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Immunogenicity of a bivalent virus-like particle norovirus vaccine in children from 1 to 8 years of age: A phase 2 randomized, double-blind study

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ABSTRACT

Background: Young children can suffer severe consequences of norovirus gastroenteritis. We performed a dose-finding study of a bivalent virus-like particle (VLP) vaccine candidate (TAK-214) in healthy 1–8-year-old children.

Methods: In this phase 2 study two age cohorts (1–3 and 4–8 years of age inclusive, N = 120 per cohort) of children enrolled from Finland, Panama and Colombia were initially randomized 1:1:1:1 to four groups which were further split into two equal subgroups, to receive one or two intramuscular doses of four TAK-214 formulations containing 15/15, 15/50, 50/50 or 50/150 μg of GI.1/GII.4c genotype VLPs and 0.5 mg $Al(OH)_3$ at 28 days interval. ELISA Pan-Ig and histoblood group antigen-blocking (HBGA) antibodies against each VLP were measured on days 1, 29, 57 and 210. Parents/guardians recorded solicited local and systemic adverse events (AE) and any unsolicited or serious AEs (SAE).

Results: All formulations were well-tolerated across both age cohorts and dosage groups with no vaccine-related SAEs reported. Solicited AEs were mostly mild-to-moderate, resolved quickly, and did not increase after the second dose. Pan-Ig and HBGA responses induced after one dose were only slightly increased by the second dose. Across dose groups at Day 29 after one dose GI.1 Pan Ig seroresponse rates (SRR) were 82–97% and 81–96% and GII.4c SRR were 79–97% and 80–91% in 1–3 and 4–8 year-olds, respectively. Respective rates were to 92–93% and 73–92% for GI.1, and 77–100% and 62–83% for GII.4c at Day 57 following two doses. HBGA responses had similar profiles. Both Pan Ig and HBGA geometric mean titers persisted above baseline up to Day 210.

Conclusions: All dosages of TAK-214 displayed acceptable reactogenicity in 1–8-year-old children and induced robust, durable immune responses after one dose which are further increased after two doses.

1. Introduction

Infection with norovirus typically results in severe but self-limiting morbidity due to acute gastroenteritis (AGE) with severe nausea, vomiting and diarrhea. However, norovirus illness in the very young and the elderly can have more serious, indeed fatal, consequences [1]. Following the introduction of rotavirus vaccine, noroviruses have emerged as the single most significant cause of epidemic outbreaks of non-bacterial AGE worldwide [1,2]. This is most apparent in high-income countries with national childhood rotavirus immunization campaigns where decreased rotavirus disease means that norovirus is the major etiology for gastroenteritis hospitalizations in children up to 4 years of age [2–7], with an estimated 14,000 hospitalizations, 281,000 emergency department visits and 627,000 outpatient visits per year in the United States [4]. In low- and middle-income countries, norovirus AGE may be

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responsible for 218,000 deaths in children under five years each year [1]. There is no specific prophylaxis other than maintaining strict conditions of sanitary hygiene, and therapy consists of ensuring adequate hydration.

Takeda Vaccines has developed a bivalent virus-like particle (VLP) vaccine (TAK-214) against norovirus that has been proven to be safe and immunogenic in several clinical studies in adults [8–11]. The vaccine consists of two VLPs representing the predominant circulating strains of norovirus which are responsible for most human disease, GI.1 and GII.4c, with aluminum hydroxide as adjuvant. The GII.4c component is a consensus sequence of three different GII.4 genotype variants—2006a (Yerseke), 2006b (Den Haag) and 2002 (Houston)—and was designed to elicit a broad response against different GII.4 variants that occur due to antigenic drift [12]. In adults, one dose of TAK-214 has been shown to elicit a rapid immune response suggestive of an amnestic response indicating that most individuals have previous immunologic experience of norovirus infection [9,10].

As the dose and dosing regimen in young children has yet to be elucidated clinically we performed an age de-escalation study in children in Colombia, Finland and Panama to assess the safety, tolerability and immunogenicity of TAK-214. In this report we present the data from the first part of this study in 1–8-year-old children, in whom we also assessed the optimal dose and dosing regimen of TAK-214.

2. Methods

This phase 2 randomized, double-blind, trial was performed in 10 centers in three countries, Finland (8 sites), Colombia (1 site) and Panama (1 site). The study protocol was approved by all the IRBs at each study center and the relevant national authorities, and performed according to the Declaration of Helsinki and ICH Good Clinical Practice guidelines. Parents or guardians supplied written informed consent for their child to be enrolled. The study objective was to assess the safety, tolerability and immunogenicity of one or two doses of different formulations of a bivalent norovirus VLP vaccine (TAK-214) in two age cohorts (1–3 and 4–8 years of age, inclusive).

2.1. Participants

Eligible participants were 1 to 8-year-old children who were in good health based on medical history, physical examination at enrolment and the investigator's judgement, and who were available for the duration of the study. Major exclusion criteria were any infection at the time of enrolment or receipt of medications within 24 h of study start, any known or suspected allergy to vaccine components, any medical condition or treatment likely to lead to alteration of immune function, or participation in any other clinical trial or recent receipt of any other vaccines, within 14 days for inactivated vaccines or 28 days for live vaccines.

2.2. Study design

After screening, 120 participants in the first age group (Cohort 1), consisting of children aged from 4 to 8 years inclusive, were enrolled and randomized (1:1:1:1) using a sponsor-supplied schedule with an interactive web response system (IWRS) to four equal groups to receive one of four different formulations of TAK-214, and further randomized into two equal subsets in each group to receive one or two doses. On Day 1 each participant received a first vaccination with the group-assigned TAK-214 formulation, and on Day 29 participants received either a second vaccination or a saline placebo injection according to their assigned subset. Following observation of a satisfactory safety profile from the older age cohort, the procedure was then repeated with Cohort 2 comprising 120 children aged 1 to 3 years.

2.3. Vaccines

The study vaccine TAK-214 (batches 3-FIN-1497, 3-FIN-1897, or 3-FIN-1858; Althea Technologies Inc., San Diego, California, USA), is composed of four different mixtures as indicated of the two VLP antigens with 500 μg $Al(OH)_3$ in each 0.5 mL dose. The four formulations were 15 μg GI.1 and 15 μg GII.4c (15/15), 15 μg GI.1 and 50 μg GII.4c (15/50), 50 μg GI.1 and 50 μg GII.4c (50/50) and 50 μg GI.1 and 150 μg GII.4c (50/150). In one dose groups the second vaccination was replaced by a saline placebo injection (West-Ward Pharmaceuticals, Eatontown, New Jersey, USA). All injections were administered in the deltoid muscle or anterolateral thigh according to national guidelines.

2.4. Safety

Children were monitored for 30 min after each vaccination and a follow-up telephone contact was made on Day 3. For 7 days after each vaccination parents/guardians completed diary cards which solicited local reactions (injection site pain, erythema, induration, swelling), and systemic adverse events (irritability/fussiness, drowsiness, loss of appetite, vomiting, and diarrhea in 1–3-year-olds; headache, fatigue, myalgia, arthralgia, vomiting, and diarrhea in 4–8-year-olds). Parents/guardians recorded their child's body temperature each day (fever was defined as a body temperature $\geq$ 38.0 °C), as well as any unsolicited adverse events until the next study visit (28 days after each vaccination). Unsolicited AEs were subsequently coded using MedDRA (version 21.0) definitions. All adverse events were graded for severity (mild, moderate or severe) and for unsolicited AEs the relationship to the vaccination was assessed by the investigator. Throughout the duration of the study (through Day 210) serious adverse events (SAE) were collected and reported immediately by the investigator to the study sponsor.

2.5. Immunogenicity

Blood samples (2 mL) were drawn on Days 1, 29, 57 and 210 for blinded assessment of immune responses at the University of Tampere. Pan Ig antibodies against the two vaccine antigens, GI.1 and GII.4c were measured by ELISA and expressed as geometric mean titer (GMT) per group at each timepoint together with seroresponse rates (SRR; group proportions with 4-fold or greater increases in titer over baseline), and geometric mean-fold rises over baseline (GMFR). Responses were also measured as histoblood group antigen-blocking (HBGA) antibodies as previously described [14], expressed as 50% blocking titers (BT_{50}) GMTs and SRR.

2.6. Statistics

This trial was designed to be descriptive and was not based on testing formal null hypotheses, and therefore the sample size was not determined based on formal statistical power calculations but was considered adequate to assess the primary and secondary endpoints to make decisions for further clinical development.

Safety analyses were descriptive. The primary safety endpoints were proportions of each study group with solicited local reactions and systemic AEs for 7 days post-vaccination, unsolicited AEs for 28 days post-vaccination, and SAEs throughout the study duration. These were analyzed in the Safety Analysis Set (SAS) which comprised all participants who received at least one dose of TAK-214 vaccine or placebo and provided post-vaccination data.

The primary immunogenicity endpoints were the SRR measured as GI.1 and GII.4c ELISA Pan Ig antibodies at Day 57 after one or two doses of TAK-214 in the Per-Protocol Analysis Set (PPS) which comprised all participants who received their planned vaccination and had no major protocol violations. Secondary immunogenicity endpoints included GI.1 and GII.4c Pan Ig GMTs and fold-increases, and GI.1 and GII.4c histoblood group antigen (HBGA) blocking titers which were analyzed in the Per Protocol Set. All statistical analyses were performed with SAS Version 9.2 or higher.

3. Results

A total of 258 children were screened of whom 18 (3 in the 4–8 years and 15 in the 1–3 age groups, respectively) were excluded before enrolment, principally due to protocol-defined exclusion criteria and 4 who were withdrawn by their parents (Fig. 1). All 120 enrollees in each age cohort received their first vaccination on Day 1 and were included in the Safety Set. Seven children did not receive their second administration of vaccine or placebo on Day 29, and a further four were withdrawn by their parents before the Day 57 blood draw (28 days after the second vaccination) (see Fig. 2).

Demographics of the study population shown in Table 1 illustrate that most groups had similar compositions in terms of sex and ethnicity, except for a lower proportion of males in the 15/50 groups. Approximately half the participants were described as White and Hispanic.

3.1. Safety

Vaccination with TAK-214 appeared to be safe and generally well-tolerated. There were no vaccine-related SAEs reported and solicited AEs reported by 46 (77%) of 4–8-year-olds and 36 (59%) of 1–3-year-olds were primarily mild or moderate in severity. One participant from the 1–3-year-old cohort (15/15 group) was withdrawn following two AEs which were considered unrelated to the vaccination; the participant had a lower respiratory tract infection and a white blood cell disorder. A total of six unrelated SAEs were reported, three in each age cohort. In the 4–8-year-olds one child had community-acquired pneumonia after the first 50/150 dose, and two children had dengue fever after their second doses of 50/50 and 50/150 TAK-241, respectively. In the younger children, a 1-year-old girl in the 50/50 group had a furuncle after her first dose, and two children in the 50/150 group had gastroenteritis, one after the second vaccination and the other after receiving a placebo injection. All cases and conditions were reported as moderate, and all children recovered and were not withdrawn from the study.

Solicited local reactions mainly consisted of injection site pain which was approximately twice as frequent in the older cohort than the younger one after the first vaccination (Table 2). Reports of pain were less frequent after the second dose of TAK-214, or the placebo injection in both age cohorts, and were unaffected by the dose level of TAK-214 administered. The other solicited local reactions, erythema, induration and swelling, were infrequent, typically occurring as single examples in each group, and mainly after the first or second vaccinations in the younger age cohort.

Solicited systemic AEs were reported after the first vaccination in 40–54% and 33–45% of the older and younger age cohorts, respectively, with no trends associated with the VLP doses (Table 3). The most frequent events were headache, fatigue and myalgia in the 4–8-year-olds and irritability, loss of appetite and drowsiness in the 1–3-year-old children. In the older children the frequency of most AEs decreased after the second vaccination or placebo injection, but headache, fatigue and myalgia remained the most prevalent. In the younger cohort rates of reported irritability, drowsiness and loss of appetite were similar after both the first and second doses.

Unsolicited AEs were reported at similar rates across the dose groups in the four weeks after each vaccination, with no consistent trend associated with VLP dose and no event was considered to be related to the vaccination (Table 4). Rates after the second doses were also similar to those in the placebo groups. Overall, unsolicited AEs were more frequent in the younger cohort than the older children. In the 4–8-year-olds an unsolicited AE was reported in approximately 25% of the children after the first dose, 20% after a second vaccination and about 28% after a placebo injection. Respective frequencies were 43%, 32% and 33% after first, second and placebo administrations in the younger cohort. Severe unsolicited AEs were rare; one case of severe upper abdominal pain was reported in the older cohort after a second 50/150 dose. In the younger age cohort four children had severe unsolicited AEs; one with the lower respiratory tract infection and white blood cell elevation described above after a first 15/15 dose, one case of severe diarrhea after a first 50/150 dose, one child with blood in their urine after a second 15/50 dose and another case of lower respiratory tract infection after a second 50/150 dose. All cases resolved spontaneously without further sequelae. Other unsolicited AEs typically consisted of childhood conditions routinely encountered in the two age cohorts and were mainly due to infections; the most frequent were mild or moderate nasopharyngitis, fatigue, gastroenteritis, headache, cough and rhinitis.

3.2. Immunogenicity - Pan Ig

At baseline both age cohorts displayed geometric mean Pan Ig titers against both VLP antigens above the lower limit of quantitation (LLOQ). These were higher in the cohort of older children than the younger ones. In all 4–8-year-olds (n = 108) across groups these were 294 EU/mL (95% CI: 201–432) for GI.1 and 1279 EU/mL (948–1725) for GII.4c. In all 1–3-year-olds (n = 122) values were 75.4 EU/mL (54.2–105) for GI.1 and 371 EU/mL (256–538) for GII.4c. One month after the first vaccination there were marked increases in all groups against both antigens illustrated by the seroresponse rates (SRR) but with no consistent trend for dose-dependent differences for either antigen in either age cohort (Fig. 3).

At Day 29, four weeks after the first vaccination, the SRR against GI.1 across dose groups were 81–96% and 82–97% in older and

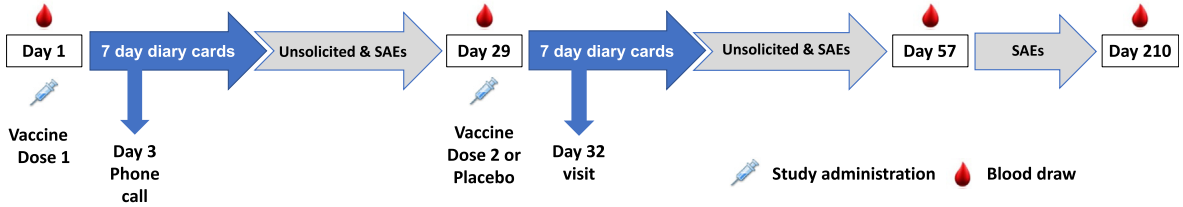

Fig. 1. Study design.

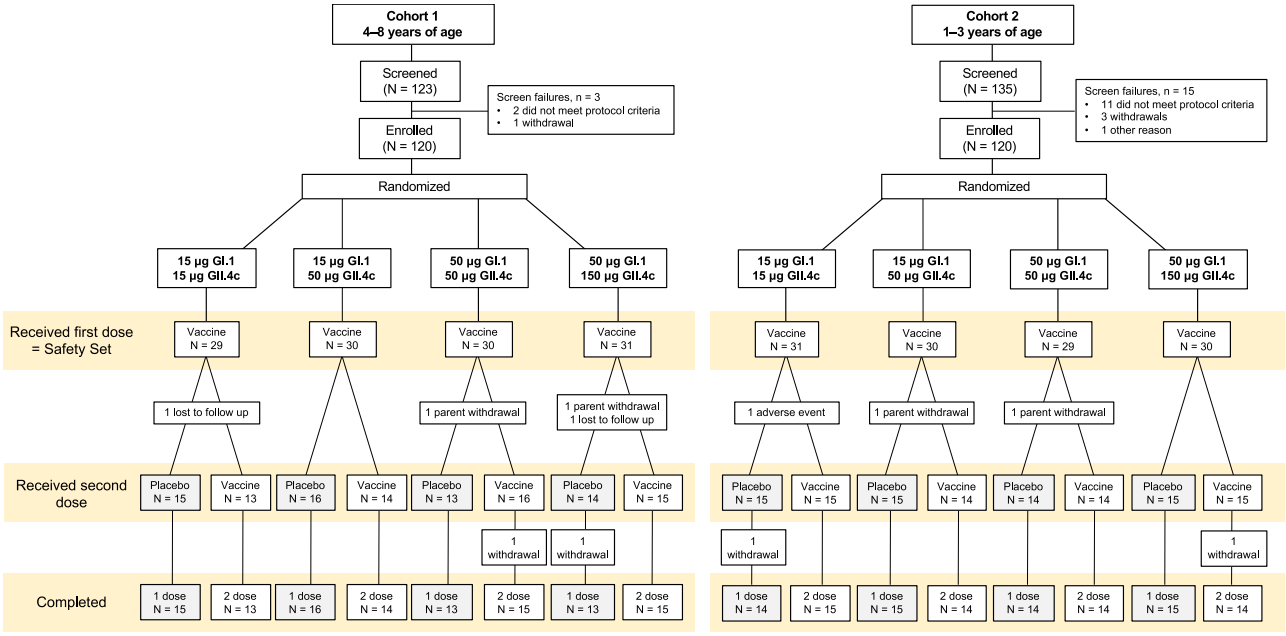

Fig. 2. Study flow charts.

Table 1
Demographics of the enrolled study populations.

	One dose groups				Two dose groups			
Group	**15/15**	**15/50**	**50/50**	**50/150**	**15/15**	**15/50**	**50/50**	**50/150**
4–8 years of age								
N =	**15**	**16**	**14**	**16**	**14**	**14**	**16**	**15**
Age ± SD (years)	5.9 ± 1.4	5.7 ± 1.1	6.3 ± 1.7	5.8 ± 1.6	5.7 ± 1.4	6.1 ± 1.4	5.9 ± 1.4	5.8 ± 1.5
Male: n (%)	7 (47)	4 (25)	8 (57)	9 (56)	10 (71)	9 (64)	7 (44)	9 (60)
Hispanic: n (%)	7 (47)	7 (44)	6 (43)	8 (50)	6 (43)	6 (43)	7 (44)	7 (47)
Non-Hispanic: n (%)	8 (53)	9 (56)	8 (57)	8 (50)	8 (57)	8 (57)	9 (56)	8 (53)
American Indian, n (%)	5 (33)	5 (31)	5 (36)	5 (31)	5 (36)	5 (36)	6 (38)	5 (33)
White, n (%)	8 (53)	9 (56)	8 (57)	8 (50)	8 (57)	8 (57)	9 (56)	8 (53)
Other, n (%)	1 (7)	2 (13)	1 (7)	2 (13)	1 (7)	1 (7)	1 (6)	2 (13)
1–3 years of age								
N =	**16**	**16**	**14**	**15**	**15**	**14**	**15**	**15**
Age ± SD (years)	2.0 ± 0.9	2.1 ± 0.8	2.0 ± 1.0	2.3 ± 0.8	2.3 ± 0.9	2.2 ± 0.9	2.0 ± 0.9	1.9 ± 0.9
Male: n (%)	9 (56)	6 (38)	7 (50)	10 (67)	9 (60)	7 (50)	10 (67)	8 (53)
Hispanic: n (%)	8 (50)	8 (50)	6 (43)	7 (47)	7 (47)	7 (50)	8 (53)	7 (47)
Non-Hispanic: n (%)	8 (50)	8 (50))	8 (57)	7 (47)*	8 (53)	7 (50)	6 (40)*	8 (53)
American Indian, n (%)	3 (19)	3 (19)	2 (14)	2 (13)	2 (13)	2 (14)	3 (20)	2 (13)
White, n (%)	8 (50)	7 (44)	8 (57)	8 (53)	8 (53)	6 (43)	7 (47)	8 (53)
Other, n (%)	5 (31)	5 (31)	4 (29)	5 (33)	5 (33)	5 (36)	5 (33)	5 (33)

* Not reported in one case each.

younger cohorts, respectively. In the older groups these rates were maintained to Day 57, but then declined by Day 210 together with a waning in GMTs (Fig. 4). In this older cohort, administration of the second dose did not increase the SRR, had only a small effect on the GMT, and did not prevent the decline in antibody titers. In the younger cohort, the SRR and GMTs were maintained at the initial Day 29 level through to Day 210, and a second vaccination resulted in small increases in GMTs which were maintained to Day 210. At Day 210 geometric mean anti-GI.1 titers across all one and two dose groups of younger children were similar to those observed in the older cohort; GMTs were 5086 EU/mL (95% CI: 4181–6187) and 4596 EU/mL (95% CI: 3843–5497) in 4–8 and 1–3-year-olds, respectively.

After one dose, Pan Ig SRR against GII.4c were 80–91% and 79–97% in older and younger cohort groups, and in the absence of a second dose these values declined to 36–54% and 31–60% by Day 210 (Fig. 3). Administration of a second dose had no impact on SRR or GMT (Fig. 4) in the older cohort, but partly delayed the decline in the younger cohort groups. By Day 210 the anti-GII.4c Pan Ig GMTs after two doses were 5014 EU/mL (95% CI: 3789–6663) in 4–8-year-olds and 3353 EU/mL (95% CI: 2147–5238) in 1–3-year-olds, with no general trend for a dose-dependent effect.

3.3. Immunogenicity - HBGA

In contrast to Pan Ig, baseline levels of HBGA against GI.1 were low and similar in both the older (19.4 $GMBT_{50}$ [95% CI:16.8–22.4]) and younger (17.6 $GMBT_{50}$ [95% CI:15.6–19.7]) cohorts, while levels against GII.4c were approximately twice as high in the older children vs. younger children: (87.4 $GMBT_{50}$ [95% CI:68.0–112]) vs. (36.7 $GMBT_{50}$ [95% CI:29.4–45.7]).

One month after the first dose 73–96% of 4–8-year-olds across the four dose groups responded to GI.1. These rates increased to 87–100% by Day 57 after one dose and were further increased to

Table 2
Incidence of solicited local reactions in the 7 days after the first and second doses of TAK-214 or placebo injection.

	First dose of TAK-214				Second dose of TAK-214				Second dose of Placebo			
Group	**15/15**	**15/50**	**50/50**	**50/150**	**15/15**	**15/50**	**50/50**	**50/150**	**15/15**	**15/50**	**50/50**	**50/150**
4–8 years of age												
N =	**29**	**30**	**30**	**31**	**14**	**14**	**16**	**15**	**15**	**16**	**14**	**16**
Any, n (%)	18 (62)	16 (53)	21 (70)	19 (61)	4 (29)	5 (36)	7 (44)	6 (40)	1 (7)	2 (13)	7 (50)	3 (19)
Pain	18 (62)	16 (53)	21 (70)	19 (61)	4 (31)*	5 (36)	7 (44)	6 (40)	1 (7)	2 (13)	7 (54)*	3 (21)**
Erythema	0	0	0	0	0	0	0	0	0	0	0	0
Induration	0	0	0	1 (3)	0	0	0	1 (7)	0	0	0	0
Swelling	0	0	1 (3)	1 (3)	0	0	0	1 (7)	0	0	0	0
1–3 years of age												
N =	**31**	**30**	**29**	**30**	**15**	**14**	**15**	**15**	**16**	**16**	**14**	**15**
Any, n (%)	12 (39)	7 (23)	8 (28)	5 (17)	6 (40)	3 (21)	2 (13)	4 (27)	3 (19)	3 (19)	1 (7)	3 (20)
Pain	12 (39)	7 (23)	7 (24)	5 (17)	6 (40)	3 (21)	2 (14)*	4 (27)	3 (20)*	3 (20)*	1 (7)	3 (20)
Erythema	2 (6)	1 (3)	1 (3)	1 (3)	1 (7)	0	0	0	0	0	0	0
Induration	0	1 (3)	0	1 (3)	0	0	0	1 (7)*	0	0	0	0
Swelling	1 (3)	1 (3)	0	0	2 (13)	0	0	0	0	0	0	0

From Tables 15.3.1.1 and 15.3.1.2.1.
* Data not available for one participant;
** Data not available for two participants.

Table 3
Incidence of solicited systemic AEs in the 7 days after the first and second doses of TAK-214, and placebo.

	First dose of TAK-214				Second dose of TAK-214				Second dose of Placebo			
Group	**15/15**	**15/50**	**50/50**	**50/150**	**15/15**	**15/50**	**50/50**	**50/150**	**15/15**	**15/50**	**50/50**	**50/150**
4–8 years of age												
N =	**29**	**30**	**30**	**31**	**14**	**14**	**16**	**15**	**15**	**16**	**14**	**16**
Any, n (%)	15 (54)*	15 (50)	15 (50)	12 (40)*	4 (31)*	5 (36)	6 (38)	5 (33)	3 (20)	3 (19)	3 (23)*	3 (21)**
Fever[a]	1 (4)	3 (10)	1 (3)	1 (3)	1 (8)	0	1 (6)	2 (13)	0	0	0	0
Headache	6 (21)	4 (13)	7 (23)	6 (20)	1 (8)	1 (7)	4 (25)	2 (13)	1 (7)	1 (6)	2 (15)	0
Fatigue	7 (25)	9 (30)	4 (13)	4 (13)	1 (8)	4 (29)	3 (19)	1 (7)	2 (13)	2 (13)	0	2 (14)
Myalgia	6 (21)	5 (17)	9 (30)	4 (13)	1 (8)	0	4 (25)	1 (7)	1 (7)	0	2 (15)	1 (7)
Arthralgia	1 (4)	1 (3)	2 (7)	1 (3)	0	0	0	0	0	0	1 (8)	0
Vomiting	3 (11)	0	1 (3)	3 (10)	0	0	0	0	1 (7)	0	0	0
Diarrhea	2 (8)	3 (10)	2 (7)	2 (7)	1 (9)	0	0	1 (7)	0	0	0	0
1–3 years of age												
N =	**31**	**30**	**29**	**30**	**15**	**14**	**15**	**15**	**16**	**16**	**14**	**15**
Any, n (%)	12 (39)	13 (43)	13 (45)	10 (33)	4 (27)	6 (43)	4 (29)*	7 (47)	5 (31)	8 (50)	1 (7)	3 (20)
Fever[a]	1 (3)	1 (3)	2 (7)	1 (3)	0	1 (7)	1 (7)	1 (7)	0	1 (7)	0	3 (21)*
Irritability	6 (19)	6 (20)	7 (24)	7 (23)	2 (13)	3 (21)	2 (13)	5 (33)	4 (27)	4 (27)	0	0
Drowsiness	5 (16)	3 (10)	5 (17)	4 (13)	1 (7)	1 (7)	2 (13)	1 (7)	1 (7)	2 (13)	0	1 (7)
Loss of appetite	1 (3)	7 (23)	9 (31)	8 (27)	1 (7)	4 (29)	2 (13)	5 (33)	3 (20)	2 (13)	1 (7)	1 (7)
Vomiting	2 (6)	1 (3)	3 (10)	0	0	0	0	1 (7)	0	0	0	1 (7)
Diarrhea	4 (13)	4 (13)	3 (10)	4 (13)	0	0	1 (7)	4 (27)	2 (13)	3 (20)	0	0

[a] Fever defined as body temperature $\geq$ 38 °C;
* Data not available for one participant;
** Data not available for two participants.

Table 4
Incidence and causality of unsolicited AEs and SAEs in the 28 days after the first and second doses of TAK-214 or placebo.

	First dose of TAK-214				Second dose of TAK-214				Second dose of Placebo			
Group	**15/15**	**15/50**	**50/50**	**50/150**	**15/15**	**15/50**	**50/50**	**50/150**	**15/15**	**15/50**	**50/50**	**50/150**
	4–8 years of age											
N =	**29**	**30**	**30**	**31**	**14**	**14**	**16**	**15**	**15**	**16**	**14**	**16**
Any, n (%)	7 (24)	7 (23)	12 (40)	5 (16)	4 (29)	2 (14)	3 (19)	3 (20)	5 (33)	5 (31)	4 (29)	3 (19)
Related	0	0	0	0	0	0	0	0	0	0	0	0
Mild	4 (14)	5 (17)	9 (30)	3 (10)	1 (7)	2 (14)	2 (13)	1 (7)	4 (27)	4 (25)	3 (21)	2 (13)
Moderate	3 (10)	2 (7)	3 (10)	2 (6)	3 (21)	0	1 (6)	1 (7)	1 (7)	1 (6)	1 (7)	1 (6)
Severe	0	0	0	0	0	0	0	1 (7)	0	0	0	0
SAEs, n (%)	0	0	0	1 (3)	0	0	1 (6)	1 (6)	0	0	0	0
	1–3 years of age											
N =	**31**	**30**	**29**	**30**	**15**	**14**	**15**	**15**	**16**	**16**	**14**	**15**
Any, n (%)	17 (39)	13 (43)	9 (31)	12 (40)	6 (40)	5 (36)	4 (27)	4 (27)	8 (50)	7 (44)	3 (21)	2 (13)
Related	0	0	0	0	0	0	0	0	0	0	0	0
Mild	12 (39)	11 (37)	6 (21)	7 (23)	5 (33)	2 (14)	4 (27)	3 (20)	6 (38)	6 (38)	2 (14)	0
Moderate	4 (13)	2 (7)	3 (10)	4 (13)	1 (7)	2 (14)	0	0	2 (13)	1 (6)	1 (7)	2 (13)
Severe	1 (3)	0	0	1 (3)	0	1 (7)	0	1 (7)	0	0	0	0
SAEs, n (%)	0	0	1 (3)	0	0	0	0	1 (7)	0	0	0	1 (7)

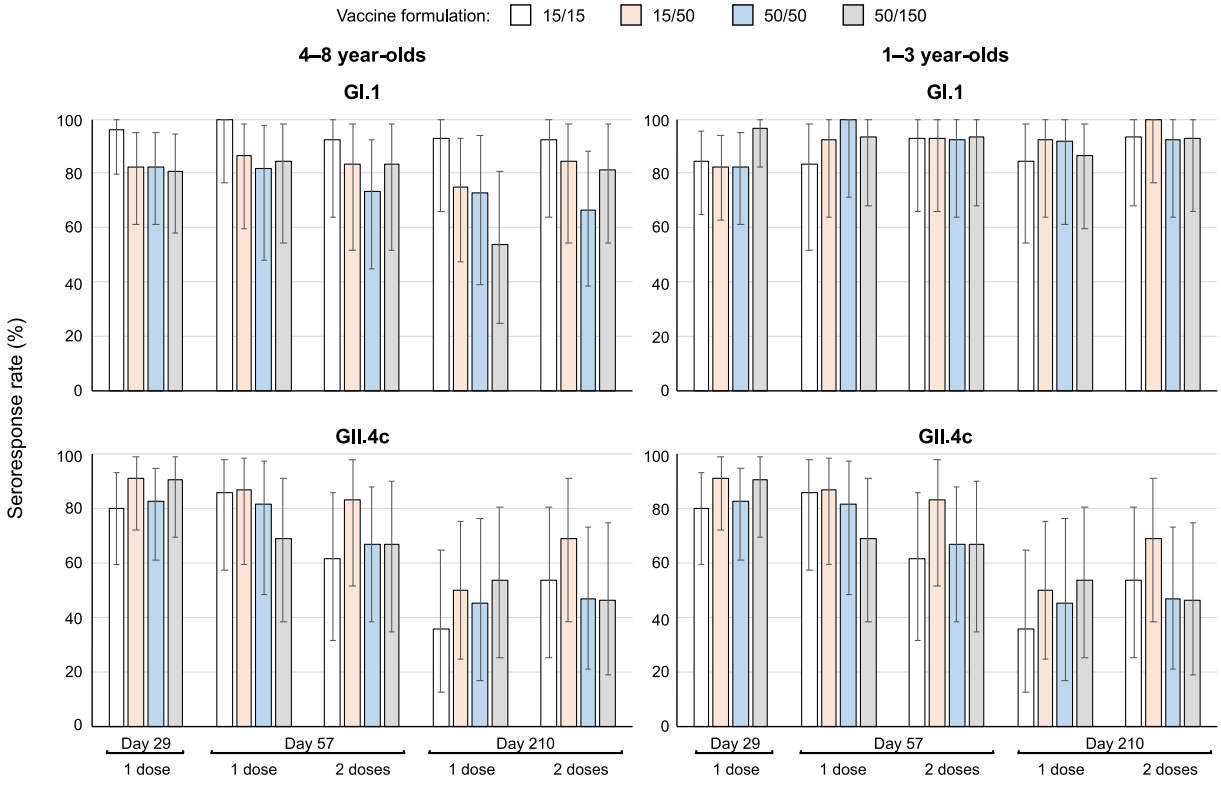

Fig. 3. Pan Ig seroresponse rates (with 95% CI) for GI.1 and GII.4c antigens in the four study groups in each age cohort.

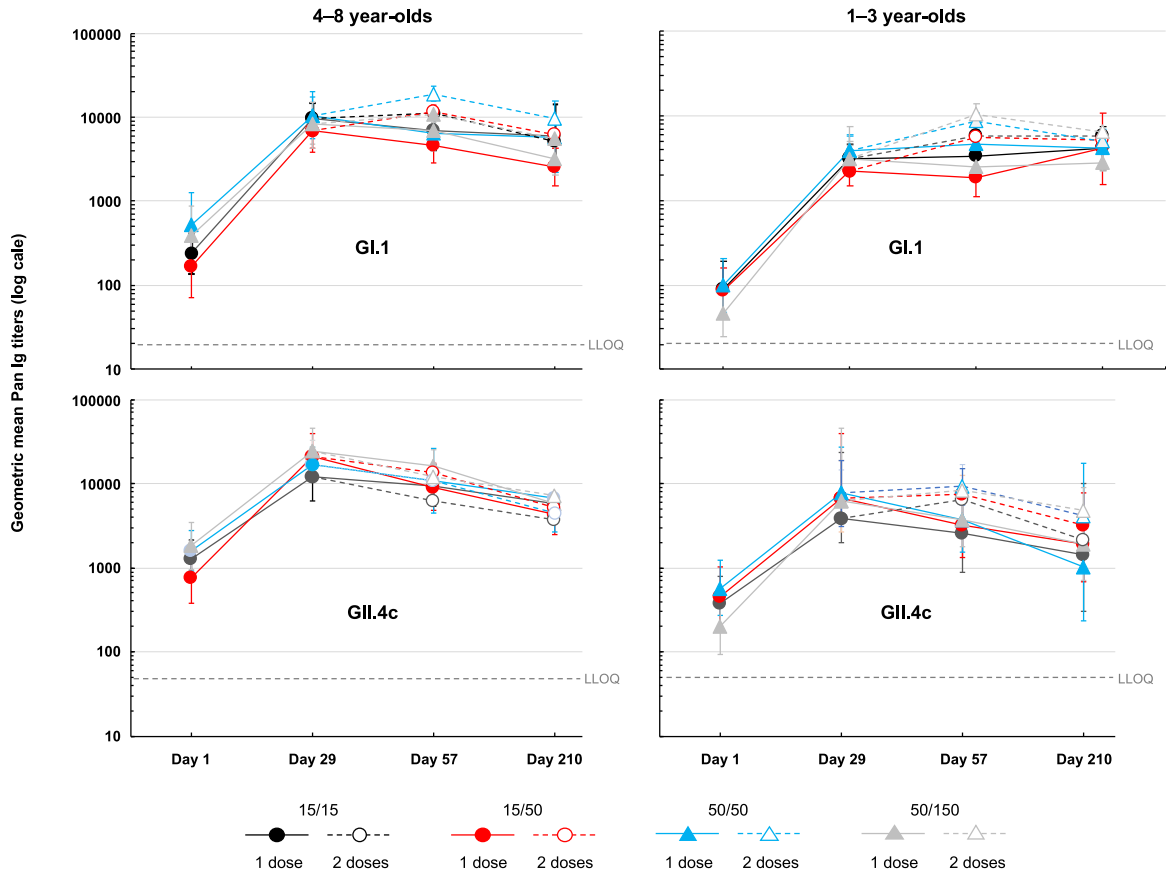

Fig. 4. Pan Ig Geometric mean titers (with 95% CI) for GI.1 and GII.4c antigens in the four study groups in each age cohort.

92–100% after a second dose (Fig. 5), with increased GMTs evident in all groups (Fig. 6). After the first dose 65–83% of the 1–3-year-olds seroresponded and the SRR persisted at 69–93% to Day 57 without a second vaccination, while 100% responded after a second vaccination with increased GMTs in all groups. SRRs in one dose groups declined by Day 210 in both older and younger cohorts, to 50–77% and 50–80%, respectively.

4. Discussion

In this first investigation of the norovirus vaccine candidate, TAK-214, in children, one or two doses displayed common (40–54%) but acceptable reactogenicity in 1–8-year-old children, with no vaccine-related serious adverse events (SAE) and mainly mild or moderate solicited local reactions and systemic AEs that were transient and rapidly resolved. Overall, reactogenicity did not increase with dosage or number of doses, being similar after the first and second doses in terms of frequency and severity of solicited AEs, but higher than placebo injections.

Substantial immune responses measured as Pan Ig antibodies against each of the vaccine antigens were already evident four weeks after one dose of any vaccine dosage in both age cohorts, with little evidence of VLP dose-dependent responses. Further increases against GI.1 after the second dose were observed in all groups; anti-GII.4c-specific responses were not consistently increased by the second dose in the older cohort but were more notable in the younger cohort while still being relatively modest compared with the responses to the first dose. Pan Ig titers against GI.1 were maintained at similarly high levels in both age cohorts six months after the second. The initial Pan Ig responses against GII.4c were similar in profile to GI.1, although there was less evidence of significant responses to second dose in either cohort; titers were waning in the older age group and were only maintained at the post-dose 1 response level in the younger children. Pan Ig levels against GII.4c then waned such that by Day 210 around 50% of children in most groups displayed a seroresponse compared with the baseline value.

The level of histoblood group antigen (HBGA) blocking antibodies has been suggested to be a correlate for protection against norovirus illness [13,14]. When measured as HBGA blocking antibodies the response profiles generally paralleled those measured as Pan Ig. There was a suggestion of an inconsistent dose-dependent enhancement of the response against GII.4c in the younger age cohort, but not the older children, and in both age groups the response against GII.4c was increased by a second vaccination. In view of the small numbers of participants in this study these results will require confirmation in larger trials, but it is also possible that the booster response after the second dose might have been larger if the time interval from the first dose was longer than 28 days.

It was apparent that both cohorts had been exposed to norovirus previously as both had preexisting antibodies against each antigen, with higher baseline Pan Ig titers against both GI.1 and GII.4c VLPs in the older cohort than the younger children. This may explain why there were robust responses to the first doses of vaccine irrespective of dose, which would represent booster responses in this population, with little or no further increase following the second dose. The ubiquitous nature of norovirus outbreaks may mean that all children who reach the age when they start to socialize with their peers will be infected and develop this baseline immunity. Observed ranges of HBGA antibody levels in these children were comparable to those previously reported in young adults. At Day 57, four weeks after a second dose the $GMBT_{50}$ (95% CI) across groups (n = 51–54) were 491 (397–608) for GI.1 and 934 (678–1285) for GII.4c in 4–8-year-olds, and 346 (298–402) for GI.1 and 515 (372–712) for GII.4c in 1–3-year-olds.

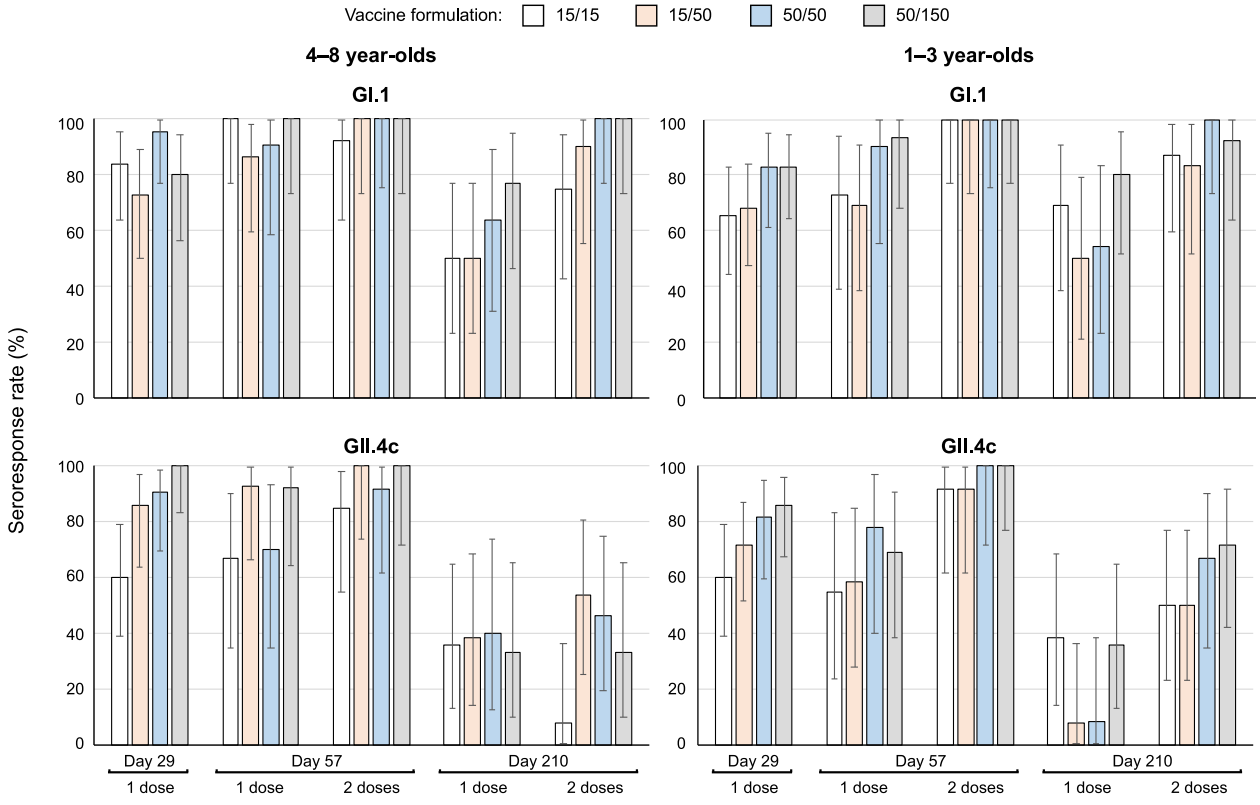

Fig. 5. HBGA seroresponse rates (with 95% CI) for GI.1 and GII.4c antigens in the four study groups in the two age cohorts.

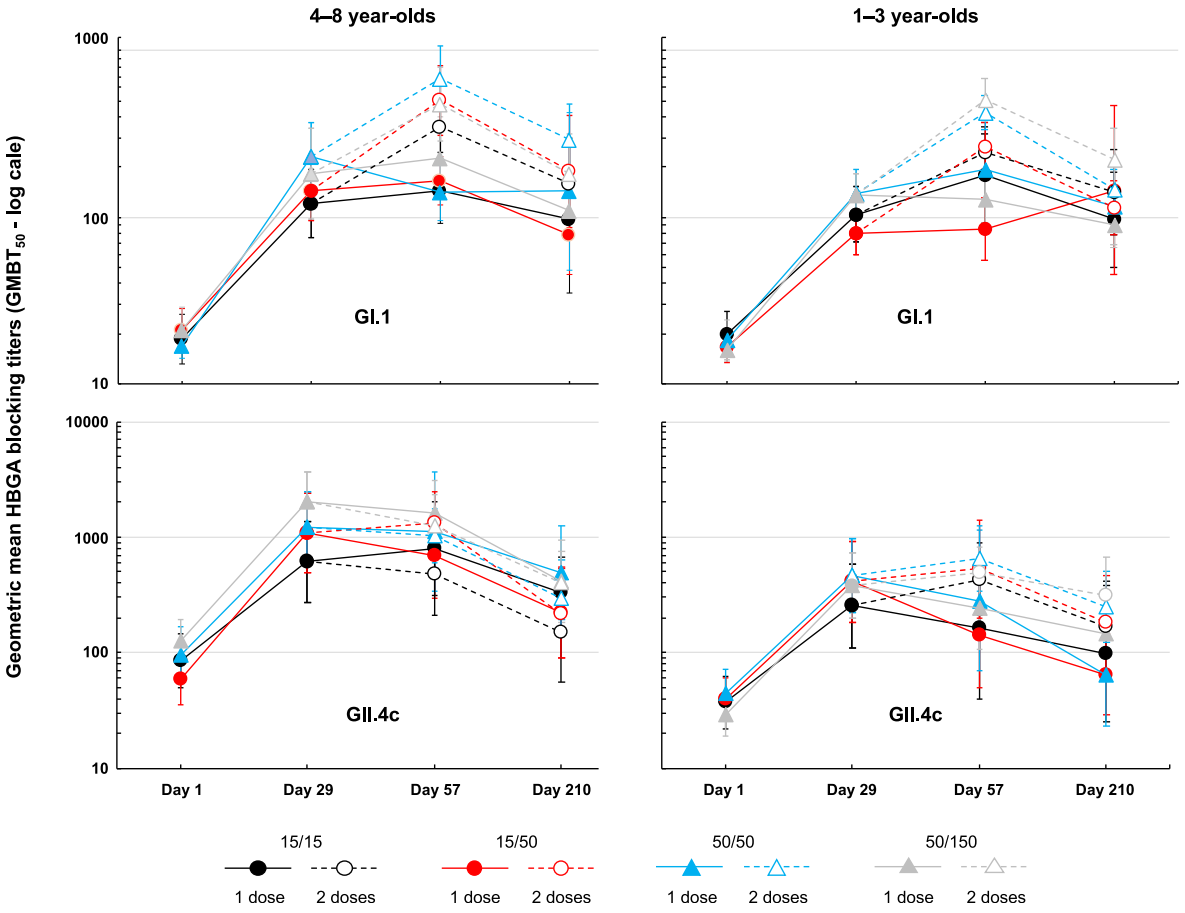

Fig. 6. Geometric mean HBGA titers (with 95% CI) for GI.1 and GII.4c antigens in the four study groups in each age cohort.

In a study in Belgian adults from 18 to 64 years of age $GMBT_{50}$ ranged from 356 to 464 for GI.1, and 427 to 604 for GII.4c after two doses of experimental formulations of TAK-214 [10].

Norovirus infections are common globally, and particularly prevalent in children, irrespective of standards of hygiene and sanitation associated with higher development status [15], although the vast majority of norovirus-associated deaths occur in developing countries [16]. In those countries where routine pediatric rotavirus vaccination has been introduced norovirus has become the leading cause of medically attended acute gastroenteritis in young children [2–7]. Circulating norovirus strains vary over time, but the GII.4 genogroup has been identified as the predominant in recent years since the mid 1990 s with different distinct strains responsible for epidemic outbreaks [17], and new variants appearing every two to three years [18]. GII strains cause more severe and persistent illness in children [19], so there is a need for a vaccine that is broadly effective against a variety of GII.4 sub-groups. This required breadth of immunity against GII.4 led to the development of the GII.4c component of TAK-214, which is a consensus sequence of three different GII.4 strains [20]. In addition to the medical need for a norovirus vaccine, there is a strong financial incentive in view of the economic burdens of norovirus infections [21]. Bartsch et al have calculated that the annual cost of norovirus infections in the United States is approximately $10.6 billion [22]. Using computer simulations the same group has shown that immunizing pre-school children in the United States with a vaccine with only 25% efficacy would be cost-effective if the vaccination costs were $445, and cost saving if those costs were $370 [23]. Use of a more efficacious vaccine would maintain these cost effective and cost saving thresholds even if the treatment costs were increased to $1,600.

The current global COVID-19 pandemic due to SARS-CoV-2 virus has had a major effect on the number of respiratory and gastrointestinal infections including norovirus cases with marked decreases around the world [24–28]. This is an obvious consequence of fewer opportunities to become infected due to less social mixing of children as schools and daycare facilities have closed. However, there is a prediction of a surge in norovirus cases once the situation returns to normal due to an increased susceptibility to norovirus [29]. Modeling suggests that a return to prepandemic behaviors will lead to a doubling of the annual incidence [29]. Despite the current decrease in the burden due to norovirus there is clearly a need for an effective pediatric norovirus vaccine to relieve societal and economic burdens of the sporadic norovirus outbreaks which occur annually around the world. The present study confirms the potential of Takeda's vaccine candidate as it shows TAK-214 provides robust immune responses in children from 1 to 8 years of age, while being generally well-tolerated, with no reports of vaccine-related SAEs and severe local or systemic AEs being relatively rare. Although the 15 μg GI.1 VLP and 50 μg GII.4c VLP formulation has been selected for adult use [8–11], further investigation will be required to confirm the final formulation and schedule required for children, notably in children under 1 year of age.

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Contribution statement

PMM, FB and AB conceived and designed the study; TV, XS-L, VB, PL and EL acquired the data; ML analyzed the data; TV, XS-L, TM, PMM, JS, FB and AB interpreted the data; JS drafted the article with the assistance of a medical writer; all authors revised the article for important intellectual content and approved the final submitted version.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: As an institution Takeda Pharmaceuticals International AG reports financial support was provided by US Army Medical Research and Materiel Command. Taisei Masuda, Paul M Mendelman, Mengya Lui, James Sherwood, Frank Baehner, Astrid Borkowski report financial support was provided by Takeda Pharmaceuticals International AG or Takeda Vaccines Inc. TM, PMM, ML, JS, FB and AB were all full-time employees of Takeda Pharmaceuticals International AG or Takeda Vaccines Inc. at the time of the study.

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The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation. The investigator(s) adhered to the policies regarding the protection of human subjects as prescribed by Code of Federal Regulations (CFR) Title 45, Volume 1, Part 46; Title 32, Chapter 1, Part 219; and Title 21, Chapter 1, Part 50 (Protection of Human Subjects).

Data statement

The datasets, including the redacted study protocol, redacted statistical analysis plan, and individual participants' data supporting the results reported in this article, will be available three months from an initial request made by researchers who provide a methodologically sound proposal. The data will be provided after its de-identification, in compliance with applicable privacy laws, data protection and requirements for consent and anonymization.

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