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Immunogenicity and safety of an inactivated enterovirus A71 vaccine in children 3–6 years and 2–35 months of age- an open-label, randomized phase IIb clinical trial

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ABSTRACT

Background: Enterovirus A71 (EV-A71) infection can cause severe debilitating complications and even death in young children. The immunogenicity and safety of an inactivated whole EV-A71 virus vaccine were assessed in children 2 months to 6 years of age.

Methods: This was an open-label, multi-center and randomized phase IIb study, which divided into part A and B. In part A, children 36 months to 6 years of age were enrolled and randomized into 3 groups, receiving 0.5 μg total viral protein (TP) with adjuvant Al(OH)3, 1.0 μg TP with Al(OH)3 or 1.0 μg TP only. Two doses of vaccines were administered at a 28-day interval and blood was taken before immunization, at week 4, 8, 28 and 52 (optional) for virus neutralization assay. Safety profiles were also monitored. After safety profiles had shown no concerns, children 2 months to 35 months of age (part B) were subsequently enrolled following the same protocol.

Results: A total of 135 children completed two doses of immunization, including 58 in part A and 77 in part B. Both adjuvanted 0.5 μg and 1.0 μg TP elicited significant raise of neutralizing antibody titers and seroconversion rate was up to 93.75–100.0% after 2 doses of immunization. Adjuvanted 1.0 μg TP induced higher titers of neutralizing antibodies than adjuvanted 0.5 μg TP. By contrast, non-adjuvanted 1.0 μg TP was not immunogenic. No major adverse events were reported.

Conclusions: This EV-A71 vaccine containing adjuvant is immunogenic and safe in children 2 months to 6 years of age.

Clinical Trials Registration. NCT03268083.

1. Introduction

Enterovirus A71 (EV-A71) is a single-stranded RNA virus, a member of the genus *Enterovirus* in the family *Picornaviridae* and currently classified into three major genotypes A, B and C (including subgenotype B1 to B5 and C1 to C5) and additional genotypes D, E and F, based on sequence variation in its structural protein VP1 [1,2]. First isolated from a child with encephalitis in California in 1969 [3], EV-A71 is a leading cause of hand, foot and mouth disease (HFMD) and herpangina in children. EV-A71 can cause severe neurological complications, involving brainstem, myocarditis, pulmonary edema or hemorrhage, leading to fatality, particularly in children under 5 years of age [4,5].

EV-A71 outbreaks have been reported worldwide, including United States, Europe, Australia and more frequently in Asia, including Malaysia, Singapore, China, Japan and Taiwan [6–8]. For example, in 1998, a major EV-A71 infection outbreak occurred in Taiwan, 405 patients developed severe complications out of a total of 129,106 cases of HFMD or herpangina, unfortunately resulting in 78 deaths [9]. A cyclical pattern of EV-A71 epidemics occurred every 2–4 years with seasonal distribution and different genotypes could be accountable. As a result, unpredictable switching of genotypes due to genetic recombination are frequently observed [10].

Several anti-EV-A71 vaccines have been developed using different components including VP1 protein, viral like particle, recombinant proteins, synthetic peptides, DNA vaccines, live attenuated virus and inactivated whole viruses [10–12]. Up to date, at least five inactivated whole EV-A71 virus vaccines have gone through different stages of clinical trials in humans. Three inactivated EV-A71 vaccines (based on subgenotype C4a) with different doses

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and manufacturing processes (by Sinovac, CAMS and Beijing Vigoo Biological) have been approved by China FDA to be used in children older than 6 months of age [12,13]. Another inactivated EV-A71 vaccine based on B2 subgenotype was developed in Singapore (Inviragen) and currently completed phase I clinical trial in humans [14].

A formalin-inactivated EV-A71 vaccine, based on Vero-cell cultured E59 strain (subgenotype B4) [15,16], was developed by Taiwan National Health Research Institute (NHRI) using roller bottle. This vaccine containing either 5 or 10 μg of total viral protein (TP) adjuvanted with $AlPO_4$ has been proved to be safe and immunogenic in 60 healthy adult volunteers in a phase I clinical trial [17].

Bioreactor was introduced to manufacture this B4 subgenotype vaccine. In this phase IIb trial, this bioreactor- generated vaccine containing either 0.5 μg or 1.0 μg inactivated EV-A71 total protein with or without adjuvant was used to immunize children in two age groups, 3–6 years and 2–35 months of age, in a sequential manner to assess its safety and immunogenicity.

2. Methods

2.1. Study design and participants

These open-label, multicenter, phase II, randomized studies were carried out in four tertiary medical centers (Taipei Veterans General Hospital, National Taiwan University Hospital, Linkou Chang Gung Memorial Hospital, China Medical University Hospital) in Taiwan between Jul 04, 2016 and Dec 12, 2017(defined as the last blood sample taken). After gaining written, informed consent from guardians, we enrolled healthy children 2 months to 6 years of age. Exclusion criteria included (1) previously known exposure to EV-A71; (2) history of herpangina, hand-foot-mouth disease, and acute hemorrhagic conjunctivitis associated with enterovirus infection in the past 3 months; (3) acute infections 7 days prior to the first dose of immunization; (4) history of hypersensitivity/allergy to vaccines or any vaccine components; (5) gestational age < 37 weeks or birth body weight < 2.5 kg; (6) severe malnutrition, dysgenopathy, major congenital defects, serious chronic illness, including perinatal brain damage, autoimmune disease, bleeding disorder or coagulopathy; (7) family history of seizures or progressive neurological disease, congenital or hereditary immunodeficiency; (8) immunization within 14 days prior to randomization; (9) immunoglobulin use or any blood product transfusion within 3 months prior to vaccination or planned use during the study period; (10) chronic use (defined as > 14 days) of immunosuppressant or other immunomodulators or systemic corticosteroids within 6 months prior to immunization; (11) use of any investigational products, such as drugs or vaccines, within 30 days before the first vaccination or planned to use during the study period. The trials were approved by Institutional Review Board (IRB)/ethics committees in all participating medical centers before enrolment. The study protocol was approved by the Taiwan Food and Drug Administration (TFDA).

2.2. Study vaccine

The tested EV-A71 vaccine was manufactured by Adimmune Corporation using bioreactor process and supplied in identical prefilled syringes. In brief, the E59 virus was grown in Vero cells with serum-free medium. Whole virion was harvested, concentrated, purified and inactivated using formalin and was formulated with aluminium hydroxide ($Al(OH)_3$). The process met the sterility and purity tests. Each 0.5 ml dose contained 0.5 μg of inactivated EV-A71 TP + 150 μg $Al(OH)_3$ (Lot number: EVCA1503), 1.0 μg of inactivated EV-A71 TP + 150 μg $Al(OH)_3$ (Lot number: EVCA1502) or 1.0 μg of inactivated EV-A71 TP only. The vaccines were stored at 2-8℃ and administration route was intramuscular injection. The production is compliant with current Good Manufacturing Practices (cGMP).

2.3. Study procedures

The trials contained two parts. Initially, children 3–6 years of age were enrolled into part A and randomized into three groups receiving different immunization formulations, including 0.5 μg TP + 150 μg $Al(OH)_3$, 1.0 μg TP + 150 μg $Al(OH)_3$, 1.0 μg TP alone. The randomization list was generated using SAS software (version 9.4) and contained sequential coding which was assigned to group A1, A2 and A3 in the ratio of 1:1:1. The demographic data for enrolled subjects were collected. Two doses of immunization were administered intramuscularly with a 28-day interval. Blood sampling was performed before the first dose of immunization (day 0), at week 4, week 8, 28 and 52 (optional). After no safety concerns were observed in part A during interim analysis by Data and Safety Monitoring Board (DSMB), children 2 months to 35 months (part B) were subsequently enrolled following the same protocol.

Immediate solicited adverse events (AE) were recorded within 30 min of injection if any. Diary cards were given to parents or guardians for recording any solicited AEs for 7 days after each immunization (day 0–6, day 28–34) and unsolicited symptoms for 28 days after immunization. Solicited local AEs include pain, tenderness, redness, swelling and ecchymosis at injection site whereas solicited systemic AEs included fever, nausea/vomiting, diarrhea, decreased appetite, headache, myalgia, joint pain, fatigue and shivering. All adverse events were coded through Medical Dictionary for regulatory activities system. Any serious AEs requiring hospitalization was to be reported throughout the study. Severity of the reported AEs and its relation to trial vaccines were assessed by investigators.

Serum neutralizing antibody (NAb) titers were tested using virus neutralization assay method as described previously [17]. In short, a two-fold serial dilutions of the serum samples were incubated with equal volumes of 100 TCID50 of EV-A71 virus in a 96-well microplate with addition of human rhabdomyosarcoma cells for five days. Cytopathic effect (CPE) was observed using crystal violet staining under a light microscope. NAb titers were defined as the reciprocal of the highest dilution showing 50% inhibition of the CPE, which was calculated using the Reed-Muench method. Seropositivity was defined as NAb titer ≥ 8 whereas seroprotection was defined as NAb titer ≥ 32 per previous studies [18–20]. Blood samples were analyzed in the central laboratory through a blinded procedure, which only the case-specific coding was labeled on the sample and the laboratory staff was not told the link between samples and immunization groups.

The Primary endpoints were to evaluate the changes between pre- and post- immunization serum neutralizing antibody titers and to calculate seroconversion rate (SCR) at week 8 and week 28. The secondary endpoints were to assess all adverse events, the incidence of EV-A71 breakthrough infection 8 weeks after immunization and neutralizing antibody titers at week 52.

2.4. Statistical analysis

All hypotheses testing was conducted at the 5% level of significance. A p-value of 0.05 or less was considered statistically significant.

NAb titers were presented as geometric mean titers (GMT) with 95% confidence interval (CI). Mann-Whitney *U* test was used to compare the difference in GMT between different dosing groups. Seroconversion rates (SCR) was defined as the percentage of sub-

jects achieving a minimum 4-fold increase of NAb titers after immunization. Fisher's exact method was used to compare SCR among immunization groups.

3. Results

3.1. Demographics

A total of 140 children were enrolled, including 59 children 3–6 years of age in part A and 81 children 2–35 months of age in part B (Fig. 1). After randomization, there were 19 children in group A1, 20 in group A2, 20 in group A3, 30 in group B1, 27 in group B2 and 24 in group B3 (Fig. 1). Consent was withdrawn in two subjects in group B2 and protocol violation occurred in 3 subjects. Therefore, a total of 135 children completed 2 doses of immunization and had blood sampling before immunization, at week 4, 8 and 28. Gender and age distribution of those 135 children were listed in Table 1.

3.2. Immunogenicity

In part A, either 0.5 μg or 1.0 μg TP + Al(OH)3 elicited significant raise of neutralizing antibody titers. Three subjects were seropositive before immunization, including one in group A1 and two in group A2, and they were excluded from immunogenicity analysis. Geometric mean titers (GMT) peaked at week 8 with GMT 289.31 (95% CI: 159.30–525.42) and 298.63 (95% CI: 232.21–384.06) for group A1 and A2, respectively. There were no statistically significant differences in GMT between group A1 and A2 either at week 8 or week 28 (*p value* 0.731 and 0.686 respectively). By contrast, without the presence of Al(OH)3, immunization with 1.0 μg of TP only (group A3) did not elicit neutralizing antibodies (Table 2).

Similarly, in part B, significantly raised NAb titers were observed in post-immunization serum from both adjuvant groups (group B1 and B2). Four subjects were seropositive before immunization, including two in group B1 and two in group B3, and they were excluded from immunogenicity analysis. GMT peaked at week 8 with GMT 199.86 (95% CI: 128.32–311.30) and 394.81 (95% CI: 260.58–598.17) for group B1 and group B2, respectively. Notebly, GMT in group B2 was nearly 2-fold higher than that in group B1 with statistically significant differences both at week 8 and week 28 (*p value* 0.045 and 0.037, respectively). In addition, 1.0 μg of TP only did not elicit neutralizing antibodies (Table 2).

As shown in Table 2, seroconversion rate (SCR) in adjuvant groups was 75–94.11% after one dose of immunization (week 4) and reached 96.43–100% at week 8. At week 28, slightly reduced SCR was observed in group A1 (down to 94.12%) whilst SCR stayed the same in group B1 (96.43%) and B2 (100.0%). There were no statistically significant differences in SCR between either group A1 and A2 or group B1 and B2. SCR was 0.0% in group A3 and B3.

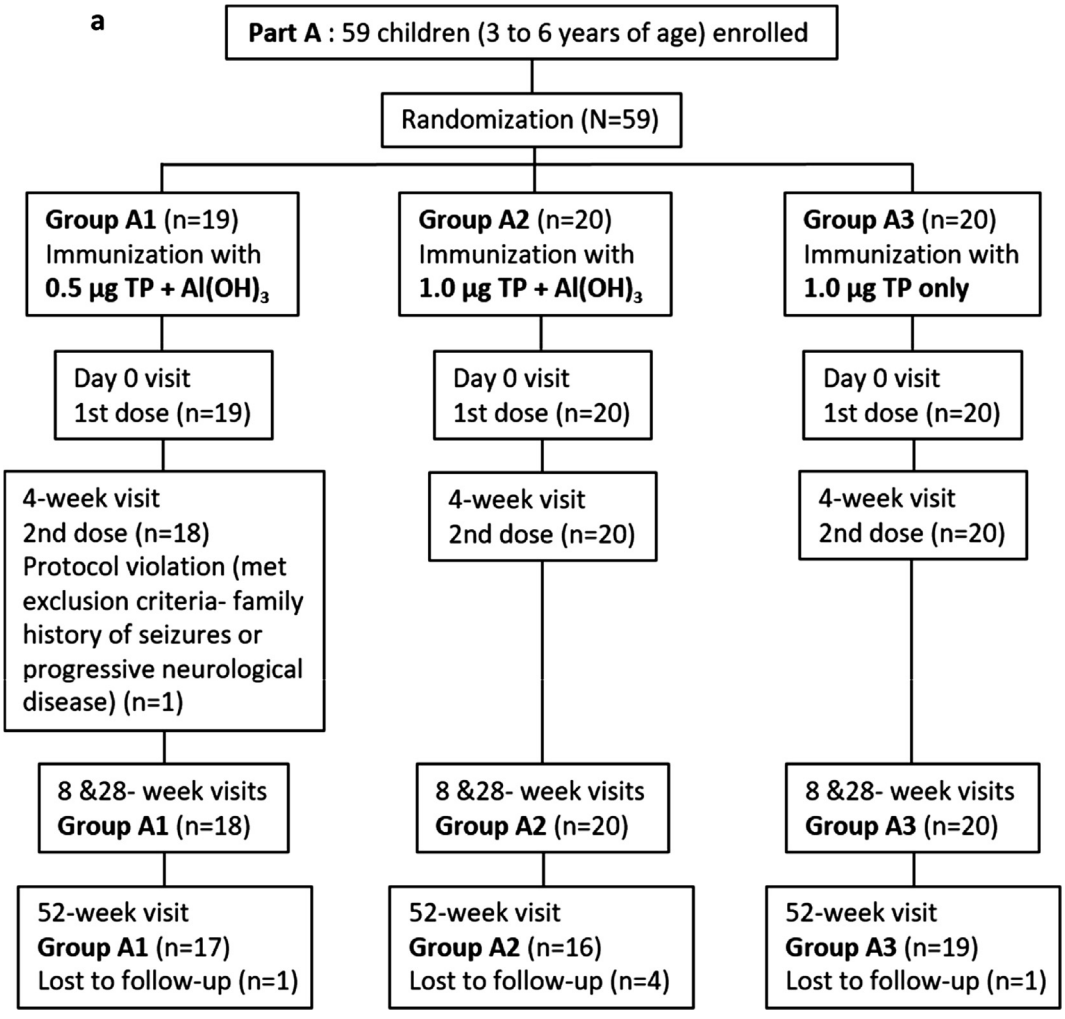

Fig. 1. (a & b) Flowchart summarizing enrollment, randomization and study completion in Part A and B.

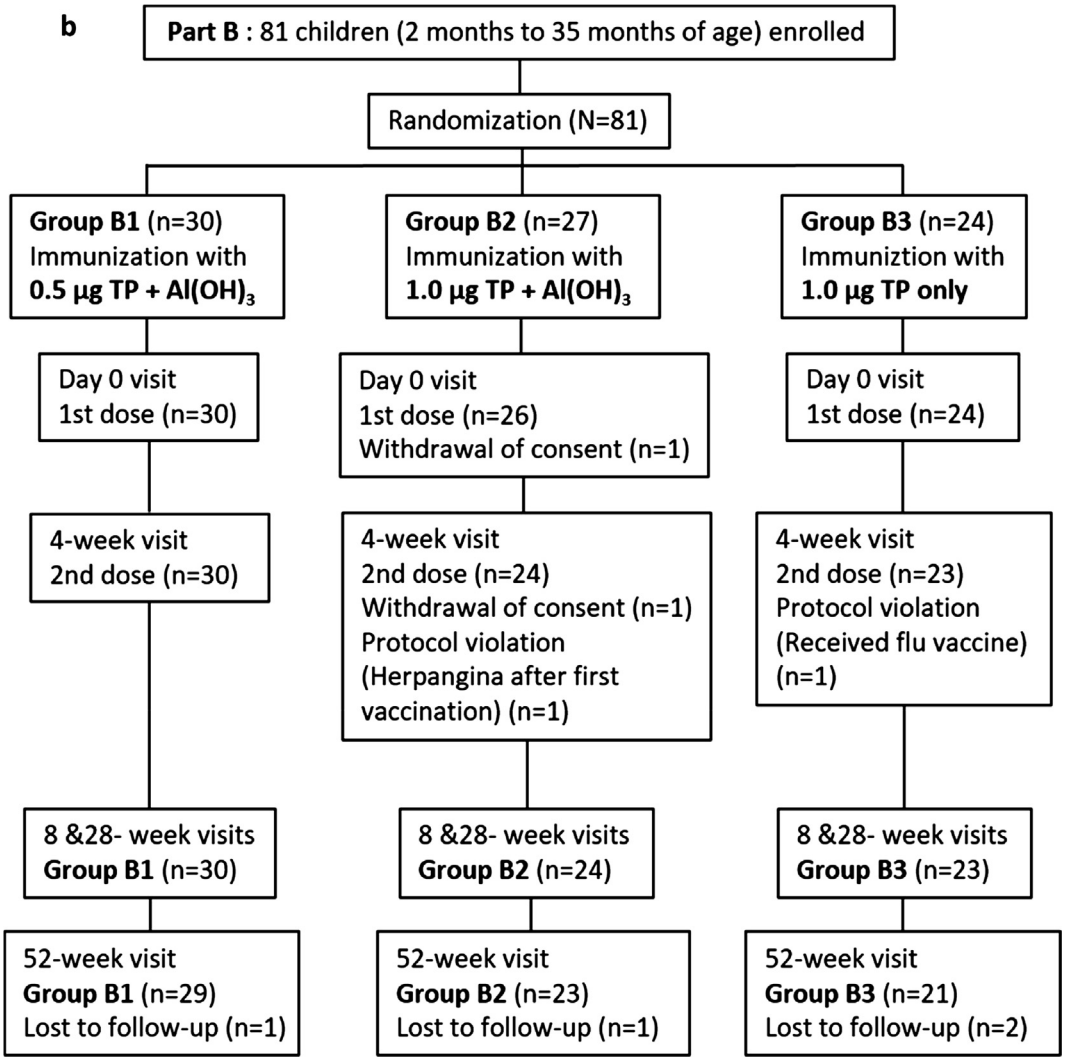

Fig. 1 (*continued*)

Table 1
Gender and age of children who completed the trial. Data are n (%).

	Part A (3–6 years old) (N = 58)			Part B (2–35 months old) (N = 77)		
	Group A1 0.5 µg TP+Al(OH)$_3$ (N = 18)	Group A2 1.0 µg TP+Al(OH)$_3$ (N = 20)	Group A3 1.0 µg TP (N = 20)	Group B1 0.5 µg TP+Al(OH)$_3$ (N = 30)	Group B2 1.0 µg TP+Al(OH)$_3$ (N = 24)	Group B3 1.0 µg TP (N = 23)
Gender						
Female	7 (38.9%)	11 (55.0%)	10 (50.0%)	9 (30.0%)	13 (54.2%)	11 (47.8%)
Male	11 (61.1%)	9 (45.0%)	10 (50.0%)	21 (70.0%)	11 (45.8%)	12 (52.2%)
Age (years)						
Mean ± SD	4.4 ± 0.9	4.8 ± 1.2	5.4 ± 1.1	1.4 ± 0.8	1.5 ± 0.8	1.7 ± 0.9
Min–Max	(3.2–6.6)	(3.0–6.9)	(3.3–6.9)	(0.3–2.8)	(0.3–2.8)	(0.3–2.9)
24–35 months old	–	–	–	9	6	9
12–23 months old	–	–	–	10	12	8
6–11 months old	–	–		7	3	3
2–5 months old	–	–	–	4	3	3

One year after immunization (week 52), optional follow-up blood sampling was done in a total of 49 subjects in part A and 69 subjects in part B (Table 2). In part A, the GMT were 107.63 (95% CI: 52.51–220.65) and 172.28 (95% CI: 110.52–268.53) for group A1 and A2, respectively. Seroprotection rates (defined as NAb titers greater or equal to 32) were 93.75% and 100.0% for group A1 and A2, respectively. In part B, the GMT were 339.53 (95% CI: 192.48–598.92) and 467.74 (95% CI: 290.04–745.31) for group B1 and B2, respectively. Seroprotection rates were 92.59% and 95.65% for group B1 and B2, respectively (Table 2). Importantly, SCR remained high at 93.75–100% among adjuvant groups whilst it remained 0.0% in non-adjuvant groups. Reverse cumulative distribution of serum NAb titers among all immunization groups is demonstrated in Fig. 2. A shift in NAb titer distribution after immunization was noted in all adjuvant groups.

3.3. Safety data

Safety data analysis was based on all children receiving either 1 or 2 doses of the trial vaccine (N = 139). As shown in Table 3,

Table 2
Geometric mean titers (GMT), seroconversion rate (SCR) and seroprotection rate (GMT ≥ 32) of anti-EV-A71 neutralizing antibodies in all groups.

	Group A1 0.5 μg TP + Al(OH)3 N = 17	Group A2 1.0 μg TP + Al(OH)3 N = 18	Group A3 1.0 μg TP N = 20	Group B1 0.5 μg TP + Al(OH)3 N = 28	Group B2 1.0 μg TP + Al(OH)3 N = 24	Group B3 1.0 μg TP N = 21
Week 4						
GMT (GSD)	69.43(4.08)	57.02(3.64)	4.14(1.17)	35.33(3.38)	36.97(4.74)	4.42(1.57)
95% CI	35.60–135.43	31.40–103.55	3.87–4.43	22.5–55.49	19.84–68.91	3.64–5.36
p value[a]	0.732			1.000		
SCR	94.11%	88.89%	0.00%	82.14%	75%	4.76%
p value[b]	1.000			1.000		
GMT ≥ 32	82.35%	77.78%	0.00%	64.29%	58.33%	4.76%
Week 8						
GMT (GSD)	289.31(3.51)	298.63(1.72)	4.76(1.46)	199.86(3.31)	394.81(2.82)	5.04(1.95)
95% CI	159.30–525.42	232.21–384.06	4.02–5.62	128.32–311.30	260.58–598.17	3.78–6.71
p value[a]	0.731			0.045*		
SCR	100%	100%	5.00%	96.43%	100%	4.76%
p value[b]	–			1.000		
GMT ≥ 32	94.12%	100%	0.00%	92.86%	100%	4.76%
Week 28						
GMT (GSD)	117.98(4.08)	118.51(2.22)	4.59(1.33)	110.33(3.17)	203.19(2.30)	4.72(2.13)
95% CI	60.49–230.10	82.02–171.24	4.06–5.20	71.99–169.12	145.51–283.72	3.41–6.52
p value[a]	0.686			0.037*		
SCR	94.12%	100%	0.00%	96.43%	100%	4.76%
p value[b]	0.486			1.000		
GMT ≥ 32	88.23529412	100%	0.00%	92.86%	100%	4.76%
Week 52						
Case number	N = 16	N = 14	N = 19	N = 27	N = 23	N = 19
GMT (GSD)	107.63(4.32)	172.28(2.33)	4.30(1.24)	339.53(4.50)	467.74(3.22)	4.00(1)
95% CI	52.51–220.65	110.52–268.53	3.90–4.75	192.48–598.92	290.04–745.31	4
p value[a]	0.306			0.435		
SCR	93.75%	100%	0.00%	100%	100%	0.00%
p value[b]	1.000			–		
GMT ≥ 32	93.75%	100%	0.00%	92.59%	95.65%	0.00%

p-value[a]: Mann-Whitney *U* test for comparing GMT at week 4, week 8, week 28 or week 52 between 0.5 μg TP + A(OH)3 and 1.0 μg TP + A(OH)3 vaccine groups within part A or part B.
p-value[b]: Fisher's exact test for intergroup SCR comparison between 0.5 μg TP + $A(OH)_3$ and 1.0 μg TP + $A(OH)_3$ vaccine groups.
*Denotes statistical significance with $p < 0.05$.
GSD is the abbreviation of geometric standard deviation.

solicited AEs were reported at least once in 27 subjects (45.8%) in part A and in 27 subjects (33.8%) in part B. In part A, commonly reported solicited AEs were pain (37.3%), tenderness (35.6%), redness (15.3%) and swelling (10.2%). In part B, pain (20.0%), redness (18.8%), tenderness (17.5%) and swelling (15.0%) were more commonly reported AEs. There was no significant difference in the incidence of local AEs among immunization groups in both Part A and Part B. The severity of all solicited local AEs was graded as mild or moderate.

Solicited systemic AEs were reported in 16 subjects (27.1%) in part A and 27 subjects (33.8%) in part B (Table 3). In part A, the most commonly reported systemic adverse event was decreased appetite (15.3%), followed by fatigue (13.6%). In part B, decreased appetite was most commonly reported (22.5%), followed by fatigue (11.3%) and diarrhea (11.3%). There was no significant difference between immunization groups within both Part A and Part B. All the systemic AEs were graded as mild to moderate in severity, except one severe AE. This subject presented with fever three days after second immunization and was later diagnosed roseola infantum.

Unsolicited AEs were reported in a total of 81 subjects (58.3%), including 32 subjects (54.2%) in Part A and 49 subjects (61.3%) in Part B (Supplementary Table 1). The severity of those unsolicited AEs was graded mild to moderate. Moreover, a total of 27 serious adverse events (SAE) (defined as requiring hospitalization) were reported in eight subjects (Supplementary Table 2). All the SAEs were deemed unrelated to the trial vaccine.

Of note, a total of 4 subjects were reported to have suspected enterovirus infections. One subject in group B2 was diagnosed herpangina on day 19, deemed protocol violation and therefore excluded from immunogenicity analysis. One subject in group A1 was diagnosed herpangina on day 42. One subject in group B2 and one subject in group B1 were diagnosed hand, foot, and mouth disease on day 31 and day 90, respectively and both were recorded as SAE due to requiring hospitalization. Throat swab and rectal swab were sent for viral culture which grew enterovirus, reactive to pan enterovirus antibody. Further virus identification using antibody against EV-A71, echovirus 4, 6, 9, 11 & 30, coxsackievirus A9, coxsackievirus A24 and coxsackievirus B1–B6 all showed negative results. Therefore, these events were considered unrelated to the immunization. Taken together, there was no evidence of breakthrough EV-A71 infection during study period.

4. Discussion

This phase II study demonstrated significant raise of anti-EV-A71 NAb titers after immunization with either 0.5 μg or 1.0 μg TP with adjuvant in children 2 months to 6 years of age. Moreover, immunization with adjuvanted 1.0 μg TP elicited higher NAb titers than immunization with adjuvanted 0.5 μg TP both in part A and part B. High seroconversion rate (>90%) at week 8 was observed in all adjuvant groups. Importantly, one year after immunization, SCR maintained at 93.75%-100% and seroprotection rate was 92.59%-100%. Interestingly, GMT values for group A2, B1 and B2 at week 52 are higher than the peak titers normally seen in all groups at week 8 (Table 2). This is intriguing as the antibody titers should have decreased by week 52. In view of incidence of enterovirus infection among the studied population, we assume that subclinical infections might have a booster effect on the antibody titers. However, further studies are needed to prove this.

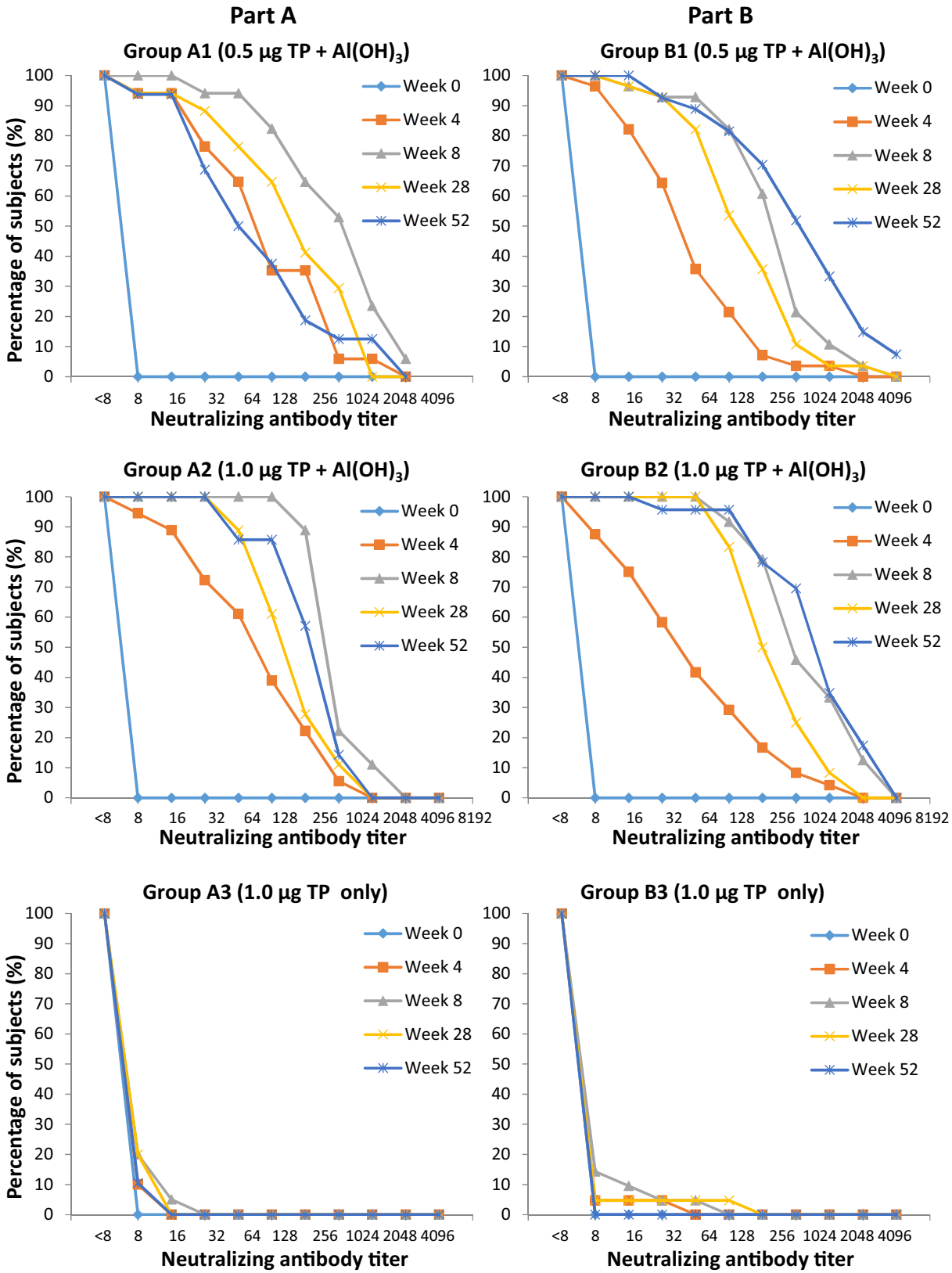

Fig. 2. Reverse cumulative distribution of serum neutralizing antibody titers in all immunization groups at week 4, week 8, week 28 and week 52. A right shift of NAb titers are present in all adjuvant groups.

Of note, this adjuvanted EV-A71 vaccine could elicit protective neutralizing antibody in infants younger than 6 months of age (n = 7) though the case number is small. One subject was seropositive (1:8) before immunization. All the seven subjects in adjuvant groups (4 in group B1 and 3 in group B2) achieved seroconversion at week 8 and sustained up to week 52. A previous study [21] has

Table 3
Solicited local and systemic adverse events by immunization groups. Data are n (%).

	Part A (3–6 years old)							Part B (2–35 months old)						
	Group A1 0.5 μg TP+Al(OH)$_3$		Group A2 1.0 μg TP+Al(OH)$_3$		Group A3 1.0 μg TP		p-value	Group B1 0.5 μg TP+Al(OH)$_3$		Group B2 1.0 μg TP+Al(OH)$_3$		Group B3 1.0 μg TP		p-value
Any Immunization	N = 19		N = 20		N = 20			N = 30		N = 26		N = 24		
Any local events	11	(57.9%)	9	(45.0%)	7	(35.0%)	0.3902	10	(33.3%)	10	(38.5%)	7	(29.2%)	0.7616
Pain	9	(47.4%)	9	(45.0%)	4	(20.0%)	0.1540	7	(23.3%)	4	(15.4%)	5	(20.8%)	0.7800
Tenderness	8	(42.1%)	8	(40.0%)	5	(25.0%)	0.4953	6	(20.0%)	5	(19.2%)	3	(12.5%)	0.8114
Redness	4	(21.1%)	3	(15.0%)	2	(10.0%)	0.6075	4	(13.3%)	8	(30.8%)	3	(12.5%)	0.1870
Swelling	2	(10.5%)	2	(10.0%)	2	(10.0%)	1.0000	5	(16.7%)	5	(19.2%)	2	(8.3%)	0.5810
Ecchymosis	1	(5.3%)	0	(0.0%)	1	(5.0%)	0.7662	2	(6.7%)	2	(7.7%)	4	(16.7%)	0.5227
Any systemic events	6	(31.6%)	7	(35.0%)	3	(15.0%)	0.3806	11	(36.7%)	6	(23.1%)	10	(41.7%)	0.3670
Fever	2	(10.5%)	1	(5.0%)	1	(5.0%)	0.6827	3	(10.0%)	3	(11.5%)	1	(4.2%)	0.6970
Nausea/Vomiting	1	(5.3%)	2	(10.0%)	1	(5.0%)	1.0000	3	(10.0%)	0	(0.0%)	1	(4.2%)	0.3123
Diarrhea	1	(5.3%)	0	(0.0%)	0	(0.0%)	0.3220	4	(13.3%)	4	(15.4%)	1	(4.2%)	0.4489
Appetite loss	3	(15.8%)	4	(20.0%)	2	(10.0%)	0.7495	8	(26.7%)	4	(15.4%)	6	(25.0%)	0.5946
Headache	3	(15.8%)	1	(5.0%)	1	(5.0%)	0.4303	1	(3.3%)	1	(3.8%)	0	(0.0%)	1.0000
Myalgia	2	(10.5%)	1	(5.0%)	1	(5.0%)	0.6827	1	(3.3%)	1	(3.8%)	0	(0.0%)	1.0000
Joint pain	0	(0.0%)	0	(0.0%)	0	(0.0%)	–	1	(3.3%)	0	(0.0%)	1	(4.2%)	0.7532
Fatigue	4	(21.1%)	3	(15.0%)	1	(5.0%)	0.3261	3	(10.0%)	2	(7.7%)	4	(16.7%)	0.6731
Shivering	1	(5.3%)	0	(0.0%)	0	(0.0%)	0.3220	2	(6.7%)	0	(0.0%)	0	(0.0%)	0.3278

shown that the majority of the transplacental maternal EV-A71 neutralizing antibodies waned down to undetectable levels at about 6 months of age whilst infants 6–11 months of age are at highest risk of contracting severe EV-A71 infections [22–24]. Hence, immunization with EV-A71 vaccine should ideally commence before 6 months of age. This trial vaccine has so far shown good immunogenicity and safety in this age group.

In comparison to other EV-A71 vaccines which have been used in children, the dose of this vaccine is comparable (1 μg by Sinovac biotech Co., Ltd. (China); 0.8 μg by Beijing Vigoo Biological (China); 0.25 μg by Chinese Academy of Medical Sciences (China); 1.25, 2.5 or 5 μg by Madigen vaccine biologicals Co. (Taiwan)) [10,20,25]. Regarding vaccine component, the 3 available EV-A71 vaccines made in China contained C4a subgenotype whereas subgenotype B4 was used in Taiwan; either aluminium hydroxide or aluminium phosphate was used in all these vaccines. Importantly, different from traditional roller bottle and cell factory method used in EV-A71 vaccine production, using bioreactor process can increase vaccine yield and reduce the production cost [26,27].

This study has several limitations. Firstly, cross-subgenotype protection was not tested. Nevertheless, vaccines made of this subgenotype B4 with alum have shown cross protection against EV-A71 subgenotypes B1, B5, B4 and C4A in volunteer adults [28] and B5, C4a, C4b and C5 in children [25]. Secondly, how long the neutralizing antibodies last was not assessed and that will guide whether a booster dose is needed after 1–2 years. Thirdly, potential interference with immunogenicity of other routine vaccines deserves further study especially if the vaccine is going to be introduced in infants <6 months of age.

In summary, this bioreactor-produced EV-A71 vaccine is immunogenic and safe in healthy children 2 months to 6 years of age. Adjuvanted 1.0 μg TP is more immunogenic than 0.5 μg TP and raised neutralizing antibodies can last for up to a year. During writing up of this manuscript, this vaccine containing adjuvanted 1.0 μg TP had entered phase III clinical trial, which will enroll thousands of children 2 months to 6 years of age. The phase III clinical trial will unveil the efficacy, immunogenicity, safety and persistence of seroconversion up to 2 years after vaccination.

Declaration of Competing Interest

All authors report no potential conflicts of interest. All authors attest they meet the ICMJE criteria for authorship. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Disclaimer

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.07.096.

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