE cases (range 0.8–25% by country) and 10% among asymptomatic controls [23,24]. Children under 2 years old had higher norovirus prevalence (18%) than children up to 5 years old (12%) [23]. A review of 38 studies from 15 countries in the Middle East and Northern Africa (MENA) region from 2000 to 2015 reported a median norovirus prevalence of 15% among AGE patients (range 0.8–37% by country) [25]. A systematic review in Latin America found an overall pooled prevalence of 15% (95% CI 13%−18%) among AGE cases [26]. Of the 29 studies reviewed, 28 focused on children less than 15 years old, with most focusing on children under 5 years of age [26]. These region-specific systematic reviews clearly show how norovirus represents a substantial portion of the AGE burden in Africa, the Middle East, and Latin America.

Other systematic reviews also show a substantial norovirus burden. A 2015 literature review in the European Union (EU) found that norovirus causes 5.7 million illnesses and 102 deaths in children under 5 years old annually [27]. A systematic review in China estimated that norovirus caused approximately 20% of AGE with higher percentages attributable to norovirus in children 6–23 months old (22.6%, 95% CI 19.1 −26.0%) and adults > 40 years old (32.4%, 95% CI 27.5–37.3%) [28]. A review of 39 studies in high- and upper-middle–income countries located in Europe, North America, Asia, the Middle East, and Australia found adults > 65 years old were at higher risk for hospitalizations and severe illness, and 10–15% of AGE deaths in this population were due to norovirus [29].

2.3. Estimates from multisite epidemiological studies

Large multisite studies have reported differences in the relative burden of norovirus as an AGE pathogen. The GEMS, a 3-year case-control study from 2007–2011 of 9,439 children in seven healthcare sites across Africa and Asia, originally found that norovirus GII was significantly associated with moderate to severe diarrhea at only one site. In The Gambia, norovirus GII had a significant attributable fraction (AF) in children 0–11 months (8.9%, 95% CI 4.3–13.4), 12–23 months (8.7%, 95% CI 5.2–12.1), and 24–59 months (9.4%, 95% CI 2.6–16.2) [30]. However, a reanalysis of stool samples from GEMS several years later using quantitative real-time PCR (qPCR) found that norovirus GII was associated with moderate-to-severe diarrhea at all seven sites, with attributable fractions ranging from 0.4 to 4.4% [31]. The Malnutrition and Enteric Disease Study (MAL-ED), a prospective cohort of 1,457 children across eight countries in Asia, Africa, and South America, found that 89% of children experienced at least one norovirus infection prior to 24 months of age; this study also identified norovirus in 22.7% of diarrheal stools and calculated an overall attributable fraction of 5.1% (95% CI 1.2–8.3) [32]. Across all MAL-ED sites, norovirus incidence ranged from 3 to 18 cases per 100 child-months. Similar to the MAL-ED study, a 2013–2014 study leveraging the global rotavirus surveillance network in 16 countries identified norovirus GII as the second most common cause of acute watery diarrhea in children under 5 years of age with an attributable fraction of 6.2% (95% CI 2.8–9.2%) [9].

2.4. Variability in burden estimates

The variability in results between burden studies may be explained by differences in setting, case definitions, and methodology. GEMS was a hospital-based case-control study focusing on children with moderate to severe diarrhea, including cases with bloody diarrhea. While the rotavirus surveillance network study was also hospital-based, it excluded children with bloody diarrhea. MAL-ED was a household-based prospective cohort study and therefore was designed to capture milder cases of AGE, including those who did not seek health care. Prospective, community-based, longitudinal birth cohorts can provide a comprehensive look at the burden of AGE, particularly for a viral pathogen such as norovirus, but are costly and time intensive [33–35].

The inclusion or exclusion of vomiting-only illness in the case definition of AGE can impact burden estimates. In a norovirus challenge study, half of the infected subjects who experienced vomiting did not also have concurrent diarrhea [36]. However, many studies do not include vomiting-only illness in their case definition of AGE. In the Ahmed et al.'s (2014) systematic review, only 32 of the 175 papers reviewed included vomiting-only illness in their definition of AGE [21]; this could lead to an underestimation of the true norovirus burden.

The prevalence of asymptomatic infections and post-symptomatic viral shedding also need to be considered when estimating norovirus burden. Nearly one-third of norovirus infections are asymptomatic and otherwise healthy individuals can shed norovirus for weeks after symptoms have resolved [37]. If asymptomatic and post-symptomatic shedding is not quantified within burden estimates, studies may overestimate the true amount of infectious norovirus. Conversely, if control groups do not appropriately exclude individuals experiencing post-symptomatic shedding, this may underestimate the true burden. Furthermore, detection of norovirus in a high proportion of asymptomatic controls does not necessarily diminish the etiologic role of norovirus when detected among AGE cases in the same setting, particularly in situations with a force of infection [38]. These factors are all important to address in surveillance and burden studies to most accurately estimate the burden of norovirus.

Despite the small number of and variability between studies estimating norovirus burden, especially more severe outcomes such as deaths, it is well established that norovirus is a leading cause of AGE worldwide. Sections 3–5 discuss the genetic diversity and immunity of noroviruses, which are important considerations for vaccine development.

3. Genotype diversity

Although GI, GII, and GIV norovirus can infect humans, GII is the predominant norovirus genogroup circulating worldwide. To track the genetic diversity of norovirus worldwide, the NoroNet network collects genetic sequences from human norovirus specimens from primarily outbreaks in 19 European countries as well as some countries in Asia, Oceania, and Africa. In an analysis of NoroNet data from 2005 to 2016, 91.7% of sequences were GII, 8.2% were GI, and <0.1% were GIV [39]. In the United States, from 2009 to 2013, 89% of norovirus outbreaks were caused by GII and 11% were caused by GI norovirus [40]. GII.

4 is the most common genotype in many parts of the world, and over the past two decades, a new GII.4 strain has emerged every 2–4 years, sometimes associated with an increased norovirus activity [5,6]. In the United States from 2009 to 2013, GII.4 was the most prominent genotype, causing 72% of norovirus outbreaks; other common genotypes included GII.12, GII.1, and GI.6, which together caused 13% of outbreaks [40].

Since an increasing number of emerging strains appear to be recombinant strains, in recent years, norovirus genotyping has expanded to also include polymerase typing, allowing for more specificity and granularity when tracking genetic shifts [41]. From 1 September 2017 to 31 March 2018, 50% of norovirus outbreaks reported to CaliciNet, a national surveillance network of federal, state, and local public health laboratories in the United States, were typed as GII.P16-GII.4 Sydney. Other common genotypes included GII.P16-GII.2, GII.P4 New Orleans-GII.4 Sydney, GII.Pe-GII.4 Sydney, GII.P12-GII.3, and GI. P6-GI.6 [42]. From 2005 to 2016, NoroNet identified 22 different recombinant genomes, including GII.P16-GII.4 Sydney, which has been identified in Europe and Asia, and GII.P17-GII.17, which was first reported in Asia in 2014 before circulating in Europe during the 2015–2016 season [39]. Systematic, ongoing surveillance to monitor the diversity of strains in different settings and populations is essential for norovirus vaccine development and implementation, as the formulation may need to be updated with the emergence of new strains. Likewise, evaluation of the potential impacts of norovirus vaccines will need to consider strain specificity.

4. Immunity

Norovirus immunity is complex. Natural susceptibility to norovirus can vary between individuals and genotypes; the duration and degree of cross-protection of acquired immunity is not well understood; and there is no single, well-established correlate of protection for norovirus infection or illness that can be used in vaccine development.

Susceptibility to norovirus infection can vary based on an individual's fucosyltransferase-2 (FUT2) gene. This gene regulates the expression of histo-blood group antigens (HBGAs), which serve as infection binding ligand on cells needed for infection [43]. Individuals with a functional *FUT2* gene ('secretors') secrete HBGAs in their body fluid and express them on the epithelial cells in their gut and are more susceptible to norovirus GII.4 infection than those who are homozygous recessive for *FUT2* ('nonsecretors') [44–47]. However, nonsecretors can still become infected with non-GII.4 norovirus strains, and being a nonsecretor only lowers, but does not eliminate, the risk of GII.4 norovirus infection [34,47]. Because of this genetic difference in susceptibility to norovirus infection and illness, it is important for vaccine trials and challenge studies to take into consideration individuals' secretor status in their design; ultimately this will aid in better understanding of the impact of secretor status on vaccine efficacy.

Natural immunity to norovirus post-infection is not well understood. Challenge studies have demonstrated short-term, strain-specific immunity, ranging between 6 months and 2 years [48–50]. More recent modeling studies have estimated that norovirus immunity lasts anywhere from 4 to 8 years post-infection [51]. A birth cohort in Peru found that children

often have repeat infections by the same genogroup, but repeat infections by the same genotype are rare, suggesting that acquired immunity is genotype specific [33].

There is no single, well-established correlate of protection for norovirus. Many candidates have been explored, including serum HBGA-blocking antibodies, serum hemagglutination inhibition antibodies, salivary, serum, and fecal immunoglobulin (Ig) A, and virus-specific IgG memory B-cells [52,53]. Many studies have focused on serum HBGA-blocking antibodies, which prevent norovirus from binding to HBGAs and subsequently prevent infection. In a challenge study, titers of HBGA-blocking antibodies were higher in individuals who did not develop AGE than those who developed symptoms [52]. In a separate norovirus challenge study, pre-challenge serum anti-GII.4 HBGA-blocking and IgA antibody levels were associated with lower rates of GII.4 infection and illness [54]. A study examining children hospitalized for norovirus found that high genotype-specific serum IgG titers and blocking antibodies correlated with protection from norovirus infection; however, this protection was genotype specific [55].

5. Vaccine development

Several norovirus vaccines using a variety of different technologies are in development, including two in clinical trials (Figure 1). These technologies include nonreplicating virus like particles (VLPs), P particles, and recombinant adenoviruses. VLPs are multi-protein structures that resemble the organization and morphology of the native virus, but contain no genetic material and are therefore noninfectious. VLPs are not only typically produced by recombinant baculovirus, but also can be produced by *E. coli, Pichia pastoris* (yeast), and plants, which, when optimized, may lower the cost of VLP production [56–59]. P particles are developed to resemble the P domain of norovirus, which is the part of the virus that binds to HBGAs [60,61]. They also can be mass-produced in laboratory settings using *E. coli* to potentially lower vaccine cost [61]. Vaccines using recombinant adenovirus expressing the norovirus major capsid protein VP1 are also being developed. Other common vaccine technologies, including killed or live-attenuated viruses, have not been pursued for norovirus due to lack of a culture system. Recently, a human intestinal enteroid culture system was described which supports human norovirus replication *in vitro* [62]. This new development opens up new avenues of norovirus research including measuring cross-reactive neutralizing antibody responses, which are required for a successful norovirus vaccine.

An important consideration for a norovirus vaccine is determining which genotypes to include in the formulation. GII.4 viruses have been the predominant strains over the past 15 years; chimeric consensus GII.4 VLPs have been designed and shown to produce a blocking immune response against homotypic and heterotypic GII.4 strains in mice [63] and are being used in the bivalent GI.1/GII.4 vaccine currently in Phase IIb clinical trials. Other vaccines in the pipeline have initially focused on GI.1 norovirus, primarily because the availability of an approved GI.1 challenge strain. However, every norovirus vaccine formulation will need to include both a GI and a GII component to provide cross protection against the more prevalent GII strains.

Section 6-7 will detail the various vaccines in development, focusing first on those that are moving forward in human clinical trials (Section 6) and then summarizing vaccines in the pre-clinical stages of research (Section 7). Trials of these various vaccine candidates have used different correlates of protection, including serum HBGA-blocking antibodies, norovirus-specific IgA antibodies, and norovirus-specific IgG memory B-cells [64–68]. Other salient differences between the candidate vaccines and their respective trial results are summarized below.

6. Vaccines in human clinical trials

6.1. Bivalent GI.1/GII.4 vaccine

The vaccine furthest along in clinical trials is a bivalent, intramuscular GI.1/GII.4 VLP vaccine developed by Takeda Pharmaceutical Company Limited, currently in Phase IIb trials [69]. This vaccine was initially developed as a monovalent GI.1 VLP intranasal vaccine before being reformulated as a bivalent GI.1/GII.4 VLP intramuscular vaccine. In Phase I trials of the GI.1 formulation, two vaccine doses 21 days apart induced 4.8- and 9.1-fold increases in norovirus-specific IgG and IgA antibodies, respectively, and significantly increased norovirus-specific IgG and IgA memory B-cells in 92–100% of participants with no serious adverse reactions [64,65]. Among participants challenged with a homologous GI.1 strain, the GI.1 VLP vaccine resulted in a 47% reduction in norovirus illness and a 26% reduction in norovirus infection [70]. The reformulated bivalent vaccine containing a chimeric GII.4 VLP and a GI.1 VLP induced broadly reactive antibodies to heterologous GI.1, GII.1, GII.3, and GIV.1 noroviruses in rabbits [71]. Further, Phase I and Phase II trials have focused on this intramuscular, bivalent GI.1/GII.4 VLP vaccine.

Phase II studies of the GI.1/GII.4 vaccine have demonstrated a rapid immune response 28 days post-vaccination, including higher pan-Ig, IgA, and HBGA-blocking antibodies against GII.4 and GI.1 VLPs when compared to baseline [66]. Longer studies have shown antigen-specific IgG memory B-cells persist until at least 180 days post-vaccination, similar to the memory B-cell response following experimental GI.1 infection [67]. Among adults aged 18–64 years, post-vaccination increases in pan-Ig, IgA, and HBGA blocking antibodies persisted above baseline through day 393 [72]. A study that challenged participants with GII.4 norovirus following two intramuscular injections of the bivalent vaccine yielded a similar point estimate for illness reduction, but failed to reach statistical significance due primarily to a low overall attack rate. Nonetheless, this challenge study demonstrated a significant reduction in illness severity; among vaccinated individuals, the average modified Vesikari score was 4.5 (compared to 7.3 among placebo recipients) and there were no reports of severe disease. Furthermore, a significant reduction in mean viral shedding 4 days after experimental infection was observed in vaccinated individuals; by day ten, only 22.4% of vaccinated individuals were still shedding virus as compared to 36.2% of unvaccinated individuals [73]. The formulation identified as a candidate for further studies includes 15 μg GI.1 VLP/50 μ g GII.4c VLP with aluminum hydroxide adjuvant, due to the immune response as measured by pan-Ig and HBGA-blocking antibodies [72]. None of the aforementioned studies reported any severe adverse effects related to vaccination.

Current trials of the bivalent vaccine include Phase II trials in infants and children (6 weeks through 8 years of age) and in elderly (those older than 60 years) populations to evaluate safety and immune responses (NCT02153112, NCT02661490). Additionally, a Phase IIb field efficacy study in military recruits is currently active (NCT02669121) and a 5-year Phase II trial in adults and the elderly to measure the length of antibody response is ongoing (NCT03039790).

6.2. Monovalent GI.1 oral vaccine

The second norovirus vaccine currently in human clinical trials employs a recombinant adenovirus expressing the norovirus GI.1 major capsid protein (VP1) in an oral tablet formulation developed by Vaxart, Inc [74]. Vaxart has demonstrated the safety and effectiveness of this oral pill technology in Phase I and Phase II trials of an oral influenza vaccine being developed [75,76]. The most significant advantage of an oral pill vaccine is that it is thermostable at ambient temperatures for up to 1 year and therefore would not require the maintenance of a cold chain for vaccine distribution [77].

Two Phase I trials on the GI.1 oral norovirus vaccine have been completed. A Phase I trial testing the safety and immunogenicity of both a low- and high-dose vaccine (NCT02868073) found the vaccine was well tolerated and reported no serious adverse events. After one administration of the high-dose vaccine (1×10^{11} IU), 78% of participants showed a two-fold increase in HBGA-blocking assays (BT50s), a significant increase over the placebo group. Vaccinated participants also showed other immune responses including increased IgA and IgG memory B-cells, and increased fecal IgA [78]. A Phase Ib dose-optimization trial (NCT03125473) was also completed in 2017, but results have not yet been published. Vaxart has reported that the vaccine was well tolerated and increased norovirus antibody titers, antigen-specific IgG and IgA responses, and memory IgA cells for up to 30 days after immunization [68,79]. No serious adverse events were reported [79]. Going forward, Vaxart reportedly plans to conduct GII.4 vaccine trials, Phase II challenge studies, and bivalent GI.1/GII.4 vaccine trials [80].

7. Vaccines in pre-clinical development

There are several norovirus vaccines in pre-clinical trials (Figure 1). Many of these vaccines are being developed as combination vaccines to protect against not only norovirus, but also other viral infections, such as rotavirus, enterovirus 71, hepatitis E, or astrovirus.

7.1. Trivalent VP6 vaccine

A trivalent norovirus GI.3, GII.4, and rotavirus vaccine currently in pre-clinical trials is being developed by the University of Tampere, Finland, the Daiichi Sankyo Company Limited, & UMN Pharma Inc., Japan [81]. The vaccine was originally formulated as a bivalent norovirus GII.4 and rotavirus VP6 protein vaccine [82]. Later, a trial in mice found a lack of a cross-protective immune response between norovirus GI and GII in monovalent vaccines and concluded that a formulation with norovirus GI.3 and GII.4 VLPs was necessary to protect against the most common pediatric norovirus strains [83]. Future research has focused on the trivalent norovirus GI.3 & GII.4, and rotavirus VP6 formulation

delivered intramuscularly. When vaccinated with the trivalent vaccine, mice showed an increase in antigen-specific IgG and type-specific blocking antibodies that persisted for 24 weeks post-vaccination [84]. Also, the addition of rotavirus VP6 protein showed an adjuvant effect, increasing cross-reactive IgG antibodies and norovirus-specific blocking antibodies, even at low levels of norovirus VLPs [85].

7.2. Bivalent GII.4 and enterovirus 71 vaccine

A VLP-based bivalent vaccine against norovirus GII.4 and enterovirus 71 (one of the viruses that causes hand, foot, and mouth disease) has been tested in mice by the Chinese Academy of Sciences in Shanghai. This combination vaccine produced significant increases in enterovirus 71 and norovirus GII.4-specific antibody responses up to 14 weeks after vaccination. These increases were comparable to those in mice vaccinated with a monovalent VLP against one of the two diseases [86], demonstrating noninterference among the VLPs.

7.3. Plant-based GII.4 VLP norovirus vaccine

Arizona State University has focused on developing a plant-based GII.4 norovirus VLP vaccine [59]. In a randomized control trial in 2000, an oral GII.4 VLP norovirus vaccine produced using transgenic potatoes generated an increase in IgA antibody-secreting cells in 95% of participants, with 20% of volunteers developing antigen-specific serum IgG and 30% of participants developing antigen-specific stool IgA [87]. Later development has focused on producing norovirus VLPs in tobacco (*Nicotiana benthamiana*) plants for use in intranasal or intramuscular vaccines. These VLPs have elicited systemic and mucosal immune responses in mice [88]. In 2014, intranasal vaccination of mice with *N. benthamiana* produced GII.4 VLPs increased VLP-specific serum IgG for 56 days [89]. These studies suggest that plant-based technology has the potential to be an inexpensive way to manufacture VLPs for a norovirus vaccine.

7.4. Trivalent hepatitis E, GII.4 norovirus, and astrovirus P particle vaccine

The Cincinnati Children’s Hospital Medical Center, University of Cincinnati, and Virginia Polytechnic Institute and State University are developing a norovirus vaccine using norovirus P particles, designed to resemble the protruding P domain of the virus [60,61]. Development began on a monovalent GII.4 norovirus vaccine, but as it progressed, hepatitis E and astrovirus antigens were added. A trial of an intranasal GII.4 norovirus P particle vaccine in gnotobiotic pigs showed a higher intestinal T-cell immune response when compared to pigs vaccinated with a GII.4 norovirus VLP vaccine [90]. In mice, an intranasal bivalent vaccine using a fused P protein from the P domains of norovirus and hepatitis E showed increased antibody titers when compared to vaccination with a mixture of P dimers from norovirus and hepatitis E [91]. Most recently, a trivalent intranasal GII.4 norovirus, hepatitis E, and astrovirus vaccine, using a fusion of the three P domains, produced a 1.9-fold higher norovirus IgG titer than immunization with norovirus P particle alone in mice [92]. These trials show the potential for a P particle vaccine to vaccinate against multiple diseases.

7.5. Recombinant adenovirus expressing norovirus GII.4

The Chinese Center for Disease Control and Prevention is developing a recombinant adenovirus vaccine expressing the norovirus GII.4 major capsid protein VP1. A study in 2008 in mice found an intranasal GII.4 vaccine increased norovirus IgG and IgA immune responses [93]. They also tested a prime-boost strategy in mice using norovirus VLPs and recombinant adenovirus expressing norovirus GII.4 capsid protein; priming with the recombinant adenovirus vaccine before VLP vaccination produced higher norovirus-specific antibody levels than priming with the VLP or multiple VLP vaccinations [94]. These studies indicate that the recombinant adenovirus expressing norovirus proteins is a technology that can produce comparable immune responses to norovirus VLP formulations.

8. Recent developments in norovirus science

While norovirus vaccines are in pre-clinical and early clinical trials, there are several recent developments that are helping push the vaccine development process forward. Notably, these include an increase in the use of multi-pathogen diagnostic panels and the advent of a new human norovirus *in-vitro* culture system.

8.1. PCR-based multi-pathogen diagnostic panels

The availability of highly sensitive PCR-based diagnostic panels for the detection of multi-enteric pathogens, including norovirus, has revolutionized testing in clinical laboratories. New multi-pathogen tests, which include the xTAG GPP (Luminex Corporation, Toronto, Canada), FilmArray GI Panel (BioFire Diagnostics Inc., Salt Lake City, UT, U.S.A.), and Verigene Enteric Pathogens Test (EP) (Nanosphere, Northbrook, IL, U.S. A.), increase testing capacity by allowing for the identification of multiple bacteria, viruses, and parasites in one test and produce results in a few hours [4,95]. A study comparing these multi-pathogen tests found they have >99% specificity and sensitivity ranging from 78.0–87.8% for norovirus detection when compared to the reference method of real time RT-PCR, which is the gold standard for norovirus detection [96]. The increased use of these tests will help to identify norovirus alongside other AGE pathogens and may help assessing the burden of norovirus disease in settings where currently few samples from AGE patients are routinely tested for norovirus.

8.2. Human norovirus intestinal enteroid culture system

A groundbreaking recent development in norovirus research is the development of an *in-vitro* culture system [97], which had remained elusive for nearly 50 years. This new culture system uses non-transformed human intestinal enteroids (HIE), also called mini-guts, and is expected to expand a number of areas of norovirus research, including testing of chlorine and alcohols [98] and antivirals. Perhaps most relevant to vaccine research, the culture system potentially opens the door to development of neutralization assays, which could yield a more definitive correlate of protection. Ultimately, this human norovirus cell culture system could lead to the development of other vaccine technologies. Additionally, while current trials are able to determine if a vaccine decreases post-symptomatic viral shedding between vaccinated and unvaccinated individuals, they have been unable to specify if the virus is infectious or not. The mini-gut cell culture system may provide more important

information for norovirus prevention through vaccination and traditional infection control practices.

9. Considerations for norovirus vaccine development

While norovirus science is advancing and norovirus vaccines are being developed, there are many other considerations vaccine developers must take into account before a norovirus vaccine can be licensed. This section will summarize these key considerations to inform current and future norovirus vaccine research and development strategies, including cost effectiveness, target population, and public acceptance of a future vaccine (Table 2).

9.1. Cost effectiveness

While norovirus carries a tremendous social and economic burden, factors such as the vaccine's cost, effectiveness, and duration of protection all impact whether a vaccine is considered cost effective. A modeling study looking at the potential impact of a norovirus vaccine in the United States found that a vaccine with 50% efficacy and a 12-month duration would avert 1.0–2.2 million illnesses per year but would cost $400 million to $1 billion annually. However, a vaccine with 50% efficacy that conferred protection for 48 months could save up to $2.1 billion annually [99]. A separate study on the cost of norovirus vaccination in the United States military concluded that a norovirus vaccine would cost more than vaccines for enterotoxigenic *E. coli,* campylobacter, and shigella. However, when vomiting-only illness was included in the analysis, the norovirus vaccine became more cost effective than those targeting the other three bacterial agents [100].

It is estimated that a bout of norovirus illness in LMICs costs $45, compared to $247 in high-income countries, given the relative differences in direct health-care expenses and the value of lost productivity [16]. Using the cost-effectiveness threshold of one times the GDP per capita per DALY (Disability Adjusted Life Year) averted, an economic analysis in Peru found that a two-dose vaccine would have to be 70% effective and cost $17 ($8.50 per dose) to be considered cost effective. This analysis did not account for indirect costs of norovirus infection, nor indirect benefits from decreased viral shedding in the community [101].

9.2. Target populations

Modeling studies have suggested that the greatest potential economic and health benefits of norovirus vaccines are in the young (under 5 years old) and the elderly (over 65 years old) [99]. A review of pediatric norovirus cases worldwide found that approximately 70% of norovirus cases occurred between 6 and 23 months of age. They concluded a norovirus vaccine schedule completed by 6 months of age could prevent up to 85% of pediatric cases, while a schedule completed by 12 months could prevent up to 50% of pediatric cases [102]. A model of the impact of a norovirus vaccine in both pediatric and elderly populations in the United States predicted that 90% pediatric coverage could avert 33–60% of norovirus cases in children under 5 years old, and 65% elderly vaccination coverage could avert 17–38% of cases in those over 65 years old, depending on vaccine effectiveness. Therefore, the authors concluded that focusing a norovirus vaccine on the pediatric population would have the greatest impact [103]. However, with a pediatric vaccine, a careful consideration of how a

norovirus vaccine would fit into the current routine childhood vaccination schedule will be fundamental to its success.

Additional populations at risk for transmission of norovirus disease include those working in health care, childcare, and food service industry, and are therefore also potential as potential groups to be considered for norovirus vaccination [104]. The full benefit of a norovirus vaccine in these populations will depend on the level of immunization coverage that can be achieved.

9.3. Public opinion and acceptance

Another important consideration is the level of public knowledge of norovirus and future acceptance of a vaccine. Despite its large burden, norovirus is not well understood by the public. A nationally representative survey of 1,051 United States adults in 2013 found that nearly half the respondents had never heard of norovirus, but 85% of respondents had heard of ‘cruise ship virus’, ‘the stomach bug’,or’the stomach flu’; many more people knew about foodborne bacteria, such as salmonella and *E. coli* [105]. Lack of knowledge about the importance of norovirus has also been documented in surveys conducted among infection prevention workers [106] and food-safety professionals [107], even though norovirus is a leading cause of outbreaks in both hospitals and food service settings [8,108]. Coordinated public health education campaigns, utilizing social media and other communication avenues, may be helpful prior to the introduction of any vaccine to emphasize the importance of vaccination against norovirus.

10. Conclusion

There are several norovirus vaccines in clinical trials. Continued research on norovirus immunogenicity, cross-protection among different genotypes, and correlates of protection will help answer crucial questions for vaccine developers and help accelerate vaccine development. The recent advent of a novel norovirus cell culture system represents a huge leap forward in this area; however, more work to simplify, optimize, and lower the cost of the culture system is still necessary. Modeling studies on the potential impact of a norovirus vaccine within different populations – including young children, older adults, and those with the potential to transmit the disease to many others – can help identify the most impactful target population(s) for a vaccine.

11. Expert commentary

Robust, current, and accurate global disease and economic burden estimates are crucial to incentivize and guide investment in the research and development of norovirus vaccines. These estimates rely on strong norovirus surveillance around the world. Current burden estimates vary widely due to the broad diversity of noroviruses, differing methodologies, and differing AGE case definitions. Particular emphasis should be focused on providing estimates for high-risk populations and in developing countries, where large data gaps currently exist. Additionally, most norovirus burden studies have been conducted in children, leaving a relative gap in knowledge about the burden of norovirus among adults. Efforts to improve existing burden estimates and obtain a better understanding of how norovirus

burden varies globally can help justify further investments in norovirus vaccine development.

Public outreach and education are also needed to increase awareness about norovirus and the appropriate prevention measures people can take to protect themselves and their families. Social media has become an increasingly important tool to disseminate accurate information about norovirus and raise the public's awareness about the disease. Concerns around vaccine safety and how a new vaccine might fit into the current vaccination schedule may need to be addressed in any public information campaigns involving future norovirus vaccines.

12. Five-year view

The next 5 years provide an exciting opportunity for meaningful advances in our understanding of norovirus immunity and cross-protection, both of which are key for a successful norovirus vaccine. Disease burden estimates will continue to improve, as more studies on norovirus prevalence and incidence in different regions of the world are completed and new surveillance studies employing the latest diagnostic tools are implemented. Progress on each of these fronts will bring us closer to a licensed norovirus vaccine with the potential to reduce the morbidity and mortality of norovirus worldwide

Acknowledgments

Copyediting and formatting assistance was provided by Mary Ann K. Hall, MPH, Office of Infectious Disease, National Center for Immunization and Respiratory Disease, Centers for Disease Control and Prevention, Atlanta, GA.

Funding

The manuscript was funded by the Centers for Disease Control and Prevention and the Oak Ridge Institute for Science and Education.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Kapikian AZ, Wyatt RG, Dolin R, et al. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J Virol. 1972;10:1075–1081. [PubMed: 4117963]
2. Hall AJ, Lopman BA, Payne DC, et al. Norovirus disease in the United States. Emerg Infect Dis. 2013;19:1198–1205. [PubMed: 23876403]
3. Pires SM, Fischer-Walker CL, Lanata CF, et al. Aetiology-specific estimates of the global and regional incidence and mortality of diarrhoeal diseases commonly transmitted through food. PLoS One. 2015;10:e0142927. [PubMed: 26632843]
4. Vinje J Advances in laboratory methods for detection and typing of norovirus. J Clin Microbiol. 2015;53:373–381. [PubMed: 24989606]
5. Siebenga JJ, Vennema H, Renckens B, et al. Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006. J Virol. 2007;81:9932–9941. [PubMed: 17609280]
6. Siebenga JJ, Vennema H, Zheng DP, et al. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001–2007. J Infect Dis. 2009;200:802–812. [PubMed: 19627248]

7. Thorne LG, Goodfellow IG. Norovirus gene expression and replication. J Gen Virol. 2014;95:278–291. [PubMed: 24243731]

8. Kirk MD, Pires SM, Black RE, et al. World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. PLoS Med. 2015;12:e1001921. [PubMed: 26633831] •• This study estimates the global burden of norovirus illnesses and deaths and identidies norovirus as the leadings cause of foodborne illness worldwide.

9. Operario DJ, Platts-Mills JA, Nadan S, et al. Etiology of severe acute watery diarrhea in children in the global rotavirus surveillance network using quantitative polymerase chain reaction. J Infect Dis. 2017;216:220–227. [PubMed: 28838152]

10. Becker-Dreps S, Bucardo F, Vilchez S, et al. Etiology of childhood diarrhea after rotavirus vaccine introduction: a prospective, population-based study in Nicaragua. Pediatr Infect Dis J 2014;33:1156–1163. [PubMed: 24879131]

11. Payne DC, Vinje J, Szilagyi PG, et al. Norovirus and medically attended gastroenteritis in U.S. children. N Engl J Med. 2013;368:1121–1130. [PubMed: 23514289]

12. McAtee CL, Webman R, Gilman RH, et al. Burden of norovirus and rotavirus in children after rotavirus vaccine introduction, Cochabamba, Bolivia. Am J Trop Med Hyg. 2016;94:212–217. [PubMed: 26598569]

13. Giersing BK, Vekemans J, Nava S, et al. Report from the World Health Organization's third Product Development for Vaccines Advisory Committee (PDVAC) meeting, Geneva, 8–10th June 2016. Vaccine. 2017 DOI: 10.1016/j.vaccine.2016.10.090

14. Cortes-Penfield NW, Ramani S, Estes MK, et al. Prospects and challenges in the development of a norovirus vaccine. Clin Ther. 2017;39:1537–1549. [PubMed: 28756066]

15. Lucero Y, Vidal R, O'Ryan GM. Norovirus vaccines under development. Vaccine. 2017 DOI: 10.1016/j.vaccine.2017.06.043

16. Bartsch SM, Lopman BA, Ozawa S, et al. Global economic burden of norovirus gastroenteritis. PLoS One. 2016;11:e0151219. [PubMed: 27115736]

17. GBD. 2013 mortality and causes of death collaborators. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the global burden of disease study 2013. Lancet. 2015;385 (9963):117–171. [PubMed: 25530442]

18. GBD. 2015 mortality and causes of death collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the global burden of disease study 2015. Lancet. 2016;388(10053):1459–1544. [PubMed: 27733281]

19. Lanata CF, Fischer-Walker CL, Olascoaga AC, et al. Global causes of diarrheal disease mortality in children <5 years of age: a systematic review. PLoS One. 2013;8:e72788. [PubMed: 24023773]

20. Hosangadi D, Smith PG, Giersing BK. Considerations for using ETEC and Shigella disease burden estimates to guide vaccine development strategy. Vaccine. 2017 DOI:10.1016/j.vaccine. 2017.09.083

21. Ahmed SM, Hall AJ, Robinson AE, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. Lancet Infect Dis. 2014;14:725–730. [PubMed: 24981041] • This systematic review estimates the proportion of AGE due to norovirus globally, in high-income, middle-income, and low-income countries. It estimates that norovirus causes approximately one-fifth of AGE worldwide.

22. Nguyen GT, Phan K, Teng I, et al. A systematic review and meta-analysis of the prevalence of norovirus in cases of gastroenteritis in developing countries. Medicine. 2017;96:e8139. [PubMed: 28984764]

23. Mans J, Armah GE, Steele AD, et al. Norovirus epidemiology in Africa: a review. PLoS One. 2016;11:e0146280. [PubMed: 27116615]

24. Kabue JP, Meader E, Hunter PR, et al. Human norovirus prevalence in Africa: a review of studies from 1990 to 2013. Trop Med Int Health. 2016;21:2–17. [PubMed: 26436862]

25. Kreidieh K, Charide R, Dbaibo G, et al. The epidemiology of norovirus in the Middle East and North Africa (MENA) region: a systematic review. Virol J. 2017;14:220. [PubMed: 29126448]

26. O'Ryan M, Riera-Montes M, Lopman B. Norovirus in Latin America: systematic review and meta-analysis. Pediatr Infect Dis J 2017;36:127–134. [PubMed: 27755462]
27. Kowalzik F, Riera-Montes M, Verstraeten T, et al. The burden of norovirus disease in children in the European Union. Pediatr Infect Dis J. 2015;34:229–234. [PubMed: 25742072]
28. Zhou HL, Zhen SS, Wang JX, et al. Burden of acute gastroenteritis caused by norovirus in China: A systematic review. J Infect. 2017;75:216–224. [PubMed: 28633888]
29. Lindsay L, Wolter J, De Coster I, et al. A decade of norovirus disease risk among older adults in upper-middle and high income countries: a systematic review. BMC Infect Dis. 2015;15:425. [PubMed: 26467099]
30. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. 2013;382(9888):209–222. [PubMed: 23680352]
31. Liu J, Platts-Mills JA, Juma J, et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. Lancet. 2016;388 (10051):1291–1301. [PubMed: 27673470]
32. Rouhani S, Penataro Yori P, Paredes Olortegui M, et al. Norovirus infection and acquired immunity in 8 countries: results from the MAL-ED study. Clin Infect Dis. 2016;62:1210–1217. [PubMed: 27013692]
33. Saito M, Goel-Apaza S, Espetia S, et al. Multiple norovirus infections in a birth cohort in a Peruvian periurban community. Clin Infect Dis. 2014;58:483–491. [PubMed: 24300042]
34. Lopman BA, Trivedi T, Vicuna Y, et al. Norovirus infection and disease in an ecuadorian birth cohort: association of certain norovirus genotypes with host FUT2 secretor status. J Infect Dis. 2015;211:1813–1821. [PubMed: 25505295]
35. Menon VK, George S, Sarkar R, et al. Norovirus gastroenteritis in a birth cohort in southern India. PLoS One. 2016;11:e0157007. [PubMed: 27284939]
36. Kirby AE, Streby A, Moe CL. Vomiting as a symptom and transmission risk in norovirus illness: evidence from human challenge studies. PLoS One. 2016;11:e0143759. [PubMed: 27116105]
37. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. N Engl J Med. 2009;361:1776–1785. [PubMed: 19864676]
38. Lopman B, Simmons K, Gambhir M, et al. Epidemiologic implications of asymptomatic reinfection: a mathematical modeling study of norovirus. Am J Epidemiol. 2014;179:507–512. [PubMed: 24305574]
39. van Beek J, de Graaf M, Al-Hello H, et al. Molecular surveillance of norovirus, 2005–2016: an epidemiological analysis of data collected from the NoroNet network. Lancet Infect Dis. 2018;18: 545–553. [PubMed: 29396001]
40. Vega E, Barclay L, Gregoricus N, et al. Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. J Clin Microbiol. 2014;52:147–155. [PubMed: 24172151]
41. Kroneman A, Vega E, Vennema H, et al. Proposal for a unified norovirus nomenclature and genotyping. Arch Virol. 2013;158:2059–2068. [PubMed: 23615870]
42. Centers for Disease Control and Prevention. CaliciNet data tables: genotype distribution of norovirus outbreaks. [cited 2018 6 05]. Available from: https://www.cdc.gov/norovirus/reporting/calicinet/data-tables.html#genotype-01
43. Harrington PR, Lindesmith L, Yount B, et al. Binding of Norwalk virus-like particles to ABH histo-blood group antigens is blocked by antisera from infected human volunteers or experimentally vaccinated mice. J Virol. 2002;76:12335–12343. [PubMed: 12414974]
44. Lindesmith L, Moe C, Marionneau S, et al. Human susceptibility and resistance to Norwalk virus infection. Nat Med. 2003;9:548–553. [PubMed: 12692541]
45. Currier RL, Payne DC, Staat MA, et al. Innate susceptibility to norovirus infections influenced by FUT2 genotype in a United States pediatric population. Clin Infect Dis. 2015;60:1631–1638. [PubMed: 25744498]
46. Nordgren J, Sharma S, Kambhampati A, et al. Innate resistance and susceptibility to Norovirus infection. PLoS Pathog. 2016;12: e1005385. [PubMed: 27115484]
47. Kambhampati A, Payne DC, Costantini V, et al. Host genetic susceptibility to enteric viruses: a systematic review and metaanalysis. Clin Infect Dis. 2016;62:11–18. [PubMed: 26508510]

48. Johnson PC, Mathewson JJ, DuPont HL, et al. Multiple-challenge study of host susceptibility to norwalk gastroenteritis in US adults. J Infect Dis. 1990;161:18–21. [PubMed: 2153184]
49. Parrino TA, Schreiber DS, Trier JS, et al. Clinical immunity in acute gastroenteritis caused by norwalk agent. N Eng J Med. 1977;297 (2):86–89.
50. Wyatt RG, Dolin R, Blacklow NR, et al. Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers. J Infect Dis. 1974;129:709–714. [PubMed: 4209723]
51. Simmons K, Gambhir M, Leon J, et al. Duration of immunity to norovirus gastroenteritis. Emerg Infect Dis. 2013;19:1260–1267. [PubMed: 23876612]
52. Reeck A, Kavanagh O, Estes MK, et al. Serological correlate of protection against norovirus-induced gastroenteritis. J Infect Dis. 2010;202:1212–1218. [PubMed: 20815703]
53. Ramani S, Estes MK, Atmar RL. Correlates of protection against norovirus infection and disease-where are we now, where do we go? PLoS Pathog. 2016;12:e1005334. [PubMed: 27115358]
54. Atmar RL, Bernstein DI, Lyon GM, et al. Serological correlates of protection against a GII.4 norovirus. Clin Vaccine Immunol. 2015;22:923–929. [PubMed: 26041041]
55. Malm M, Uusi-Kerttula H, Vesikari T, et al. High serum levels of norovirus genotype-specific blocking antibodies correlate with protection from infection in children. J Infect Dis. 2014;210:1755–1762. [PubMed: 24970849]
56. Huo Y, Wan X, Ling T, et al. Expression and purification of norovirus virus like particles in Escherichia coli and their immunogenicity in mice. Mol Immunol. 2017;93:278–284. [PubMed: 28802994]
57. Parker SA, Maloy MH, Tome-Amat J, et al. Optimization of norovirus virus-like particle production in Pichia pastoris using a realtime near-infrared bioprocess monitor. Biotechnol Prog. 2016;32:518–526. [PubMed: 26748930]
58. Tome-Amat J, Fleischer L, Parker SA, et al. Secreted production of assembled norovirus virus-like particles from Pichia pastoris. Microb Cell Fact. 2014;13:134. [PubMed: 25201129]
59. Huang Z, Chen Q, Hjelm B, et al. A DNA replicon system for rapid high-level production of virus-like particles in plants. Biotechnol Bioeng. 2009;103:706–714. [PubMed: 19309755]
60. Tan M, Fang P, Chachiyo T, et al. Noroviral P particle: structure, function and applications in virus-host interaction. Virology. 2008;382:115–123. [PubMed: 18926552]
61. Tan M, Jiang X. Norovirus P particle: a subviral nanoparticle for vaccine development against norovirus, rotavirus and influenza virus. Nanomedicine (Lond). 2012;7:889–897. [PubMed: 22734641]
62. Ettayebi K, Crawford SE, Murakami K, et al. Replication of human noroviruses in stem cell-derived human enteroids. Science. 2016;353(6306):1387–1393. [PubMed: 27562956] •• This paper describes the human intestinal enteroid model that was developed and is used for the in-vitro culture system for norovirus.
63. Debbink K, Lindesmith LC, Donaldson EF, et al. Chimeric GII.4 norovirus virus-like-particle-based vaccines induce broadly blocking immune responses. J Virol. 2014;88:7256–7266. [PubMed: 24741081]
64. El-Kamary SS, Pasetti MF, Mendelman PM, et al. Adjuvanted intranasal Norwalk virus-like particle vaccine elicits antibodies and antibody-secreting cells that express homing receptors for mucosal and peripheral lymphoid tissues. J Infect Dis. 2010;202:1649–1658. [PubMed: 20979455]
65. Ramirez K, Wahid R, Richardson C, et al. Intranasal vaccination with an adjuvanted Norwalk virus-like particle vaccine elicits antigen-specific B memory responses in human adult volunteers. Clin Immunol. 2012;144:98–108. [PubMed: 22710446]
66. Atmar RL, Baehner F, Cramer JP, et al. Rapid responses to 2 virus-like particle norovirus vaccine candidate formulations in healthy adults: a randomized controlled trial. J Infect Dis. 2016;214:845–853. [PubMed: 27354368]
67. Ramani S, Neill FH, Ferreira J, et al. B-cell responses to intramuscular administration of a bivalent virus-like particle human norovirus vaccine. Clin Vaccine Immunol. 2017;24(5):e00571–16. [PubMed: 28249841]

68. Tucker S Oral immunization of a rAd vector expressing norovirus VP1 elicits a potent mucosal immune response without an increase in anti-vector immunity Paper presented at: International Congress on Infectious Diseases; 2018 3 3; Buenos Aires: Argentina.
69. Takeda initiates world's first norovirus vaccine field trial [Internet]; Osaka, Japan: Takeda; 20 Jun 2016 [cited 2018 5 9]. Available from: https://www.takeda.com/newsroom/newsreleases/2016/takeda-initiates-worlds-first-norovirus-vaccine-field-trial/
70. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk virus illness. N Engl J Med. 2011;365:2178–2187. [PubMed: 22150036] A randomized, double-blind, placebo-controlled challenge trial of a GI.1 norovirus VLP vaccine in healthy adults, which showed a reduction of norovirus illness and infection among vaccine recipients.
71. Parra GI, Bok K, Taylor R, et al. Immunogenicity and specificity of norovirus consensus GII.4 virus-like particles in monovalent and bivalent vaccine formulations. Vaccine. 2012;30:3580–3586. [PubMed: 22469864]
72. Leroux-Roels G, Cramer JP, Mendelman PM, et al. Safety and immunogenicity of different formulations of norovirus vaccine candidate in healthy adults: a randomized, controlled, double-blind clinical trial. J Infect Dis. 2017;217:597–607.
73. Bernstein DI, Atmar RL, Lyon GM, et al. Norovirus vaccine against experimental human GII.4 virus illness: a challenge study in healthy adults. J Infect Dis. 2015;211:870–878. [PubMed: 25210140] • A phase II vaccine challenge study that demonstrated a reduction in norovirus illness severity and mean viral shedding, but not norovirus infection or illness, after vaccination.
74. Tucker SN, Tingley DW, Scallan CD. Oral adenoviral-based vaccines: historical perspective and future opportunity. Exp Rev Vaccines. 2008;7:25–31.
75. Liebowitz D, Lindbloom JD, Brandl JR, et al. High titre neutralising antibodies to influenza after oral tablet immunisation: a phase 1, randomised, placebo-controlled trial. Lancet Infect Dis. 2015;15:1041–1048. [PubMed: 26333337]
76. Peters W, Brandl JR, Lindbloom JD, et al. Oral administration of an adenovirus vector encoding both an avian influenza A hemagglutinin and a TLR3 ligand induces antigen specific granzyme B and IFN-gamma T cell responses in humans. Vaccine. 2013;31:1752–1758. [PubMed: 23357198]
77. Vaxart. Technology: advantages [Internet]. South San Francisco, CA: Vaxart; 2018 [cited 2018 5 10]. Available from: http://vaxart.com/TechAdvantages.html
78. Kim L, Liebowitz D, Lin K, et al. Safety and immunogenicity of an oral tablet norovirus vaccine, a phase I randomized, placebo-controlled trial. JCI Insight. 2018;3:e121077.A phase I vaccine trial of a GI.1 norovirus oral tablet vaccine that demonstrated the vaccine was safe, well-tolerated, and elicited an immune response as measured by mucosal IgA and serum-blocking antibody titers.
79. Vaxart announces positive results for its oral norovirus vaccine in Phase 1b clinical study: first oral tablet norovirus vaccine progresses to Phase 2 challenge study [Press release]. South San Francisco, CA: Vaxart; 2017 Oct 30 [cited 2018 5 10]. Available from: http://vaxart.com/NRfiles/VaxartAnnouncesNorovirusPhase1bResults.pdf
80. Tucker S Novel norovirus vaccine approaches Paper presented at: NoroCORE Final Showcase Meeting; 2018 3 19-21; Atlanta, GA.
81. Daiichi Sankyo Company Limited, UMN Pharma Inc. Daiichi Sankyo and UMN pharma enter into research agreement for norovirus vaccine [Internet]. Tokyo, Japan: Daiichi-Sankyo; 2014 Feb 14 [cited 2018 5 9]. Available from: https://www.daiichisankyo.com/media_investors/media_relations/press_releases/detail/006078.html
82. Blazevic V, Lappalainen S, Nurminen K, et al. Norovirus VLPs and rotavirus VP6 protein as combined vaccine for childhood gastroenteritis. Vaccine. 2011;29:8126–8133. [PubMed: 21854823]
83. Malm M, Tamminen K, Lappalainen S, et al. Genotype considerations for virus-like particle-based bivalent norovirus vaccine composition. Clin Vaccine Immunol. 2015;22:656–663. [PubMed: 25903355]
84. Tamminen K, Lappalainen S, Huhti L, et al. Trivalent combination vaccine induces broad heterologous immune responses to norovirus and rotavirus in mice. PLoS One. 2013;8(7):e70409. [PubMed: 23922988]

85. Blazevic V, Malm M, Arinobu D, et al. Rotavirus capsid VP6 protein acts as an adjuvant in vivo for norovirus virus-like particles in a combination vaccine. Hum Vaccin Immunother. 2016;12:740–748. [PubMed: 26467630]
86. Wang X, Ku Z, Dai W, et al. A bivalent virus-like particle based vaccine induces a balanced antibody response against both enterovirus 71 and norovirus in mice. Vaccine. 2015;33:5779–5785. [PubMed: 26424606]
87. Tacket CO, Mason HS, Losonsky G, et al. Human immune responses to a novel norwalk virus vaccine delivered in transgenic potatoes. J Infect Dis. 2000;182:302–305. [PubMed: 10882612]
88. Santi L, Batchelor L, Huang Z, et al. An efficient plant viral expression system generating orally immunogenic Norwalk virus-like particles. Vaccine. 2008;26:1846–1854. [PubMed: 18325641]
89. Mathew LG, Herbst-Kralovetz MM, Mason HS. Norovirus narita 104 virus-like particles expressed in Nicotiana benthamiana induce serum and mucosal immune responses. Biomed Res Int. 2014;2014:807539. [PubMed: 24949472]
90. Kocher J, Bui T, Giri-Rachman E, et al. Intranasal P particle vaccine provided partial cross-variant protection against human GII.4 norovirus diarrhea in gnotobiotic pigs. J Virol. 2014;88:9728–9743. [PubMed: 24920797]
91. Wang L, Cao D, Wei C, et al. A dual vaccine candidate against norovirus and hepatitis E virus. Vaccine. 2014;32:445–452. [PubMed: 24291540]
92. Xia M, Wei C, Wang L, et al. A trivalent vaccine candidate against hepatitis E virus, norovirus, and astrovirus. Vaccine. 2016;34:905–913. [PubMed: 26778421]
93. Guo L, Wang J, Zhou H, et al. Intranasal administration of a recombinant adenovirus expressing the norovirus capsid protein stimulates specific humoral, mucosal, and cellular immune responses in mice. Vaccine. 2008;26:460–468. [PubMed: 18160189]
94. Guo L, Zhou H, Wang M, et al. A recombinant adenovirus prime-virus-like particle boost regimen elicits effective and specific immunities against norovirus in mice. Vaccine. 2009;27:5233–5238. [PubMed: 19589399]
95. Cardemil CV, Parashar UD, Hall AJ. Norovirus infection in older adults: epidemiology, risk factors, and opportunities for prevention and control. Infect Dis Clin North Am. 2017;31:839–870. [PubMed: 28911830]
96. Chhabra P, Gregoricus N, Weinberg GA, et al. Comparison of three multiplex gastrointestinal platforms for the detection of gastroenteritis viruses. J Clin Virol. 2017;95:66–71. [PubMed: 28889082]
97. Zou WY, Blutt SE, Crawford SE, et al. Human intestinal enteroids: new models to study gastrointestinal virus infections. Methods Mol Biol. 2017. doi:10.1007/7651_2017_1.
98. Costantini VP, Morantz EK, Browne H, et al. Human norovirus replication in human intestinal enteroids as a model to evaluate virus inactivation. Emerg Infect Dis. 2018;24(8).
99. Bartsch SM, Lopman BA, Hall AJ, et al. The potential economic value of a human norovirus vaccine for the United States. Vaccine. 2012;30:7097–7104. [PubMed: 23026689]
100. Tallant A, Porter CK, Putnam SD, et al. Relative cost-effectiveness of a norovirus vaccine in the deployed military setting compared to a vaccine against Campylobacter sp., ETEC, and Shigella sp. Vaccine. 2014;32:5156–5162. [PubMed: 25086264]
101. Mirelman AJ, Ballard SB, Saito M, et al. Cost-effectiveness of norovirus vaccination in children in Peru. Vaccine. 2015;33:3084–3091. [PubMed: 25980428]
102. Shioda K, Kambhampati A, Hall AJ, et al. Global age distribution of pediatric norovirus cases. Vaccine. 2015;33:4065–4068. [PubMed: 26051514]
103. Steele MK, Remais JV, Gambhir M, et al. Targeting pediatric versus elderly populations for norovirus vaccines: a model-based analysis of mass vaccination options. Epidemics. 2016;17:42–49. [PubMed: 27821278]
104. Aliabadi N, Lopman BA, Parashar UD, et al. Progress toward norovirus vaccines: considerations for further development and implementation in potential target populations. Exp Rev Vaccines. 2015;14:1241–1253.
105. Cates SC, Kosa KM, Brophy JE, et al. Consumer education needed on norovirus prevention and control: findings from a nationally representative survey of U.S. adults. J Food Prot. 2015;78:484–490. [PubMed: 25719870]

106. Kosa KM, Cates SC, Hall AJ, et al. Knowledge of norovirus prevention and control among infection preventionists. Am J Infect Control. 2014;42:676–678. [PubMed: 24837119]

107. Kosa KM, Cates SC, Hall AJ, et al. Gaps in food safety professionals' knowledge about noroviruses. J Food Prot. 2014;77:1336–1341. [PubMed: 25198594]

108. Rhinehart E, Walker S, Murphy D, et al. Frequency of outbreak investigations in US hospitals: results of a national survey of infection preventionists. Am J Infect Control. 2012;40:2–8. [PubMed: 22300590]

Key issues

- Norovirus is an important pathogen that poses a significant disease and economic burden globally.
- Noroviruses are genetically and antigenically very diverse, with more than 25 genotypes across three genogroups infecting humans. GII.4 is the most prominent norovirus genotype worldwide.
- Norovirus causes approximately one-fifth of AGE cases globally.
- Children under 5 years old and the elderly over 65 years old are most frequently affected and suffer the most severe outcomes due to norovirus disease.
- Burden estimates vary between studies and regions. Additional and more robust burden studies are needed to better quantify the impacts of norovirus globally.
- Norovirus immunity is not well understood and there is currently no single well-established correlate of protection that can be used in vaccine trials.
- Two vaccines currently in human clinical trials include a bivalent GI.1/GII.4 intramuscular VLP vaccine in Phase IIb and a monovalent GI.1 oral pill recombinant adenovirus vaccine in Phase I trials.
- Cost effectiveness is a key aspect of acceptability of a norovirus vaccine.
- Public awareness of norovirus is relatively low, suggesting need for public outreach and education to maximize uptake of future vaccines.
- A licensed norovirus vaccine has the potential to save lives and prevent a significant proportion of diarrheal illnesses worldwide.

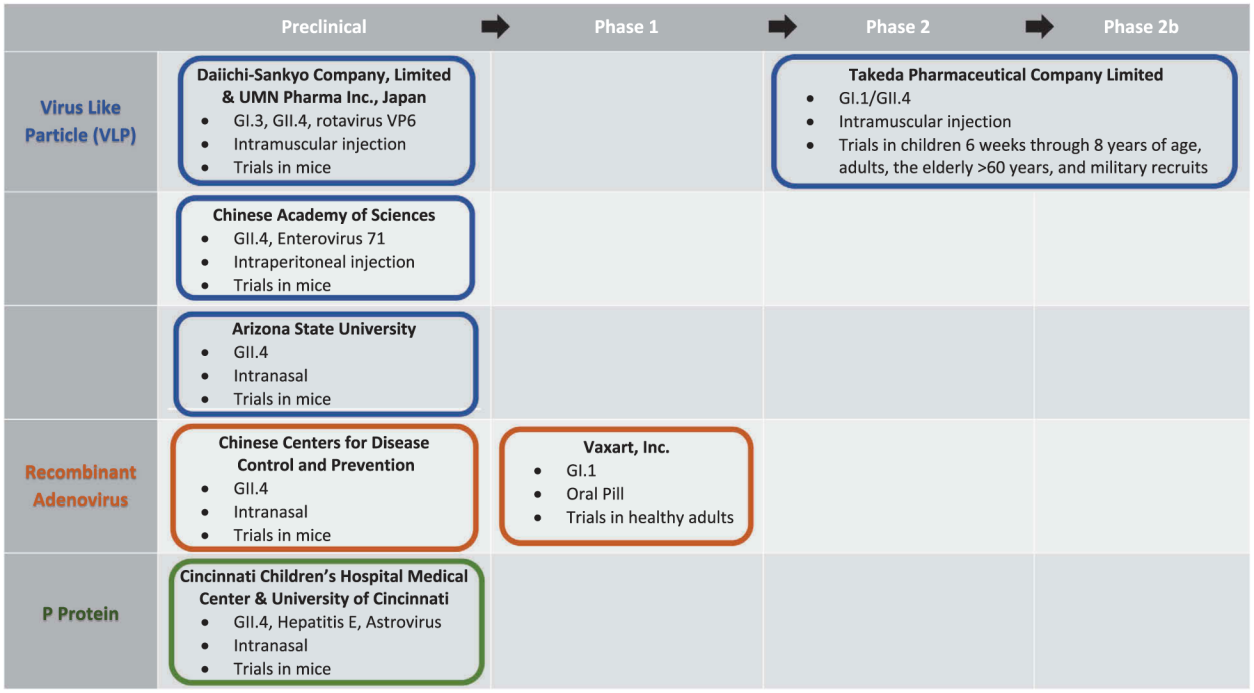

Figure 1.
Vaccine candidates in development, by type and pre-clinical or clinical phase.

Table 1.

Global estimates of deaths due to diarrheal diseases and norovirus (adapted from Hosangadi et al. 2017 [20]).

Study	WHO Foodborne Disease Burden Epidemiology Reference Group (FERG) [8]	Child Health Epidemiology Reference Group (CHERG) [19]	Global Burden of Disease 2013 [17]	Global Burden of Disease 2015 [18]
Publication Year	2015	2013	2015	2016
Time period	2010	2011	2013	2015
Diarrheal deaths: <5 years of age	91,621 (95% UI * 62,442–132,707)	712,000 (95% UR ** 491,000–1,049,000)	<1 month: 44,800 (95% UI 36,800–53,300) 1−59 months: 474,900 (95% UI 398,100–545,000)	498,900 (95% UI 447,500–557,600)
Diarrheal deaths: all ages	715,196 (95% UI 603,325–846,397)	–	1,264,100 (95% UI 1,151,200–1,383,200)	1,312,100 (95% UI 1,233,600–1,391,300)
Norovirus deaths: <5 years of age	8,992 (95% UI 4,251–19,347) ****	71,000 (95% UR 39,000–113,000) ***	1800 (95% UI 700–3,100)	14,800 (95% UI 4,200–33,700)
Norovirus deaths: all ages	212,489 (95% UI 160,595–278,420)	–	1800 (95% UI 700–3,200)	29,700 (95% UI 4,800–67,600)

* Uncertainty Interval

** Uncertainty Range

*** Estimates of all calicivirus deaths, including norovirus GI, GII, and sapovirus

**** Estimate of foodborne norovirus deaths only

Table 2.

Key public health considerations for norovirus vaccine development.

Considerations	Existing knowledge	Remaining questions	Future studies and work needed
Norovirus Burden & Immunity	Norovirus is a leading cause of AGE worldwide.	How does the disease burden vary at national and sub-national levels? How does burden vary in developed versus developing countries?	More local and country-specific norovirus burden estimates, particularly in developing settings.
	Globally, the burden of norovirus disease is high and circulating genotypes are diverse, but estimates vary by study design, setting, country, and population.	What is the most appropriate method to account for asymptomatic norovirus infection in disease burden studies? What are the major circulating genotypes of norovirus?	Increased testing for norovirus genotypes among those with AGE. Improved differentiation of etiologic infection from asymptomatic shedding.
	Norovirus immunity is short term, generally homotypic, and influenced by host genetics.	How long can a vaccine confer protection, and will protection vary based on circulating strains?	Accurate estimates of the duration of immunity and degree of cross-protection afforded by natural norovirus infection and vaccination.
	There is no single, well-established correlate of immunity.	Can a correlate of immunity be identified?	More norovirus immunologic studies to further identify correlates of immunity.
Cost Effectiveness	Norovirus disease causes significant health care and societal costs.	What is the total economic impact of norovirus? Does the economic impact vary between countries?	Estimates of the direct and indirect costs of norovirus in developed and developing countries
	Cost effectiveness is highly dependent on vaccine efficacy and length of protection.	What will be the efficacy and length of protection for a norovirus vaccine? Will a norovirus vaccine be affordable for the populations that need it most?	Accurate estimates of the safety, efficacy and length of protection of norovirus vaccine candidates.
Target Population	The disease and economic burden is highest among children and the elderly. Indirect effects of norovirus vaccine in nontarget groups may be substantial.	How will vaccination impact the disease burden in children and the elderly? What are the relative direct and indirect impacts of different vaccination strategies?	Modeling studies of the impact of vaccination by age group and setting, including indirect effects.
	Childhood vaccination schedules already contain many different vaccines.	How effective and acceptable will a vaccine be if administered at the same time as other vaccines?	Trials of pediatric-formulated vaccines to evaluate efficacy and potential interference when administered at the same time as other vaccines.
Public Knowledge & Acceptance	The majority of the public does not know what norovirus is and perceives AGE as a mild illness.	What are the most effective ways to raise public knowledge about the severity and prevalence of norovirus?	Surveys on public knowledge of norovirus and potential norovirus vaccine acceptance.
			Public awareness and educational campaigns about the severity and prevalence of norovirus.