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Prospects and Challenges in the Development of a Norovirus Vaccine

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Abstract

Norovirus is the leading cause of acute epidemic gastroenteritis among children under the age of five years and adults in the US and in adults worldwide, accounting for an estimated 20% of episodes of acute gastroenteritis across all ages. No effective vaccine is currently available. Two candidate vaccines have reached clinical trials, and a number of other candidates are in preclinical stages of development. This article provides an overview of the current state of norovirus vaccine development, emphasizing barriers and challenges to the development of an effective vaccine, correlates of protection used to assess vaccine efficacy and the results of clinical trials of the major candidate vaccines.

Keywords

Norovirus; vaccine; acute gastroenteritis; calicivirus; prevention

Introduction

Norovirus is the leading cause of epidemic acute gastroenteritis in children and adults in the United States, resulting in more than 19–21 million episodes of illness, a thousand reported outbreaks, 2 million office visits, 70,000 hospitalizations, and up to 800 deaths in the US each year, with up to 50% increases in these numbers during years in which a new pandemic strain emerges [1–2]. Surveillance studies suggest that norovirus causes approximately 20% of acute gastroenteritis in US children under the age of 5, and that norovirus gastroenteritis in this age group now exceeds the rate of rotavirus gastroenteritis and incurs an estimated 273 million USD in annual treatment costs [3–4]. Globally, norovirus is estimated to cause 699 million illnesses and 219,000 deaths annually, representing one-fifth of episodes of

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Conflicts of Interest

NWCP and SR report no conflicts of interest.

acute gastroenteritis across all ages and resulting in over 4 billion USD in direct medical costs and over 60 billion USD in indirect societal costs, the latter of which are overwhelmingly due to lost productivity [5–6]. Rates of illness requiring medical care and mortality rates are particularly high among children under the age of 5 and adults over 65, respectively [1].

Given this burden of illness, analyses modeling cost-effectiveness and economics of a norovirus vaccine are unsurprisingly favorable. Bartsch and colleagues [7] demonstrated that vaccinating 95% of the US population with a human norovirus vaccine costing $25 and conferring protection from norovirus in 75% of recipients for at least 48 months would have the potential to reduce net healthcare expenditures in the US by up to 2.1 billion dollars, while also preventing more than 2 million episodes of norovirus gastroenteritis annually. Based on the vaccine cost, the proportion of vaccine recipients protected, and the duration of protection conferred, the cost per case averted varied significantly, from -$303 to $3315. Tallant and colleagues [8] estimated that vaccinating US troops against norovirus would either be cost-neutral or possibly cost-saving and could be more cost-effective than vaccination for common bacterial causes of gastroenteritis. With the potential to reduce overall healthcare costs while preventing a significant burden of human disease, norovirus is an attractive target for vaccine development.

We review the current status of and existing challenges to the development of an effective norovirus vaccine, describe meaningful correlates of protective efficacy for norovirus vaccine candidates, and examine the status of the major norovirus vaccine candidates currently in development.

Norovirus Virology and Possible Barriers to the Development of an Effective Norovirus Vaccine

Noroviruses are single-stranded RNA viruses in the family *Caliciviridae* and are divided into seven genogroups based on phylogenetic analysis of the entire virus genome or of individual virus genes. Genogroups I (GI) and II (GII) are the most prevalent causes of human disease (Table 1). Each genogroup is further divided into genotypes based phylogenetic analysis of capsid (Figure 1) and polymerase gene sequences [9]. Mutations as well as recombination within and between norovirus genotypes in co-infected patients lead to the periodic emergence of new norovirus variants as well as broad genetic and antigenic diversity of circulating norovirus strains. This genetic diversity poses a potential challenge to the development of a broadly protective norovirus vaccines, as some studies show that immunization and natural infection with a norovirus may elicit immunity specific to that norovirus' genogroup [10–11]. The emergence of GII genotype 4 (GII.4) variants that have caused new global pandemics suggests that evolution of the capsid gene can help the virus escape immunity induced by infection or vaccination [12]. However, serologic studies have shown that many individuals have cross-reactive, putatively protective serum antibodies that recognize new norovirus variants years before such variants emerge [13–14]. Genogroup II (GII) noroviruses cause over 90% of norovirus disease in the US, with GII.4 noroviruses causing 50–80% of disease from year-to-year [2]. Accordingly, while some of the early

vaccine efforts were based solely on the first discovered human norovirus, genogroup I genotype I (GI.1), current vaccine development efforts have been directed towards GII.4 noroviruses, with or without the inclusion of a GI norovirus antigen.

The norovirus genome contains three open reading frames, encoding a large polyprotein encompassing the nonstructural norovirus proteins, the major capsid protein VP1, and a minor stabilizing protein VP2, respectively [15]. Norovirus VP1 contains a highly conserved shell (S) domain stabilizing the viral particle's icosahedral structure and a variable protruding (P) domain, which is composed of a moderately conserved P1 subdomain and a highly variable P2 subdomain facilitating the norovirus particle's binding to host cell glycans and other receptors [16]. When expressed in eukaryotic cells, the norovirus VP1 capsid protein spontaneously self-assembles into virus-like particles (VLPs), which are morphologically and antigenically similar to the complete viral particle [17]. These VLPs have been shown to induce norovirus-specific antibodies when administered by the parenteral, oral or intranasal routes, and are the basis for several candidate norovirus vaccines [18–20]. When a modified P domain of the norovirus capsid protein that has been end-linked with oligopeptide containing cysteine residues is expressed in cell culture, it can dimerize and further aggregate into a subviral particle (P particle) that has also been proposed as a candidate vaccine [21].

The host receptor for human noroviruses has not been definitively determined. However, histo-blood group antigens (HBGAs) have been identified as attachment cofactors critical to the establishment of norovirus infection and clinical disease. HBGAs are present on the surface of all human mucosal epithelial cells and are essential for norovirus entry into host cells. Individuals who lack specific HBGAs due to the absence of a functional fucosyltransferase 2 (FUT2) enzyme are innately resistant to infection with GII.4 and GI.1 noroviruses, although they can be susceptible to infection with other genotypes, reflecting the diversity of glycan binding between noroviruses [22]. The presence of antibodies that block the interaction between norovirus particles or VLPs and HBGAs has been proposed as a correlate of protection from norovirus infection; this is discussed in greater detail below.

There are several possible barriers to the development of an effective norovirus vaccine. Circulating noroviruses are antigenically diverse and continually evolving, which could limit the durability of protection conferred by a vaccine that does not elicit broadly neutralizing antibodies. Differences in immune response to natural norovirus infection, discussed in detail below, suggest that immunizations eliciting a robust immune response in adults may not be equally efficacious in children. Similarly, severe complications of norovirus disease, including death, are more likely to occur among the elderly and patients with immunocompromising conditions, two groups for which the patient's underlying impairments in immune function may limit vaccine efficacy. Finally, as epidemiologic studies suggest that immunity after natural norovirus infection may be limited to as little as two months, developing a vaccine that confers a duration of protection adequate to make vaccination cost-effective may be challenging [23]. More recent modeling studies using age-specific norovirus incidence data from the UK suggest that the probable duration of post-exposure norovirus immunity may range from 4 to 9 years [24], although no clinical study has assessed persistence of norovirus immunity over this period. The duration of a norovirus

vaccine's protective efficacy is also likely to depend on the outcome used to define efficacy (e.g. the development of detectable infection, the development of diarrhea, or the development of clinically severe diarrhea requiring clinical evaluation or hospitalization).

Preclinical investigations of therapeutics for human noroviruses have long been limited by the lack of an affordable and relevant small animal model and by the lack of an *in vitro* culture system for human norovirus infection. Recent breakthroughs have overcome the latter obstacle. We have recently demonstrated that human noroviruses replicate and produce infectious progeny particles in the differentiated enterocytes of human intestinal enteroids, and that based on viral genotype, infection either requires or is enhanced by bile acids [25]. This norovirus culture system has been used to demonstrate successful inactivation of norovirus by heat and radiation as well as abrogation of norovirus infectivity by the addition of human serum containing HBGA-blocking antibodies. Karst and colleagues have also reported replication of human noroviruses in the BJAB B cell line, which requires the addition of bacterial cofactors supplied either in unfiltered norovirus stool inocula, by addition of heat-inactivated *Enterobacter cloacae* to the B cell media during infection, or by infection of B cells cultured below polarized HT-29 cells grown on transwells; this system has also been used to demonstrate viral inactivation [26–27]. These efforts are enabling the development of assays to determine whether antibodies induced by vaccination can abrogate human norovirus infectivity *in vitro*, which in turn may help speed the progress of candidate vaccines from preclinical to clinical trials.

Correlates of Norovirus Vaccine Protection

Identification of an immune correlate of protection, defined as a surrogate marker of immune function known to correlate with protection from disease following vaccination or natural infection [28], can greatly facilitate vaccine development and has been useful to norovirus vaccine development as a surrogate clinical endpoint measure. Experimental human infection studies found that although total serum norovirus-specific antibodies increase following infection, pre-infection antibody levels were not associated with protection from disease [23, 29]. However, total levels of serum norovirus-specific antibodies, as measured by ELISA, were inversely associated with the development of norovirus gastroenteritis in at least one epidemiological study, suggesting that these antibodies may be protective against norovirus disease or may serve as a marker of prior exposure and protection against reinfection induced by another mechanism [30]. Given that earlier challenge studies were performed prior to the recognition that carriage of a functional FUT2 enzyme and HBGA expression profile mediate host susceptibility, a potential explanation for the lack of correlation is that the results may have been confounded by the inclusion of individuals who had low serum antibody titers to norovirus because they were innately (genetically) resistant to norovirus infection.

The first clear correlate of protection for norovirus gastroenteritis, identified using samples from a norovirus challenge study, was the presence of antibodies blocking binding of norovirus VLPs to HBGAs, detected either by an ELISA glycan blocking assay or by a hemagglutination inhibition assay (Table 2) [31–32]. A norovirus challenge study among recipients of a VLP-based Norwalk virus vaccine further confirmed acquisition of serum

HBGA-blocking and norovirus-specific IgA antibodies following vaccination as correlates of protection [33]. Interestingly, although prechallenge levels of serum norovirus-specific IgA and HBGA-blocking antibodies correlated with disease protection among placebo and vaccine recipients in this study, the threshold titer for disease protection was higher for vaccine recipients than for placebo recipients, raising the possibility that the serum pool of HBGA-blocking antibodies may not be a mechanistic correlate of protection against infection. Similar differences in thresholds of serum antibodies acquired naturally and following vaccination have been reported for hemagglutination inhibition antibodies in influenza [34].

Levels of norovirus-specific IgA in saliva and feces and norovirus-specific memory IgG cells have also been identified as correlates of protection (Table 2). In a norovirus human experimental infection study, higher prechallenge levels of salivary IgA and virus-specific memory IgG cells were associated with a decreased frequency in the development of gastroenteritis following norovirus challenge, while prechallenge fecal IgA levels were associated with a lower peak fecal viral load after norovirus [35].

Immunization of mice with norovirus VLPs has demonstrated that GI VLPs fail to produce antibodies with blocking activity against GII noroviruses and vice versa [10]. A genogroup-specific induction of HBGA-blocking antibodies has also been demonstrated after natural infection in children [11]; however, infection with Norwalk virus (GI.1) in adults induces modest heterotypic HBGA-blocking activity against multiple genotypes including GII.4 [14]. In a cross-challenge study, norovirus-infected volunteers were immune 6–15 weeks later to rechallenge with the same inoculum but not to inocula of unrelated norovirus strains, suggesting a limited role for cross-protection in natural infection [36]. On the other hand, in a recent phase 1 clinical trial, immunization with a multivalent GI.1 and GII.4 VLP-based vaccine elicited broadly reactive antibodies with activity against norovirus strains not included in the vaccine and fold-changes in antibody titer for non-vaccine genotypes similar to those induced to GI.1 and GII.4 noroviruses [37]. It remains an open question, then, whether antigenic drift and the emergence of new epidemic norovirus genotypes will limit a norovirus vaccine’s efficacy or require regular updating of the norovirus vaccine to match circulating virus strains, as is done with the vaccine for seasonal influenza, or if the multivalency of some candidate norovirus vaccines will induce adequately broad norovirus immunity.

The duration of a norovirus vaccine’s protective efficacy also is an open question, one that will have a significant impact on the value of a norovirus vaccine from a cost-effectiveness standpoint [7]. In subjecting human volunteers to repeated challenge with Norwalk virus, Parrino et al demonstrated that naturally acquired immunity to noroviruses appears to last between two months and two years, and the development of serum antibodies specific for norovirus does not necessarily confer protection from recurrent infection; however, these findings should be interpreted with caution, as levels of HBGA-blocking antibodies were not specifically assessed, and the inclusion of non-secretors innately nonsusceptible to Norwalk virus infection may have confounded the study [23]. A later study rechallenging volunteers with Norwalk virus at regular six-month intervals demonstrated that protective immunity develops from repeated exposure and is present six months after the last episode of illness;

interestingly, in this study disease protection was associated with increases in norovirus-specific serum antibodies [29]. However, it is not evident that the duration of protection demonstrated by these challenge studies, in which each dose was likely several magnitudes greater than the typical infectious dose to which individuals are exposed in the community, is correlated with the duration of protection from community-acquired norovirus disease. Epidemiologic data suggest that natural infection with GII.4 noroviruses in children may protect against clinically evident acute gastroenteritis with recurrent infection [38]; however, another study found that infection with one norovirus genotype did not confer protection from or obviate clinical symptoms during infection with other norovirus genotypes [39–40]. Given that cross-protective immunity appears variable between norovirus genogroups and genotypes, the duration of immunity conferred in practice would likely vary depending on the persistence versus drift in the circulating norovirus strains.

Candidate Norovirus Vaccines in Development

Several norovirus vaccine candidates are in development; a selection of these vaccines, which have either progressed to clinical trials or have numerous preclinical investigations reported in the published literature, are presented in Table 3. Two groups are developing VLP-based vaccines; one, based on a combination of GI.1 and GII.4 VLPs, is under development by Takeda Pharmaceuticals and is in human trials, while the other, based on a mixture of GI.3 and GII.4 VLPs, remains in preclinical development. Two other groups have developed vaccines based on recombinant adenovirus serotype 5 vectors expressing norovirus VP1; one, being developed by Vaxart, Inc, is based on a G1.I norovirus sequence and is currently in phase 1 clinical trials, while the other, developed by a group at the Chinese Center for Disease Control and Prevention, is based on a GII.4 sequence. A fifth group has published extensive preclinical data on a GII.4 P-particle based vaccine, which has not yet progressed to human trials [41]. More limited preclinical research into possible norovirus vaccines has been conducted into other vaccine vectors and in the production of VLPs by a variety of methods, including expression in mammalian and plant cells, bacteria, and yeast. Live attenuated and inactivated norovirus particle vaccines have not yet been created, in large part due to the prior lack of an *in vitro* culture system for human noroviruses. With two norovirus candidate vaccines in human trials and *in vitro* culture of human noroviruses still quite expensive, development of a vaccine based on complete norovirus particles seems unlikely in the immediate future. Select candidate vaccines are discussed individually below.

GI.1/GII.4 VLP vaccines

The initial experience with a norovirus VLP-based vaccine in humans consisted of oral administration of unadjuvanted GI.1 norovirus VLPs; 83% of recipients had a four-fold increase in virus-specific serum IgG titers, and no adverse reactions occurred [42]. This led to development of a VLP vaccine adjuvanted with monophosphoryl lipid A (MPL) and the mucoadhesin chitosan, formulated for intranasal (IN) delivery as two divided doses administered twice three weeks apart. This vaccine initially was studied in a phase 1, double-blind, placebo-controlled study and was found to induce norovirus-specific IgG and

IgA memory B cells as well as norovirus-specific IgG and IgA; no serious vaccine-related adverse events occurred [43–44].

A randomized double-blinded, placebo-controlled trial studied the ability of the IN-delivered vaccine to prevent infection and illness [33]. After subjects received two doses of vaccine or placebo, each was challenged with ~10 human infectious doses of Norwalk virus. Receipt of the vaccine was associated with a 32% absolute reduction in risk of gastroenteritis (37% vs. 69%, p=0.006). Of note, this protection correlated with increases in norovirus-specific antibody levels including IgA, as well as increases in serum HBGA-blocking antibodies. Vaccine recipients who developed gastroenteritis had a longer incubation period from challenge to symptom onset versus placebo recipients (4.3 hours, p=0.02), but there was no reduction in total duration of symptoms among ill infected participants. Local nasal symptoms such as nasal discharge, stuffiness, itching and sneezing were more common after the second dose in the vaccine arm versus placebo; however, there were no serious or severe vaccine-associated adverse events.

The next approach studied was delivery of VLP vaccine by the intramuscular route, chosen for ease of administration and the potential to elicit a more rapid and robust immune response. In addition, the prevalence of GII.4 noroviruses led to the addition of a GII.4 VLP component to the GI.1 VLPs to make a bivalent vaccine. Preclinical studies showed that the addition of a GII.4 “consensus” VLP, designed from sequences of 3 different previously isolated GII.4 norovirus strain variants, to the GI.1 Norwalk VLP created a vaccine that induced broadly reactive antibodies able to recognize heterologous GI.3, GII.1, GII.3, and GIV.1 noroviruses [45].

The bivalent GI.1 and consensus GII.4 VLP vaccine was adjuvanted with MPL and aluminum hydroxide and reformulated as a series of two intramuscular injections [46]. In a randomized, placebo-controlled clinical trial of this vaccine, immunization lead to development of G1.1 and GII.4 specific serum antibody responses that peaked at day 7; most subjects had increases in HBGA-blocking antibody titers. Vaccination also elicited plasmablasts, antibody-secreting cells, and memory B cells specific to the norovirus vaccine strains [47–48]. Dose escalation did not provide higher levels of norovirus-specific antibodies. High levels of HBGA-blocking antibodies developed in all age groups (18–49, 50–64, and 65–85 years old) after the first vaccine dose, with little additional boosting in titers after the second dose. No serious adverse events with vaccination were observed. Analysis of sera from the subjects in this trial demonstrated that immunization could produce antibodies with broad activity against GII.4 noroviruses, including novel strains not included in the consensus sequence [37].

The bivalent vaccine was evaluated in a challenge study to assess its protective efficacy to a GII.4 norovirus [49]. In this randomized, double-blinded, placebo-controlled trial, 63 persons received the norovirus vaccine, and 64 received a placebo vaccine; of these, 56 of 63 and 53 of 64 individuals participated in the challenge phase, during which subjects were challenged with 4400 reverse transcription polymerase chain reaction units of a GII.4 norovirus variant not included in the consensus GII.4 sequence. Vaccine recipients developed increases in total norovirus-specific Ig levels. Although the primary endpoint of

the challenge portion of the study was not achieved, the infection and illness attack rates were much lower than expected in the placebo group (62.5% and 37.5%, respectively), adversely affecting the power of the study to detect differences between vaccine and placebo groups. Receipt of the norovirus vaccine versus placebo was associated with modest decreases in the incidence of severe, moderate, and mild gastroenteritis, all of which trended towards but none of which reached statistical significance. However, using a modified Vesikari score as a global assessment of disease severity, vaccination was associated with milder symptoms after norovirus challenge (4.5 +/− 2.1 points in the vaccine group versus 7.3 +/− 2.0 points in the placebo group). Neither time from challenge to symptom onset nor duration of norovirus illness was reduced among vaccine recipients. No severe adverse events were reported.

In summary, these studies demonstrate that a norovirus VLP-based vaccine can prevent norovirus illness, and possibly infection, when administered intranasally or intramuscularly. Takeda Vaccines, Inc., is continuing to pursue a bivalent GI.1/GII.4 vaccine in Phase II clinical trials. Different formulation parameters are being tested, including the ideal dosing of GI.1 and GII.4 VLPs to balance the vaccine's tolerability and immunogenicity, as well as the need for the adjuvants [50]. Vaccine safety and immunogenicity are also being evaluated in different age groups (children and elderly) (NCT02153112, NCT02661490, NCT03039790), and a phase IIb field efficacy study is currently underway in military recruits (NCT02669121).

Adenovirus vector-based GI.I VP1 vaccine

Guo and colleagues at the Chinese Center for Disease Control and Prevention demonstrated that intranasal vaccination with a recombinant serotype 5 adenovirus vector expressing a GII.4 norovirus VP1 was immunogenic in mice, producing norovirus-specific IgG and IgA in serum as well as in feces and intestinal and respiratory mucosa [51]. They further demonstrated that combining vaccination with their adenovirus vector with booster vaccination using norovirus VLPs could enhance this immune response [52].

Vaxart, Inc., is developing an oral adenovirus vector-based norovirus vaccine. VXA-GI.1-NN uses a replication-defective adenovirus serotype 5 vaccine vector expressing the norovirus major capsid protein VP1 from the GI.I Norwalk virus. No published data are currently available regarding the safety, immunogenicity, or protective efficacy of VXA-GI.1-NN in preclinical or clinical trials. However, Vaxart has reported on the safety and immunogenicity of an oral vaccine to H1N1 influenza vaccine expressed in the same adenovirus-vectored platform [53]. A press statement from Vaxart recently reported completion of their Phase 1 trial of VXA-GI.1-NN (NCT02868073; results not yet publically available) and states that the vaccine reached its safety and immunogenicity endpoints, with no serious adverse events and significant increases in serum norovirus blocking antibody titers among recipients of a single dose of the vaccine [54]. Because the Vaxart vaccine is based on a GI.1 norovirus VP1, a genotype responsible for a minority of the episodes of norovirus illness in the US, and because other clinical studies have not consistently shown that immunization induces a robust immune response to heterotypic

norovirus strains, development of a multivalent vaccine is likely to be necessary to confer broad protection against norovirus infection.

Vaccines and other efforts in preclinical development

Another VLP-based norovirus vaccine is under development by Vesikari and colleagues. The initial formulation of the vaccine, consisting of a VLP derived from a 2010 GII.4\2009 (New Orleans) norovirus, was first described in 2011, when it was shown to be effective in stimulating a norovirus-specific antibody response in mice [55]. Vaccination resulted in elevation of norovirus-specific IgG levels in serum and feces as well as elevations in titers of HBGA-blocking antibodies including HBGA-blocking activity against heterologous norovirus strains. A subsequent animal study using a trivalent vaccine including GII.4 and GI.3 norovirus VLPs in addition to rotavirus VP6 antigen, delivered by intramuscular route, also showed immunogenicity [56]. Of note, antibodies blocking HBGA binding to GI.3 norovirus VLPs were not induced when mice were immunized with GII.4 VLPs alone and vice-versa in this study, suggesting that multivalent vaccination may be necessary to ensure a broadly protective immune response.

The incorporation of rotavirus VP6 nanotubes as a delivery vehicle for norovirus VLPs is a unique feature of the vaccine being developed by Vesikari and colleagues. These nanotubes facilitate entry of vaccine components into antigen-producing cells as well as the elaboration of proinflammatory cytokines that might aid in acquisition of protective immunity, potentially reducing the need for other adjuvants while allowing a single vaccine to confer immunity to multiple gastrointestinal pathogens [57].

Like norovirus VLPs, P particles can produce norovirus-specific antibodies including antibodies blocking VLP binding to HBGAs; however, unlike VLPs, P particles can be easily produced in *E.coli* [41]. This ease of particle production at scale offers potential cost advantage over VLP-based vaccines and may expand the proportion of the population for whom receipt of a norovirus vaccine would be cost-effective. Additionally, the P particle is at least somewhat permissive to alteration of the norovirus P-domain, a feature that has been exploited to induce immunity to viruses other than norovirus. In one study, chimeric P particles produced by insertion of the rotavirus VP8* into the norovirus VP1 P domain produced HBGA-blocking antibodies in immunized mice while also inducing a neutralizing antibody response to rotavirus, suggesting that P particles may be an alternative avenue of development for a dual rotavirus-norovirus vaccine [58]. Another P-particle construct inserting the influenza M2e gene in the norovirus VP1 P domain broadened the spectrum of influenza immunity conferred when coadministered with a traditional inactivated influenza vaccine in chickens [59–60]. A third P-particle construct combining the P domains of norovirus and hepatitis E virus viral capsids effectively induced both HBGA-blocking antibodies and hepatitis E virus-specific antibodies when given to mice as an intranasal immunization [61].

A challenge study of human norovirus infection in gnotobiotic pigs evaluated the P-particle based norovirus vaccine. Kocher and colleagues [62] administered either VLPs or P-particles, both derived from GII.4 strain VA387, to newborn animals in three intranasal

doses, or alternately caused a primary infection with an oral norovirus challenge. A subset of animals from each group received a GII.4 oral norovirus challenge. Pigs immunized with either VLPs or P particles had postchallenge occurrence of diarrhea reduced by 46.7% and 60%, respectively, comparable to the 82.9% reduction in diarrhea among animals that had received natural immunity by prior infection. However, vaccination did not result in statistically significant reductions in mean duration of diarrhea among diarrheic animals. The P-particle vaccine has not yet been evaluated in human subjects.

A number of groups are pursuing vaccine development efforts other than the generation of new vaccine candidates. Norovirus VLPs have been successfully generated in embryonated chicken eggs, mammalian cells, plant cells, and yeasts in various systems [63–68]. In many of these reports, the VLPs produced were used in animal vaccination studies and shown to be immunogenic as has been previously shown with traditionally produced VLPs [63, 65, 69]. At least one of these constructs has been tested in human subjects. In 2000, Tacket et al administered transgenic potatoes expressing norovirus capsid protein that spontaneously assembled into VLPs within the tuber to human volunteers; after 2–3 doses, 95% of subjects had significant increases in norovirus-specific IgA antibody secreting cells, and 30% had had norovirus-specific IgA detected in stool [70]. Such technology may lead to more efficient and inexpensive means to produce large quantities of VLPs, potentially increasing both the cost-effectiveness and global reach of a norovirus vaccine.

Adjuvant technologies of specific interest to norovirus vaccine development are also being actively explored. Toll-like receptor (TLR) agonists other than MPL have been examined as possible adjuvants to increase the mucosal immune response of intranasal norovirus VLP vaccines. Velasquez and colleagues showed that the addition of the imidazoquinoline-based TLR7 agonist gardiquimod to an intranasally delivered GI.I norovirus vaccine enhanced production of norovirus-specific serum IgG as well as norovirus-specific IgA in saliva, small intestine, feces, and respiratory and genitourinary tracts in immunized mice [69]. These findings suggest that addition of adjuvants that direct the host immune response toward IgA/mucosal immunity might further enhance the efficacy of existing candidate vaccines. One limitation to intranasal routes of vaccination is mucociliary clearance, a host defense which may limit the host's duration of exposure to the antigen. However, addition of an in situ gelling dry powder produced from inert *Aloe vera* polysaccharides (GelVac) to an intranasally delivered norovirus vaccine has been shown to increase production of norovirus-specific serum and mucosal antibodies in immunized animals versus liquid formulation [71]. Immune response to the dry powder coformulated VLP vaccine was not further enhanced by addition of gardiquimod, suggesting that a GelVac-based norovirus VLP vaccine may be effective and potentially better tolerated than adjuvanted alternatives.

Conclusion and Future Directions for Norovirus Vaccine Development

Recombinant expression of norovirus capsid protein in numerous systems has led to several promising norovirus vaccine candidates based on virus-like particles and P-particles. Animal and human studies of bivalent VLP vaccines suggest that multivalent vaccination may be an effective strategy to induce broadly neutralizing antibodies protective against challenge with novel and heterologous norovirus strains. Future studies will need to address whether current

vaccine candidates induce protection against disease caused by heterologous noroviruses (i.e., by strains not included in the vaccine) and whether the breadth and robustness of the vaccine's response will be adequate to contend with the diversity and shifts in circulating norovirus genotypes. Future work will also need to address whether these vaccines are equally efficacious in different age groups and in persons with immune compromising conditions and whether the immunity conferred will be sufficiently long-lasting to justify (clinically or economically) the vaccine's adoption. Immune correlates of protection against norovirus infection and disease need to be further developed to facilitate these studies, as most of the currently identified correlates of protection have not been validated in large challenge studies, nor have the degrees to which these correlates covary been assessed.

The burden of norovirus disease globally and in the US is substantial. An effective norovirus vaccine has accordingly great potential to reduce the direct and indirect societal costs of acute gastroenteritis and relieve human suffering. Additional trials of candidate norovirus vaccines intended to confirm their tolerability and efficacy in human subjects are indicated and are underway.

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MKE is named as an inventor on patents related to cloning of the Norwalk virus genome and has served as a consultant to Takeda Vaccines, Inc. RLA has received research grant funding from and is an unpaid consultant to Takeda Vaccines, Inc.

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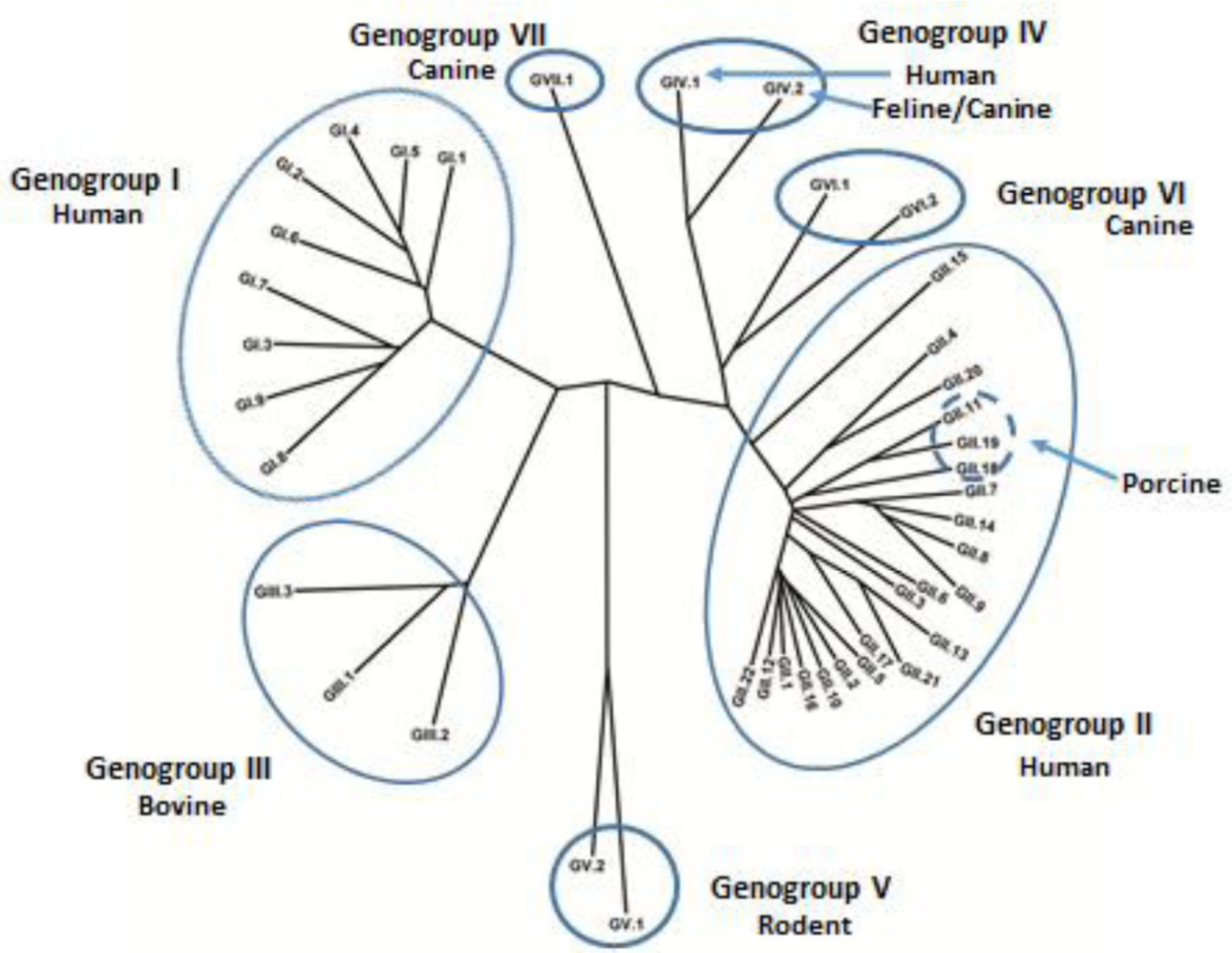

Figure 1.

Norovirus phylogeny, based on amino acid sequencing of the major capsid protein VP1. Norovirus host range is largely determined by genogroup, although host specificity varies between genotypes for genogroup II (human, porcine) and genogroup IV (human, feline, and canine) noroviruses.

Table 1

Norovirus Genogroups and Genotypes Associated With Human Disease

Genogroup	Genotypes
I	GI.1 – GI.9
II	GII.1 – GII.10, GII.12-GII.17, GII.20-GII.22
IV	GIV.1

Table 2

Immune Correlates of Protection From Human Noroviruses

Immune Correlate	Prevention of or reduction in clinical illness	Prevention of or reduction in documented infection	References
Serum HBGA-blocking antibody	√	√	24, 63
Serum hemagglutination inhibition antibody	√		23
NV-specific salivary IgA	√		26
NV-specific fecal IgA		√	26
NV-specific memory IgG cells	√		26

Table 3

Select Human Norovirus Vaccine Candidates

Investigators	Vaccine Candidate	Route of Administration	Norovirus Genotypes Included in Vaccine	Current Status:
In clinical trials:				
Takeda Vaccines	Norovirus VLP	Intramuscular (previously formulated as intranasal)	GI.1, GII.4	Phase IIb clinical trials
Vaxart	Recombinant Adenovirus expressing norovirus VP1	Oral	G1.1	Phase I clinical trials
Undergoing preclinical investigation:				
Vesikari and colleagues	Norovirus VLP	Intramuscular or Intradermal	G1.3, GII.4	Preclinical (animal studies)
Guo and colleagues	Recombinant Adenovirus	Intranasal	GII.4	Preclinical (animal studies)
Jiang and colleagues	Norovirus P particle	Intranasal	GII.4	Preclinical (animal studies)