MAJOR ARTICLE

Norovirus Vaccine Against Experimental Human GII.4 Virus Illness: A Challenge Study in Healthy Adults

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(See the editorial commentary by Vesikari and Blazevic on pages 853–5 and the major article by Sakon et al on pages 879–88.)

Background. Vaccines against norovirus, the leading cause of acute gastroenteritis, should protect against medically significant illness and reduce transmission.

Methods. In this randomized, double-blind, placebo-controlled trial, 18- to 50-year-olds received 2 injections of placebo or norovirus GI.1/GII.4 bivalent vaccine-like particle (VLP) vaccine with 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL) and alum. Participants were challenged as inpatients with GII.4 virus (4400 reverse transcription polymerase chain reaction [RT-PCR] units), and monitored for illness and infection.

Results. Per protocol, 27 of 50 (54.0%) vaccinees and 30 of 48 (62.5%) controls were infected. Using predefined illness and infection definitions, vaccination did not meet the primary endpoint, but self-reported cases of severe (0% vaccinees vs 8.3% controls; $P = .054$), moderate or greater (6.0% vs 18.8%; $P = .068$), and mild or greater severity of vomiting and/or diarrhea (20.0% vs 37.5%; $P = .074$) were less frequent. Vaccination also reduced the modified Vesikari score from 7.3 to 4.5 ($P = .002$). Difficulties encountered were low norovirus disease rate, and inability to define illness by quantitative RT-PCR or further antibody rise in vaccinees due to high vaccine-induced titers. By day 10, 11 of 49 (22.4%) vaccinees were shedding virus compared with 17 of 47 (36.2%) placebo recipients ($P = .179$).

Conclusions. Bivalent norovirus VLP vaccine reduced norovirus-related vomiting and/or diarrhea; field efficacy studies are planned.

Clinical Trials Registration. NCT01609257.

Keywords. norovirus; vaccine; challenge; acute gastroenteritis.

Noroviruses are the leading cause of epidemic and sporadic outbreaks of gastroenteritis worldwide [1], and the most important cause of foodborne illness [2]. They cause up to 71 000 hospitalizations and 800 deaths annually [3]. Since rotavirus vaccine introduction, noroviruses have become the leading cause of medically attended acute gastroenteritis in US children, associated with nearly 1 million healthcare visits annually [4].

Norovirus is a genus within the Caliciviridae family, classified into at least 6 genogroups (GI–GVI) and further into 29 genotypes based on the major viral capsid protein (VP1), including the 3 genogroups that infect humans (GI, GII, and GIV) [5]. Vaccine development is a challenge because of the diversity and low cross-reactivity between genogroups, which is further hampered

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by the inability to culture the virus and the lack of small animal models that mimic human disease [6].

Experimental norovirus vaccines composed of virus-like particles (VLPs) expressing VP1 are generally well tolerated and immunogenic when administered either orally [7, 8], intramuscularly [9], or intranasally [10]. A norovirus GI.1 monovalent VLP vaccine protected against illness and infection following challenge with homologous GI.1 Norwalk virus [11], prompting development of a bivalent GI.1/GII.4 VLP vaccine [12], as these 2 genogroups account for the majority of norovirus outbreaks [13–17]. The GII.4 component is a consensus VLP derived from 3 naturally occurring GII.4 variants that induced broadly reactive antibody responses in animal studies and may induce a multivalent response against different genotypes [12].

METHODS

Study Design

We conducted a randomized, double-blind, placebo-controlled trial at 5 sites in the United States from May 2012 to July 2013. The study protocol was approved by the institutional review board of each site, registered at ClinicalTrials.gov (NCT01609257), and performed according to current Good Clinical Practices and Declaration of Helsinki guidelines. Co–primary objectives were to (1) compare the rates of norovirus acute gastroenteritis (AGE) following challenge with a norovirus GII.4 strain in vaccine and placebo recipients, and (2) evaluate the safety and tolerability of 2 doses of the investigational vaccine.

Subjects

Eligible subjects were healthy adults 18–50 years of age of either sex, able to provide written informed consent, and available for the study follow-up visits. Exclusion criteria included working in healthcare, food services, or childcare; being pregnant or nursing; close contact with persons at risk of complications from norovirus infection; abnormal results of physical and common laboratory evaluations; positive test results for human immunodeficiency virus, hepatitis B virus, hepatitis C virus, or bacterial and parasitic stool infections. To ensure susceptibility to the challenge virus, only subjects with a functional *FUT2* gene determined by the presence of salivary histo-blood group antigens (HBGAs) were included, after screening for enzyme-linked immunosorbent assay (ELISA) antibodies to the P domain of the challenge strain, with a titer cutoff of ≤1:1600 for eligibility [18].

Vaccine

The vaccine formulation contained 100 μg of norovirus VLPs (50 μg each of GI.1 and GII.4 VLPs), produced in a baculovirus expression system and adjuvanted with 50 μg of 3-*O*-desacyl-4′-monophosphoryl lipid A adjuvant (3-O-desacyl-4′-monophosphoryl lipid A, GlaxoSmithKline) and 0.5 mg of aluminum hydroxide (Brenntag Biosector A/S, Denmark) per dose, administered in 0.5 mL by intramuscular injection. The GI.1 component was a Norwalk virus VLP [11], the GII.4 component was designed by aligning 3 GII.4 capsid protein sequences and determining the "consensus" amino acid residues at each position as previously described [12]. Placebo was sterile saline (0.5 mL).

Norovirus Challenge Strain and Challenge Procedure

The challenge strain was from a 2003 clinical sample previously identified by reverse transcription polymerase chain reaction (RT-PCR) as a GII.4 norovirus strain (GII.4 Farmington Hill variant) that bound to the A, B, and H HBGAs, and was free of other pathogens [18]. Virus concentration of the final challenge pool was approximately 4.4×10^4 RT-PCR units/mL. Based on unreported studies, a challenge dose of 4.4×10^3 RT-PCR units was chosen, as this dose produced similar rates of infection (26/34 [76.5%]) and illness (19/34 [55.9%]) as a 10-fold higher dose [18].

Eligible participants were randomized (1:1) to receive study vaccine or placebo, stratified by clinical site, with 2 doses injected in the deltoid 4 weeks apart. Solicited adverse events (AEs) were collected for 7 days and unsolicited AEs for 28 days after each study dose. Serious adverse events (SAEs) were collected for 1 year after last study dose. Serum samples for immunogenicity analyses were collected before administration of each dose and 4 weeks after the second study dose, and serum samples for laboratory safety analyses were collected 14 days after each immunization.

At least 28 days after the second study dose, eligible participants were admitted to an inpatient challenge facility. Eighty percent were challenged by day 42, with a range up to day 196. They were given a clear liquid meal 5 hours before challenge, and could have clear liquids until 90 minutes before challenge when all intake was stopped. Approximately 2 minutes after drinking 60 mL of 2% sodium bicarbonate solution to buffer stomach acids, subjects drank the challenge virus in 80 mL of sterile water, followed 5 minutes later by another 60 mL of 2% sodium bicarbonate solution. They then refrained from eating and drinking for at least 90 minutes.

After challenge, subjects remained in the inpatient facility for a minimum of 96 hours and until they were free of vomiting or diarrhea for ≥18 hours. They were evaluated twice daily by an investigator, and the severity of each prespecified sign and symptom was graded by the subject on a scale of 0 (no change in health) to 3 (incapacitating or unable to perform usual activities). A prespecified modification of the Vesikari scoring scale was also applied, based on the number of episodes and duration of vomiting and diarrhea, the presence of fever, and the need for intravenous rehydration [11]. All stool and emesis specimens were collected, graded, and weighed. Aliquots from 1 representative stool sample per subject were collected on days 1–4, 10, and 30 postchallenge. The stools were then processed and stored for testing for norovirus shedding. Additional serum samples taken before and 30 days after challenge were tested for

anti-GII.4 norovirus antibodies. All subjects had access to oral rehydration solution. Before discharge, abnormal clinical laboratory analyses were repeated, and followed to resolution or stability.

Illness Definitions

Acute gastroenteritis was predefined as diarrhea (≥3 loose or liquid stools or >400–600 g of loose or liquid stools produced in any 24-hour period) or vomiting (≥2 vomiting episodes in any 24-hour period), or 1 vomiting episode plus any loose or liquid stool in any 24-hour period, or 1 vomiting episode plus at least 2 of the following 5 events: nausea, oral temperature ≥37.6°C, abdominal cramps or pains, abdominal gurgling or bloating, or myalgia in any 24-hour period. Norovirus GII.4 infection was to be confirmed by seroresponse (≥4-fold rise in anti-GII.4 norovirus P particle antibody titer from prechallenge to postchallenge) or fecal virus excretion detected by quantitative RT-PCR (qRT-PCR) on any day postchallenge. Norovirus disease met both criteria.

Laboratory Assays

Monoclonal antibody–based saliva binding assays using antibodies specific for salivary Le^b, Le^y, and H type 1 HBGAs (Covance Inc) were used to select secretor-positive subjects [19, 20]. Serum antibodies to the GII.4 P particle were measured for screening and determination of infection [18, 21], and seroresponses to the vaccine were measured using a total serum antibody ELISA [11].

Whole stool specimens were tested for the presence of the norovirus challenge strain (GII.4 Farmington Hills) with a strain-specific TaqMan-based qRT-PCR assay targeting the P2 region of the major capsid gene, with viral load determined against a standard curve of RNA transcripts.

Statistical Analysis

A sample size of 60 per group was selected to have approximately 45 subjects per group challenged, which would provide ≥95% power in comparing AGE illness rates between groups assuming a 50% illness rates in placebo recipients ([17] and unpublished data) and an efficacy of 70%. Illness rates and infection rates were compared between groups by Fisher exact test. Start and stop times of AGE were determined by a committee review of coded listings of individual subject clinical signs and symptoms. Severity of illness was based on a previously reported modification of the Vesikari scale, with a maximum possible score of 17 [11]. Severity and duration were compared between groups by the Wilcoxon rank-sum test. Time to onset was compared between groups using a log-rank test and was limited to those who were ill and infected. Viral shedding was assessed by analysis of variance of the logarithmic transformation of the area under the curve defined by viral load by day postchallenge.

Geometric mean titers (GMTs) and geometric mean fold rises of antibodies were compared between groups by Wilcoxon rank-sum test, and seroresponse rates (percentages of subjects with ≥4-fold rises) were compared between groups by Fisher exact test. All reported *P* values are 2-sided. All data analyses and statistical computations were conducted with SAS software (version 9.3).

RESULTS

Study Population and Demographics

Of the 132 secretor-positive volunteers who were enrolled and randomized, 127 (63 vaccine and 64 placebo) received both study injections; 23 of these subjects did not participate in the

Table 1. Study Subject Enrollment and Disposition[a]

Characteristic	Vaccine	Placebo	Total
Enrolled and randomized	67	65	132
Received 2 doses of vaccine or placebo			
Yes	63	64	127
No	4	1	5
Reasons for discontinuation			
Adverse event (rash on forearm and abdomen)	0	1	1
Voluntary discontinuation by subject	2	0	2
Lost to follow-up	2	0	2
Challenged			
Yes	56	53	109
No	7	11	18
Reasons not challenged			
Adverse events (elevated glucose, LFTs)	2	1	3
Voluntary discontinuation by subject	2	3	5
Investigator decision based on protocol exclusion criteria: (history of bulimia, IV infusion, started taking oral antibiotics for acne treatment)	1	2	3
Lost to follow-up	1	2	3
Other	1	3	4
3 subjects unable to comply			
1 no-show for challenge			
Per-protocol challenge population	50	48	98
Lost to follow-up		1	1
No prechallenge serum samples, or sera collected out of window	2	1	3
Missing >1 required stool sample	2		2
Emesis immediately following challenge	1		1
Bicarbonate not received prior to challenge	1		1
Illness while traveling outside of country		1	1
No postchallenge serum sample		1	1
Early inpatient discharge		1	1

Abbreviations: IV, intravenous; LFTs, liver function tests.

[a] Total screened, n = 1007; total not randomized, n = 875.

Table 2. Demographics and Subject Disposition in the Study Stages

	All Vaccinated Subjects		Per Protocol, Challenged Subjects	
Characteristic	Vaccine (n = 67)	Placebo (n = 65)	Vaccine (n = 50)	Placebo (n = 48)
Sex, No. (%)				
Male	35 (52)	33 (51)	29 (58)	25 (52)
Female	32 (48)	32 (49)	21 (42)	23 (48)
Ethnicity, No. (%)				
Non-Hispanic	65 (97)	63 (97)	48 (96)	47 (98)
Hispanic	2 (3)	2 (3)	2 (4)	1 (2)
Race, No. (%)				
American Indian/Alaskan Native	1 (1.5)	0	0	0
Asian	4 (6.0)	2 (3.1)	2 (4.0)	2 (4.2)
Hawaiian/Pacific Islander	0	0	0	0
Black/African American	37 (55.2)	36 (55.4)	31 (62.0)	26 (54.2)
White	25 (37.3)	26 (40.0)	17 (34.0)	19 (39.6)
Multiracial	0	1 (1.5)	0	1 (2.1)
Other/unknown	0	0	0	0
Age at challenge, y				
Mean (SD)	32.8 (10.2)	32.1 (9.3)	33.2 (10.3)	33.0 (9.3)
Median	31.0	29.0	32.0	32.0
Min, Max	(18, 49)	(18, 49)	(18, 49)	(18, 49)

Abbreviation: SD, standard deviation.

challenge stage. Subsequently, 109 (56 vaccine and 53 placebo) subjects were challenged with oral norovirus GII.4, and 98 were eligible for the per-protocol analysis as identified by a blinded review by the clinical evaluation committee. Reasons for all attritions are shown in Table 1. Results in per-protocol and modified intent-to-treat populations were similar, so only per-protocol evaluations are presented except for lab abnormalities.

Demographic data for vaccine and placebo groups were similar for both the vaccine stage and challenge stage, with a slight predominance of male sex and black race (Table 2).

Safety and Tolerability

There were no SAEs reported throughout the study period (excluding severe acute gastroenteritis due to challenge virus). Injection site reactions, predominantly tenderness, were frequent in vaccinees (34/50 [68.0%]) compared with placebo recipients (21/48 [43.8%]), but were mainly mild to moderate, lasting <4 days (Figure 1*A*). Systemic reactions were reported by 21 of 50 (42.0%) vaccinees and 18 of 48 (37.5%) placebo recipients (Figure 1*B*). Fever was detected in 1 vaccinee (2.0%) and 2 placebo recipients (4.2%). Clinical laboratory abnormalities were infrequent and occurred in similar proportions in vaccine and placebo groups. During the vaccination stage, 10 laboratory abnormalities (5 in vaccinees and 5 in placebo recipients) in 6 subjects were considered clinically significant. Only 3 of these were considered to be possibly related to vaccination (all occurred in the same subject): grade 3 elevated direct and total bilirubin and grade 1 elevated alanine aminotransferase occurring at the day 14 follow-up after the second dose. All laboratory abnormalities returned to normal.

Vaccine Immunogenicity

The first immunization produced robust responses against GI.1 and GII.4 as measured 28 days after the immunization (Table 3). Notably, the fold increases in ELISA GMTs after the first vaccination were greater for GI.1 (62.2; 95% confidence interval [CI], 42.8–90.5) than for GII.4 (10.8; 95% CI, 7.3–15.8). There was little, if any, detectable increase in antibody titers following the second vaccination. After 1 dose, the GI.1 seroresponse rate was 100% (49/49), and remained 100% after the second dose, compared with only 2.1% (1/48) of the placebo recipients. Against GII.4, the seroresponse rate of 83.7% (41/49) after 1 dose increased to 89.8% (44/49). After the second vaccine dose, only 1 of 48 (2.1%) placebo recipients developed a seroresponse.

Efficacy

After challenge, 57 of the 98 subjects were infected, as evidenced by detection of the challenge virus in fecal specimens: 27 of 50 (54.0%) vaccinees and 30 of 48 (62.5%) placebo recipients (Table 4). Seroresponses (4-fold increase in immunoglobulin G P-particle titers) after challenge were detected in 26 of 30 (86.7%) placebo recipients who shed virus, but in only 4 of 27 (14.8%) vaccinees who shed virus. Using the predefined definitions of norovirus gastroenteritis, based on the number of episodes and volume of diarrhea and vomitus as well as selected

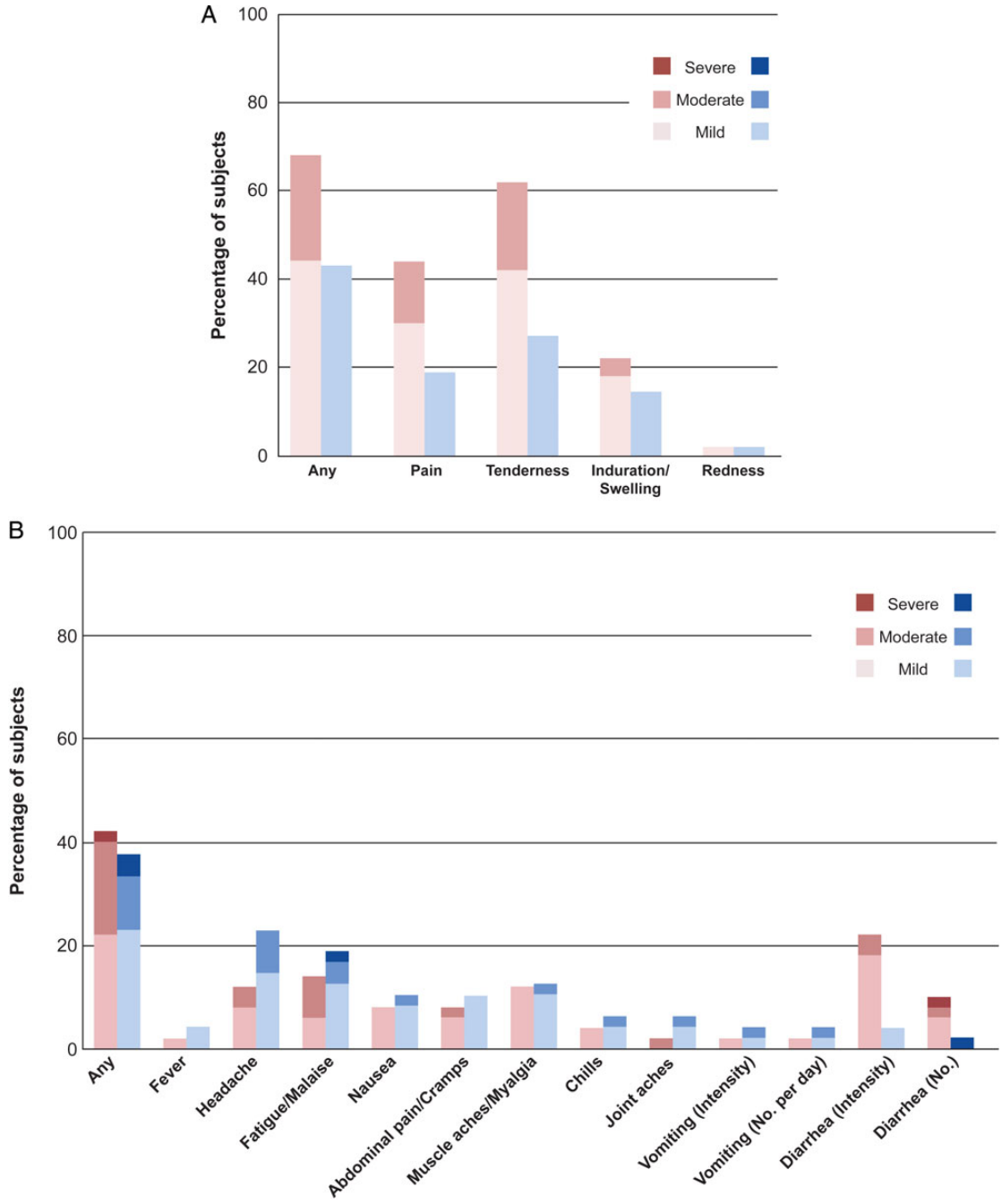

Figure 1. Reactions to vaccination shown as percentage of subjects with solicited local (*A*) and systemic (*B*) adverse reactions. Reaction severity is indicated by shading. Column 1, vaccine recipients; column 2, placebo recipients.

symptoms, the vaccine did not significantly reduce the incidence of protocol-defined illness, with 13 (26.0%) cases among vaccinees and 16 (33.3%) in placebo recipients (Table 4). Vaccination appeared to reduce the incidence of acute norovirus gastroenteritis based on vomiting and diarrhea reported by the subjects (Table 4): Severe vomiting or diarrhea was reported by 4 of 48 (8.3%) placebo recipients and 0 of 50 vaccinees ($P = .054$), moderate or severe vomiting or diarrhea by 9 of 48 (18.8%) placebo recipients and 3 of 50 (6.0%) vaccinees ($P = .068$), and vomiting or diarrhea of mild or greater severity by 18 of 48 (37.5%) placebo recipients and 10 of 50 (20.0%) vaccinees ($P = .074$).

To eliminate any bias in identification of norovirus in each group, we also compared the incidence of all reported illnesses regardless of meeting the criteria for norovirus infection (Table 4). The results were the same except for the comparison of vomiting

Table 3. Immune Responses as Total Enzyme-Linked Immunosorbent Assay Immunoglobulin Against the Norovirus GI.1 and GII.4 Serotypes Before and After Vaccination, and After Challenge in Placebo Recipients

	GI.1			GII.4			Infected Placebo Recipients 30 d Postchallenge	
	First Dose		Second Dose	First Dose		Second Dose		
	Day 0 Predose	Day 28 Postdose 1	Day 56 Postdose 2	Day 0 Predose	Day 28 Postdose 1	Day 56 Postdose 2	GI.1	GII.4
Total Ig GMTs (95% CI)								
Vaccine	n = 50	n = 49	n = 49	n = 50	n = 49	n = 49		
	2023 (1399–2923)	128 819 (100 421–165 248)	120 023(95 697–150 533)	5412 (3928–7456)	60 011 (45 410–79 307)	54 354 (43 360–68 135)		
Placebo	n = 48	n = 47	n = 48	n = 48	n = 47	n = 48	n = 30	n = 30
	1759 (1186–2607)	1551 (3323–6609)	1590 (1045–2418)	4496 (3254–6212)	4686 (3323–6609)	5120 (3524–7440)	1769 (1111–2815)	40 024 (28 211–56 786)
Geometric mean fold rise from baseline (95% CI)								
Vaccine	. . .	62.2 (42.8–90.5)	58.0 (41.2–81.6)	. . .	10.8 (7.3–15.8)	9.9 (7.1–13.8)		
Placebo	. . .	0.9 (.8–1.0)	0.9 (.8–1.0)	. . .	1.1 (.9–1.2)	1.1 (1.0–1.4)	1.6 (1.2–1.9)	9.8 (6.4–15.2)
Seroresponse rate (95% CI)								
Vaccine	. . .	100 (92.7–100)	100 (92.7–100)	. . .	83.7 (70.3–92.7)	89.8 (77.8–96.6)		
Placebo	. . .	0 (0–7.5)	2.1 (.1–11.1)	. . .	2.1 (.1–11.3)	2.1 (.1–11.1)	13.3 (3.8–30.7)	86.7 (69.3–96.2)

Abbreviations: CI, confidence interval; GMTs, geometric mean titers; Ig, immunoglobulin.

or diarrhea of mild or greater severity, which was now significant ($P = .028$). Notably, all cases of moderate or greater vomiting or diarrhea were confirmed to be infected. Using the modified Vesikari scale as a global assessment, the severity of disease was reduced from 7.3 ± 2.0 in the placebo group to 4.5 ± 2.1 in the vaccine group in those who became ill and infected ($P = .002$). Neither the duration of norovirus illness nor the time to onset of norovirus illness was significantly different in vaccinees with breakthrough disease compared with placebo recipients.

The primary study endpoint required confirmation of norovirus infection by at least 1 of 2 different assays. Quantitative RT-PCR identified infection in both study groups, but at a rate that was somewhat lower than in previous challenge studies [11, 18], and both symptomatic and asymptomatic infections were identified. One subject who self-reported mild illness did not become positive for infection by qRT-PCR until the outpatient phase of the study. As prechallenge antibody titers were high in vaccine recipients, apparently peaking at a higher level than that induced by the challenge, a 4-fold rise in GII.4 antibodies following norovirus challenge could only be demonstrated in placebo recipients; only 2 vaccinees vs 16 placebo recipients (88% reduction) had protocol-defined illness plus a 4-fold rise in GII.4 antibody in the per-protocol population ($P < .001$). Seven subjects (2 vaccinees and 5 placebo) met 1 of the protocol definitions of illness but were negative for norovirus infection by either the stool qRT-PCR or serological assay.

Fecal shedding of challenge virus (Figure 2) peaked on days 3–4 after challenge. The quantity of virus was higher in those who became ill compared with those who remained asymptomatic according to protocol definitions after infection (analysis of variance log [area under the curve]; $P = .005$), although both symptomatic and asymptomatic infected subjects shed virus over a wide range of values. One vaccine recipient was negative for viral shedding by qRT-PCR during the inpatient phase of the study, but positive for a 4-fold increase in antibody titer. The subject later became positive by qRT-PCR during the outpatient phase. Vaccinees appeared to clear virus more rapidly so that mean concentrations on day 4 and later were lower than those in placebo recipients. On day 10, 11 of 49 (22.4%) vaccinees were shedding virus compared with 17 of 47 (36.2%) placebo recipients ($P = .179$).

DISCUSSION

In this first efficacy evaluation of a candidate bivalent VLP norovirus vaccine, the vaccine was found to be well-tolerated and immunogenic, and appeared to reduce acute gastroenteritis (vomiting and/or diarrhea) induced by challenge with GII.4 norovirus as reported by the subjects. Similarly, the vaccine reduced the severity of disease when evaluated using the modified Vesikari score, from 7.3 to 4.5 ($P = .002$).The vaccine was more protective against severe forms of acute gastroenteritis than

Table 4. Measurements of Vaccine Efficacy (Per-Protocol Populations)

Measurement	Vaccine (n = 50)	Placebo (n = 48)	Relative Reduction, % (95% CI)	*P* Value
NV infected[a]	27 (54.0%)	30 (62.5%)	13.6 (–21.0 to 38.3)	.420[b]
NV infected and ill[a]	13 (26.0%)	16 (33.3%)	22.0 (–44.3 to 57.8)	.509[b]
Severity of norovirus vomiting or diarrhea by subject assessment				
Severe vomiting and/or diarrhea and infected	0	4 (8.3%)	100	.054[b]
Moderate to severe vomiting and/or diarrhea and infected	3 (6.0%)	9 (18.8%)	68 (–11.2 to 90.8)	.068[b]
Vomiting and/or diarrhea any severity and infected	10 (20%)	18 (37.5%)	47 (–3.6 to 72.5)	.074[b]
Severity of vomiting or diarrhea by subject assessment				
Severe vomiting and/or diarrhea	0	4 (8.3%)	100	.054[b]
Moderate to severe vomiting and/or diarrhea	3 (6.0%)	9 (18.8%)	68 (–11.2 to 90.8)	.068[b]
Vomiting and/or diarrhea, any severity	10 (20%)	20 (41.7%)	52.0 (8.3–74.9)	.028[b]
Modified Vesikari severity score, mean (SD)				
NV infected	3.0 (2.1)	4.9 (3.0)		.016[c]
NV infected and ill	4.5 (2.1)	7.3 (2.0)		.002[c]
NV illness duration, h, mean (SD)	33.6 (17.1)	42.9 (20.1)		.154[c]
Time to NV illness onset, h, mean (SD)	33.2 (13.0)	36.9 (15.4)		.538[d]

Data are presented as No. (%) unless otherwise specified.

Abbreviations: CI, confidence interval; NV, norovirus; SD, standard deviation.

[a] Infection = norovirus identified by polymerase chain reaction and or antibody rise, illness as defined per protocol.

[b] Fisher exact test.

[c] Wilcoxon test.

[d] Log-rank test.

against mild disease, consistent with other vaccines. The increasing efficacy against more severe disease is similar to what has previously been seen with rotavirus vaccines [22]. Part of the inability to demonstrate efficacy using the predefined criteria for gastroenteritis might reflect the fact that some subjects met the predefined criteria but did not self-report the presence of any vomiting or diarrhea. Furthermore, some subjects who met the predefined illness criteria were not confirmed to be norovirus-infected by either the qRT-PCR stool assay or the serological assay. This illustrates the complexity of conducting a challenge study and objectively measuring infection and illness. Vaccination offered several potentially beneficial effects for public health, including fewer subjects with symptomatic disease, and an observed decrease in viral shedding, both in terms of duration and quantity of virus shed.

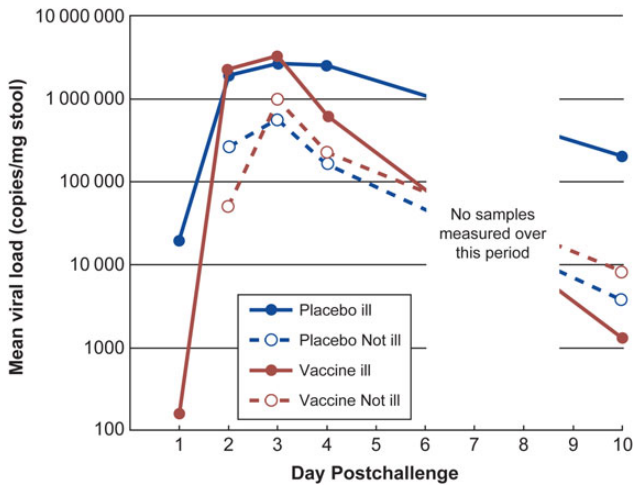

Figure 2. Viral load in stools (as viral count per milligrams of stool) in vaccine and placebo subjects according to whether they were ill (per-protocol definition) over the 10 days of postchallenge with norovirus GII.4.

Previously, the GI.1 component of the vaccine was shown to be efficacious against challenge with a GI.1 challenge strain, Norwalk virus, when administered by the nasal route [11]. In that study, vaccine reduced the incidence of Norwalk virus gastroenteritis by 47% (95% CI, 15%–67%), and reduced the severity of disease as assessed by the modified Vesikari score from 5.5 to 3.6 ($P = .009$) [11]. There are several important differences between the studies of monovalent vaccine and GI.1 challenge compared with the bivalent vaccine and GII.4 challenge reported here. These include (1) the route of immunization (intranasal vs intramuscular); (2) the challenge virus (homologous to vaccine vs heterovariant; the GII.4 challenge strain and the GII.4 antigen in the vaccine differed by 19 amino acids in the hypervariable domain of the norovirus capsid protein); (3) the definition of disease; and (4) the incidence of disease in the control group.

There are several issues in the epidemiology and immunology of norovirus that may be critical to vaccine development [23]. Genetic diversity of norovirus both between and within

genogroups might present a major challenge. Therefore, the evaluated candidate vaccine is bivalent to extend protection to both GI.1 and GII.4 viruses. Furthermore, the GII.4 component was derived using a consensus sequence with the intent of providing multivalent protection; previous work has suggested that infection with Norwalk virus (GI.1) can induce antibody that can block binding of other GI viruses [25]. Guinea pigs immunized with the consensus GII.4 VLP used in the present study induced antibodies that recognized other GII.4 VLPs from clinical samples representing 30 years of genetic drift [12]. Importantly, serum samples from other subjects who received the same bivalent vaccine have been found to have cross-blocking antibody to the GII.4 Sydney 2012 strain that emerged after vaccinations were completed (L. Lindesmith and R. Baric, personal communication). Additional studies will be required to determine the extent to which the candidate vaccine will provide multivalent protection against genetically drifted norovirus strains and genotypes not included in the vaccine. The optimization of illness definitions and diagnostic assays to attribute illness to norovirus will be important aspects of these future studies.

Remaining questions for the development of a safe and effective norovirus vaccine include immunogenicity and protection in different age groups, and the duration of protection that can be provided by vaccination. Mathematical modeling studies of community norovirus transmission suggest that protection from natural infection lasts 4.1–8.7 years [24]. Although duration of protection following vaccination remains to be determined, even short-lived protection could be beneficial for controlling symptoms and transmission in populations that are clustered in confined spaces, such as military personnel in barracks or cruise ship passengers, or in outbreak situations such as those which occur in nursing homes or hospitals.

There is increasing attention on norovirus-associated burden of illness and healthcare costs, with estimated hospitalization costs for pediatric norovirus infections of $3918 and norovirus-related emergency room visits at $435, with even higher costs in older adults [4]. A vaccine preventing moderate to severe disease may be expected to decrease hospitalizations and emergency room visits, and reduce associated healthcare costs, as seen with rotavirus vaccines. The vaccine used in the present trial may fulfill these criteria, but large field efficacy studies are needed to confirm the degree to which protection can be achieved against norovirus illness in the community, including vaccine efficacy studies in adults, children, and the elderly.

Notes

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Potential conflicts of interest. R. G., A. B., R. C., and P. M. M. are all full-time employees of the study sponsor; M. K. E. is a named inventor on patents related to cloning of the Norwalk virus genome and is a consultant to Takeda Vaccines (Montana) Inc; and D. I. B. and M. K. E. are consultants to Takeda. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. N Engl J Med **2009**; 361:1776–85.
2. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis **2011**; 17:7–15.
3. Centers for Disease Control and Prevention. Norovirus. http://www.cdc.gov/norovirus/trends-outbreaks.html. Accessed 24 April 2014.
4. Payne DC, Vinje J, Szilagyi PG, et al. Norovirus and medically attended gastroenteritis in U.S. children. N Engl J Med **2013**; 368:1121–30.
5. Green KY. Caliciviridae: the noroviruses. In: Knipe DM, Howley P, eds. Fields virology. 6th ed. Philadelphia: Lippincott Williams & Wilkins, **2013**, 582–608.
6. Richardson C, Bargatze RF, Goodwin R, Mendelman PM. Norovirus virus-like particle vaccines for the prevention of acute gastroenteritis. Exp Rev Vaccines **2013**; 12:155–67.
7. Ball JM, Graham DY, Opekun AR, Gilger MA, Guerrero RA, Estes MK. Recombinant Norwalk virus-like particles given orally to volunteers: phase I study. Gastroenterology **1999**; 117:40–8.
8. Tacket CO, Sztein MB, Losonsky GA, Wasserman SS, Estes MK. Humoral, mucosal, and cellular immune responses to oral Norwalk virus-like particles in volunteers. Clin Immunol **2003**; 108:241–7.
9. Treanor JJ, Atmar RL, Frey SE, et al. A novel intramuscular bivalent norovirus virus-like particle vaccine candidate-reactogenicity, safety, and immunogenicity in a phase 1 trial in healthy adults. J Infect Dis **2014**; 210:1763–71.
10. 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**; 202:1649–58.
11. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk virus illness. N Engl J Med **2011**; 365:2178–87.
12. 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**; 30:3580–6.
13. Mai H, Jin M, Guo X, et al. Clinical and epidemiologic characteristics of norovirus GII.4 Sydney during winter 2012–13 in Beijing, China following its global emergence. PLoS One **2013**; 8:e71483.
14. Hoa Tran TN, Trainor E, Nakagomi T, Cunliffe NA, Nakagomi O. Molecular epidemiology of noroviruses associated with acute sporadic gastroenteritis in children: global distribution of genogroups, genotypes and GII.4 variants. J Clin Virol **2013**; 56:185–93.

15. Lindesmith LC, Beltramello M, Donaldson EF, et al. Immunogenetic mechanisms driving norovirus GII.4 antigenic variation. PLoS Pathog **2012**; 8:e1002705.
16. Desai R, Hembree CD, Handel A, et al. Severe outcomes are associated with genogroup 2 genotype 4 norovirus outbreaks: a systematic literature review. Clin Infect Dis **2012**; 55:189–93.
17. Noel JS, Ando T, Leite JP, et al. Correlation of patient immune responses with genetically characterized small round-structured viruses involved in outbreaks of nonbacterial acute gastroenteritis in the United States, 1990 to 1995. J Med Virol **1997**; 53:372–83.
18. Frenck R, Bernstein DI, Xia M, et al. Predicting susceptibility to norovirus GII.4 using a human challenge model. J Infect Dis **2012**; 206:1386–93.
19. Huang P, Farkas T, Marionneau S, et al. Noroviruses bind to human ABO, Lewis, and secretor histo-blood group antigens: identification of 4 distinct strain-specific patterns. J Infect Dis **2003**; 188:19–31.
20. Marionneau S, Ruvoen N, Le Moullac-Vaidye B, et al. Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. Gastroenterology **2002**; 122:1967–77.
21. Yang Y, Xia M, Tan M, et al. Genetic and phenotypic characterization of GII-4 noroviruses that circulated during 1987 to 2008. J Virol **2010**; 84:9595–607.
22. Vesikari T. Rotavirus vaccination: a concise review. Clin Microbiol Infect **2012**; 18(suppl 5):57–63.
23. Atmar RL, Estes MK. Norovirus vaccine development: next steps. Exp Rev Vacc **2012**; 11:1023–5.
24. Simmons K, Gambhir M, Leon J, Lopman B. Duration of immunity to norovirus gastroenteritis. Emerg Infect Dis **2013**; 19:1260–7.
25. Lindesmith LC, Donaldson E, Leon J, et al. Heterotypic humoral and cellular immune responses following Norwalk virus infection. J Virol **2010**; 84:1800–15.