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Enhanced and persistent antibody response against homologous and heterologous strains elicited by a MF59®-adjuvanted influenza vaccine in infants and young children

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ABSTRACT

Background: Non-adjuvanted seasonal influenza vaccines show only modest efficacy in young children. This study compared the immunogenicity, reactogenicity and safety of the MF59®-adjuvanted trivalent subunit vaccine (aTIV) with two non-adjuvanted trivalent vaccines, TIV-1, the non-adjuvanted version of aTIV, and TIV-2, a split virion vaccine.
Methods: 6078 children received two doses of aTIV ($n = 3125$), TIV-1 ($n = 1479$), or TIV-2 ($n = 1474$) four weeks apart (Days 1 and 29). Children aged 6 to <36 months and 36 to <72 months received 0.25 mL and 0.50 mL doses, respectively. Immunogenicity was assessed by hemagglutination inhibition (HI) assay ($n = 2435$) on Days 1, 29, 50 and 209. Safety was assessed up to Day 394.
Results: After the second vaccination (Day 50), the aTIV group showed significantly higher geometric mean HI titers and seroconversion rates than the TIV-1 or TIV-2 groups against all homologous and heterologous strains. The difference was enhanced at HI titers ≥110. aTIV elicited a faster, more persistent antibody response, with significantly higher titers in the aTIV group after one vaccination (Day 29) and after six months (Day 209) than in either TIV group. aTIV was more reactogenic than were TIV-1 and TIV-2 but rates of severe adverse events were very low for all three vaccines.
Conclusion: In infants and young children, the MF59-adjuvanted vaccine induced substantially faster (after one dose), higher, persistent HI titers than the non-adjuvanted vaccines, with consistently higher seroprotection rates at increased threshold HI titers.
This trial is registered at clinicaltrials.gov: NCT01346592.

1. Introduction

Influenza rates are highest in children, with young children, especially those under two years old, constituting one of the high-risk groups for infection and associated complications [1–5]. Most influenza infections in children are self-limiting, but influenza may be severe, particularly in young children and those with chronic medical conditions [3].

Abbreviations: AE, adverse event; aTIV, adjuvanted trivalent influenza vaccine; CI, confidence interval; FAS, full analysis set; GMT, geometric mean titer; HI, hemagglutination inhibition; PPS, per protocol set; TIV, trivalent influenza vaccine.

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In the US, annual influenza vaccination has been recommended for all children from six months to 18 years of age since the 2008–2009 influenza season, when there were 90 million new cases of influenza worldwide in children under five years old [6,7]. However, vaccine coverage rates among children are relatively low: according to the US Centers for Disease Control only about 40% of children received an influenza vaccine in 2012–2013 [8]. One of the reasons for this poor uptake is that healthcare professionals consider currently available non-adjuvanted seasonal influenza vaccines to only have modest efficacy in young children [9,10].

The influenza virus evolves rapidly, evading immune responses by antigenic shift and drift leading to frequent emergence of novel strains [11]. Thus vaccines developed for seasonal influenza often contain mismatched strains to the ones circulating in the population the following year, further reducing vaccine effectiveness [12,13]. Most licenced influenza vaccines are inactivated vaccines, with split virion and subunit vaccines having been administered to millions of people worldwide. Split vaccines are generated by disruption of the inactivated whole virion using detergents whereas subunit vaccines are enriched for hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins [14]. Enhanced antibody responses to seasonal influenza vaccines have been shown using MF59®, an oil-in-water emulsion containing squalene, as an adjuvant [15–17]. MF59 acts locally by activating monocytes, macrophages and dendritic cells, which release a mixture of cytokines attracting phagocytic cells to the injection site, resulting in more efficient antigen-transport to the lymph nodes [18,19]. MF59 has also been shown to enhance the immune response by increasing the binding strength of antibody to the influenza virus [16,17] Influenza vaccines containing MF59 have also consistently demonstrated an established safety profile [20–23]. The MF59-adjuvanted seasonal influenza vaccine, Fluad®, is recommended in numerous countries for adults over the age of 65, but further research is particularly necessary to assess the effectiveness and safety of inclusion of MF59 adjuvant in seasonal vaccines for children. In this study we compared the immunogenicity against both homologous and heterologous influenza strains, reactogenicity and safety of an MF59-adjuvanted trivalent vaccine, aTIV (a subunit vaccine), with that of two non-adjuvanted trivalent influenza vaccines, TIV-1 (the non-adjuvanted version of aTIV) and TIV-2 (a split particle vaccine) in children aged 6 to <72 months.

2. Materials and methods

2.1. Study design and objectives

This Phase III, observer-blind, stratified, randomized study was conducted at eight sites in Argentina, five in Australia, two in Chile, 12 in The Philippines, and five in South Africa, between April 2011 and July 2012. The primary immunogenicity objective was to demonstrate the non-inferior immunogenicity of two doses of aTIV compared with TIV-1 and TIV-2, measured by seroconversion rates and geometric mean titers (GMTs) against three homologous strains, in children aged 6 to <72 months. An additional primary objective was to demonstrate the non-inferiority of two doses of TIV-1 to TIV-2 in children aged 6 to <36 months. Secondary objectives were: to demonstrate the superiority of aTIV to TIV-1 and TIV-2 against homologous strains in children aged 6 to <24 months and 6 to <72 months, to evaluate immunogenicity against heterologous strains in children aged 6 to <72 months, and to assess reactogenicity and safety of all three vaccines. The protocol was approved by Ethics Review Committees in each institution, and the study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. Written informed consent was obtained from the parents/legal guardians of all children before enrolment. The study was registered with ClinicalTrials.gov: NCT01346592.

2.2. Children

6104 healthy children aged from 6 to <72 months were enrolled. The main exclusion criteria were: history of Guillain–Barré syndrome or severe convulsion; fatal prognosis for an underlying medical condition; history of serious vaccine reactions; hypersensitivity to any vaccine component; receipt of another investigational agent within four weeks prior to enrollment or before completion of the study; impairment/alteration of immune function; fever (≥38.0 °C); receipt of licensed vaccines within 14 days (inactivated) or 28 days (live attenuated) prior to enrolment; planned surgery.

2.3. Study procedures

Children were stratified into three age groups (6 to <24 months, 24 to <36 months and 36 to <72 months) and randomly assigned using a web-based system to receive aTIV, TIV-1 or TIV-2, with allocation ratios 3:2:2 (6 to <24 months and 24 to <36 months) or 4:1:1 (36 to <72 months). Vaccines were administered on Days 1 and 29: children aged 6 to <36 months received a 0.25 mL dose and children aged 36 to <72 months received a 0.5 mL dose. For immunogenicity analyses, a subset (n = 2655) of children from each age and vaccine group was used. Blood samples (5 mL) were collected at baseline (Day 1), prior to the second vaccination (Day 29), and 21 (Day 50) and 180 (Day 209) days after the second vaccination. Subject disposition and study design are illustrated in Fig. 1.

2.4. Vaccines

A single 0.5 mL dose of the trivalent MF59®-adjuvanted seasonal egg-derived subunit vaccine, aTIV (Fluad®, Novartis Vaccines) (Lots: A52P14H1A/A52P15H1A/A52P16H1A/B52D21N1/B52D21N1A/B52D21N1B), the trivalent seasonal egg-derived subunit vaccine, TIV-1 (Agriflu®, Novartis Vaccines) (Lots: B51D01N1/B51D04N1A/B51D04N1B), and the trivalent seasonal egg-derived split vaccine TIV-2 (Fluzone®, Sanofi Pasteur) (Lots: U3792BA/U3641BA), all contained 15 μg of each of A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008 hemagglutinin (HA), as recommended by the World Health Organization for the Southern Hemisphere influenza season 2011. All vaccines were injected intramuscularly into the deltoid muscle of the non-dominant arm, using a one inch Luer lock needle.

2.5. Immunogenicity assessment

Blood samples were collected by venipuncture, centrifuged, immediately stored at −18 °C or below and later transported to the Novartis Vaccines Clinical Sciences Laboratory in Marburg, Germany, where antibody responses against both homologous and heterologous strains were assessed, in terms of GMTs, seroconversion and seroprotection rates, by hemagglutination inhibition (HI), based on the methods of Stephenson et al. [24]. Cross-reactive antibody responses were tested against the following heterologous strains: A/New Jersey/8/1976 (H1N1), A/Uruguay/716/2007 (H3N2) and B/Malaysia/2506/2004. All three heterologous strains were found to be antigenically distinct (>4–8 fold difference) from the vaccine strains, when analyzed using antibody responses to ferret antisera: >64-fold difference in HI for the A/H1N1 and A/H3N2

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strains, and a >16-fold difference for the B strains [25]. Alignment of the HA sequences between the vaccine strain and the heterologous subtype test strain show the following percentages of identity: 90.6% (A/H1N1), 94.9% (A/H3N2) and 99% (B strain). HI titers were expressed as the reciprocal of the highest dilution at which hemagglutination was totally inhibited. Seroconversion (or significant increase) was defined as: Day 50 HI titers ≥40 for children seronegative at baseline [HI titer <10] or a minimum 4-fold increase in HI titer by Day 50 for children seropositive at baseline [HI titer ≥10]. Seroprotection rates were also estimated at higher threshold HI titers (≥110 to ≥629), which have been shown to be a more meaningful correlate of protection in children [23].

2.6. Reactogenicity and safety assessment

Children were observed for approximately 30 min after vaccination for immediate reactions. Solicited local and systemic adverse reactions up to Day 7 and unsolicited adverse events (AEs) up to Day 50 were recorded on diary cards by the child's parent or guardian. All serious AEs (SAEs), AEs of special interest (AESIs), new onset of chronic disease (NOCD) and AEs leading to study withdrawal were collected up to Day 394.

2.7. Statistical analysis

2.7.1. Data sets

The per-protocol set (PPS) was used to analyze the non-inferiority objective, i.e. children who were vaccinated and had no major protocol violations. For persistence of response, the full analysis persistence set was used, i.e. children who were vaccinated and provided evaluable sera at all time points. For cross-reactive antibody response, the heterologous full analysis persistence set was used. For all other analyses, the full analysis set (FAS) was used, i.e. children who were vaccinated and provided evaluable sera at Days 1 and 50. Vaccine group differences in seroconversion and seroprotection at increasing threshold HI titers were estimated using a categorical model with adjustment for age strata; ratios between vaccine group GMTs, referred to throughout as GMT ratios, were estimated using a general linear model with adjustment for age strata and baseline titer.

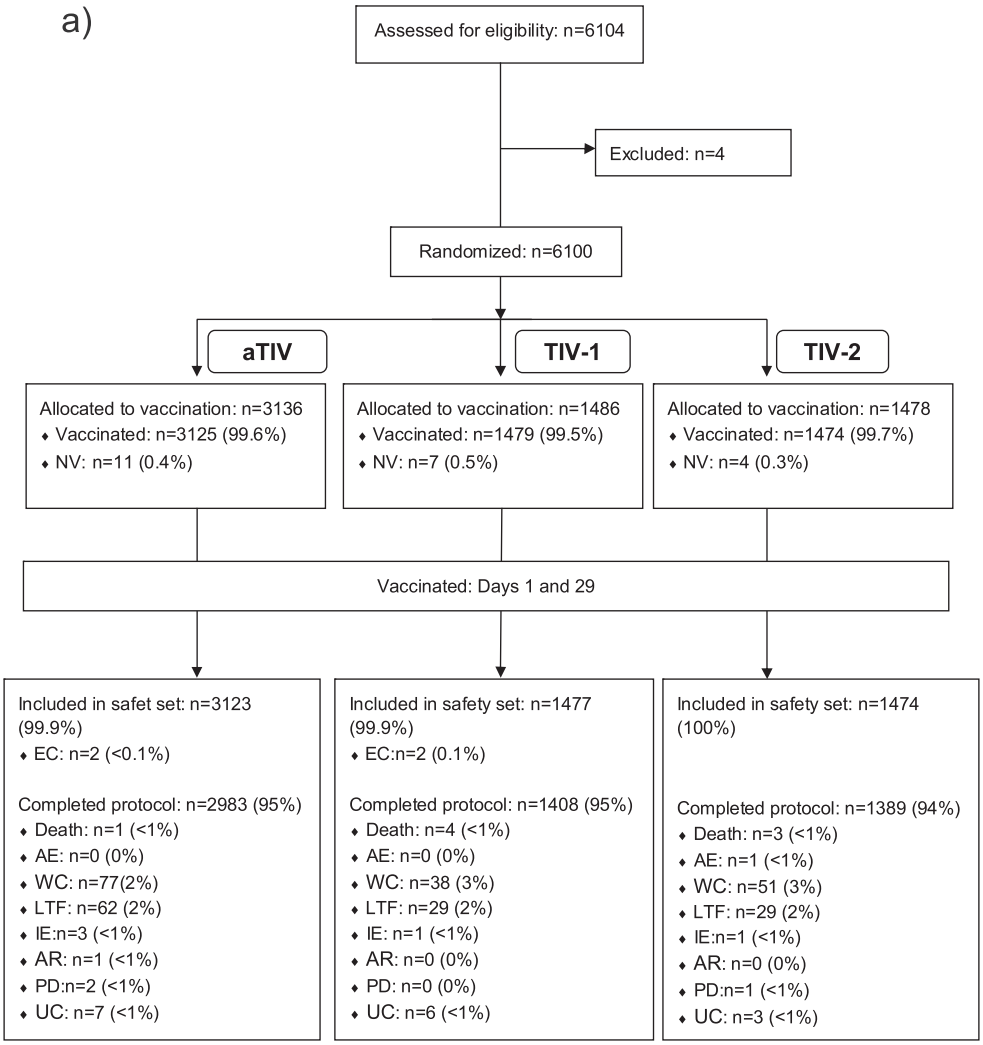

Fig. 1. Study design and subject disposition for (a) the safety set and (b) the immunogenicity subsets for homologous and heterologous strains.

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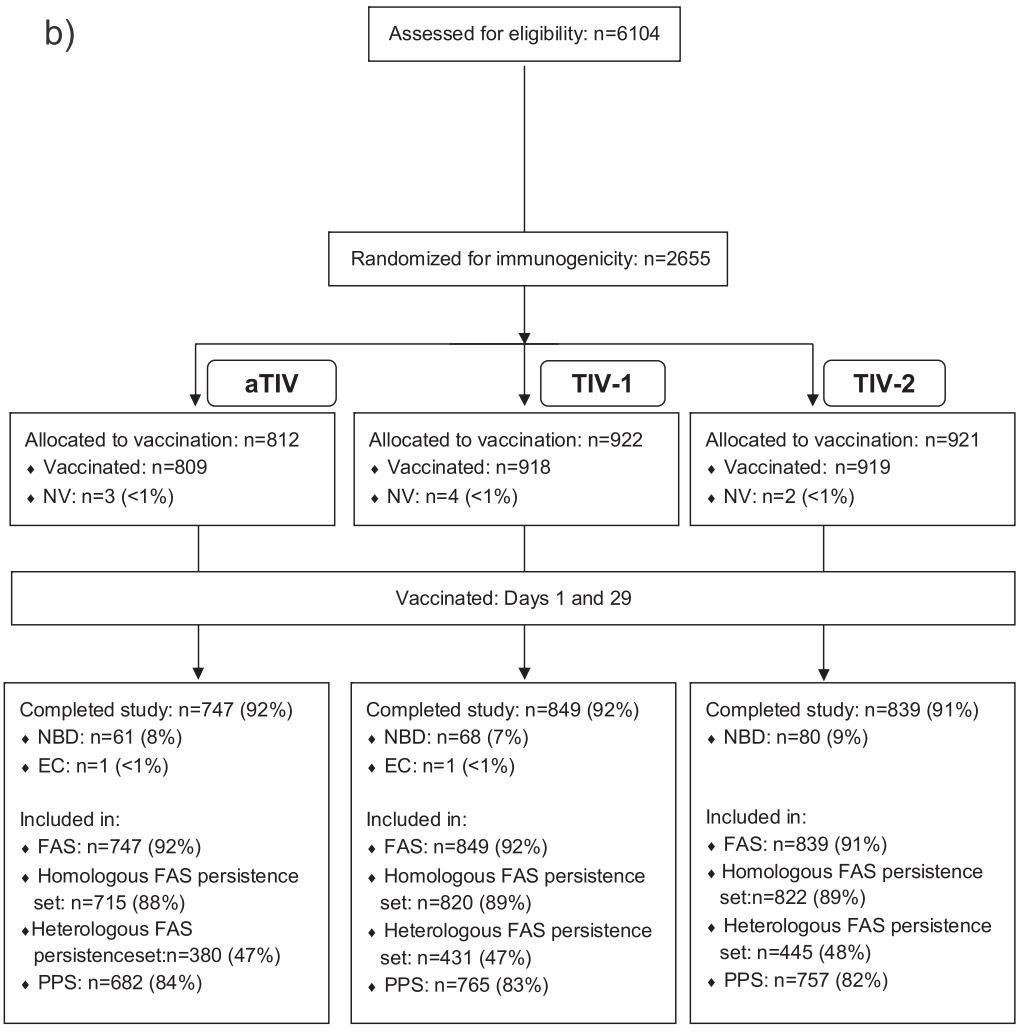

Fig. 1. (*Continued*)

2.7.2. Statistical significance

A significantly higher response was concluded if, for GMTs, the lower bound of the 95% confidence interval (CI) around the group ratio was >1.0, and, for seroconversion rates, if the lower bound of the 95% CI (Clopper–Pearson) around the group difference was >0%.

2.7.3. Non-inferiority

Non-inferiority was concluded if the lower bound of multiplicity-adjusted two-sided CI (97.6% for aTIV and 97.4% for TIV-1 non-inferiority objectives) exceeded the corresponding margin (0.667 for the GMTs and -10% for seroconversion) in all 12 comparisons for aTIV versus both TIVs (2 endpoints × 3 strains × 2 comparators), and in all six comparisons for TIV-1 versus TIV-2 (2 endpoints × 3 strains).

2.7.4. Superiority

Superiority was concluded if the multiplicity adjusted one-sided *p*-value was ≤1.2% for two out of three strains, with superiority margins of 1.5 for group GMT ratios and 10% for group differences in seroconversion rates.

2.7.5. Reactogenicity and safety

Reactogenicity and safety data were evaluated descriptively and expressed as the percentage of children with adverse reactions/events in each group, which were categorized as mild, moderate or severe. AEs were also classed as not related, possibly related or probably related to study vaccination.

3. Results

3.1. Study population

Of the 6104 children enrolled in the study, 6100 were randomized and 6078 were vaccinated: 3125 children received aTIV, 1479 children received TIV-1, and 1474 children received TIV-2. Demographic and other characteristics were balanced between the vaccine groups (Table 1), except for mean age, which was higher in the aTIV group. This imbalance was addressed in immunogenicity analyses by statistical adjustment for age group.

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3.2. Immunogenicity

3.2.1. Responses to homologous strains

By Day 29, children in the aTIV group had significantly higher GMTs and seroconversion rates against all homologous strains than children in either the TIV-1 or the TIV-2 groups (Fig. 2, Supplementary Table). The difference in GMTs between aTIV and the TIVs had increased by Day 50 and remained significantly higher six months after vaccination. For children aged 6 to <72 months, aTIV met the statistical criteria for non-inferiority to both TIVs against all three strains, for both GMT ratios and seroconversion rates at Day 50 (Table 2). aTIV also met the pre-defined superiority threshold against both TIVs in terms of GMTs, but not in terms of seroconversion rates. For the subset of youngest children (6 to <24 months), aTIV achieved superiority to TIV-1 in both GMTs and seroconversion rates (Table 3). GMT ratios between aTIV and TIV-2 were significantly higher than the superiority margin (p-value <0.0001) but differences in seroconversion rates did not meet the 10% threshold for superiority against any strain in this subset.

For the entire study population, the magnitude of the difference in seroprotection between aTIV and the TIVs was statistically significant at the ≥40 threshold titer, and increased at higher titers, with the largest differences seen at titers of ≥330 and above for the A strains, and of ≥110 and above for the B strain (Fig. 3). All differences at titers of ≥110 and above were significant ($p < 0.01$) for all homologous strains.

Although there was evidence of TIV-1 being non-inferior to TIV-2 in terms of both seroconversion rates and GMTs for the A/H3N2 and B strains, non-inferiority was not met for the A/H1N1 strain in terms of either seroconversion rate or GMTs (Table 4), hence the overall non-inferiority as defined in study objective (i.e. non-inferiority met for all six measures) could not be concluded.

3.2.2. Responses to heterologous strains

When tested against all three heterologous strains, significantly higher GMTs and seroconversion rates were seen in the aTIV group compared with TIV groups at all time points post-vaccination, except at Day 29 against the A/H1N1 strain for aTIV compared with TIV-1 (Fig. 4, Supplementary Table). The greatest differences between aTIV and the two comparator vaccines were observed against the heterologous A/H3N2 strain after one vaccination (Day 29), and against the heterologous B strain following the complete two-vaccination schedule (Day 50).

3.3. Reactogenicity

Reported solicited adverse reactions were generally mild to moderate and transient in nature. More of the older children (36 to <72 months) in the aTIV group reported local adverse reaction, including injection site pain, than in either TIV group (Table 5). After the first vaccination, more of the older children in the aTIV group reported solicited systemic adverse reactions, including fever, than in the other vaccine groups, though the rates of fever in the youngest age group was similar across the vaccine groups (Table 6). After the second vaccination, similar levels of systemic adverse reactions were recorded across all vaccine groups, but the incidence of fever remained slightly higher in the aTIV group for all age groups. While low, the rate of severe reactions among children in the aTIV group (0.38%) was also higher than in the TIV-1 (0.07%) or TIV-2 (0.14%) groups.

Table 1
Demographics by age and vaccine group for children enrolled and randomized.

	6 to <24 months			6 to <36 months			36 to <72 months			All ages (6 to < 72 months)		
	aTIV (n = 846)	TIV-1 (n = 573)	TIV-2 (n = 571)	aTIV (n = 1525)	TIV-1 (n = 1050)	TIV-2 (n = 1040)	aTIV (n = 1611)	TIV-1 (n = 436)	TIV-2 (n = 438)	aTIV (n = 3136)	TIV-1 (n = 1486)	TIV-2 (n = 1478)
Mean age ± SD (months)	13.9 ± 5.5	14.1 ± 5.3	13.9 ± 5.4	20.8 ± 9.0	21.1 ± 8.9	20.9 ± 9.1	52.5 ± 10.2	52.2 ± 10.0	51.6 ± 10.4	37.1 ± 18.6	30.2 ± 16.9	30.0 ± 16.9
Male (%)	48	50	49	49	51	50	52	50	49	51	51	50
Mean weight ± SD (kg)	9.2 ± 2.0	9.2 ± 2.0	9.1 ± 1.9	10.5 ± 2.5	10.6 ± 2.5	10.6 ± 2.6	15.8 ± 3.2	15.9 ± 3.1	15.7 ± 3.3	13.2 ± 3.9	12.1 ± 3.6	12.1 ± 3.7
Mean height ± SD (cm)	73 ± 6.4	73 ± 6.6	73 ± 6.6	79 ± 8.6	79 ± 8.6	79 ± 8.8	100 ± 7.6	101 ± 7.5	100 ± 8.0	90 ± 13.5	85 ± 12.8	85 ± 12.8
Asian (%)	83	83	83	83	82	82	63	61	61	72	76	76
Black (%)	10	10	11	10	10	10	18	18	18	14	13	13
Caucasian (%)	5	5	5	5	5	5	12	12	12	9	7	7
Hispanic (%)	2	2	2	2	3	3	7	8	9	4	4	5
Other (%)	<1	0	0	<1	<1	0	<1	0	0	<1	<1	0
BMI	17.3 ± 2.7	17.1 ± 2.6	17.1 ± 2.6	16.8 ± 2.6	16.8 ± 2.6	16.8 ± 2.5	15.6 ± 2.1	15.7 ± 1.9	15.7 ± 2.1	16.2 ± 2.4	16.5 ± 2.4	16.5 ± 2.5
Previous influenza vaccination (%)	<1	<1	<1	1	2	1	6	5	5	4	3	3

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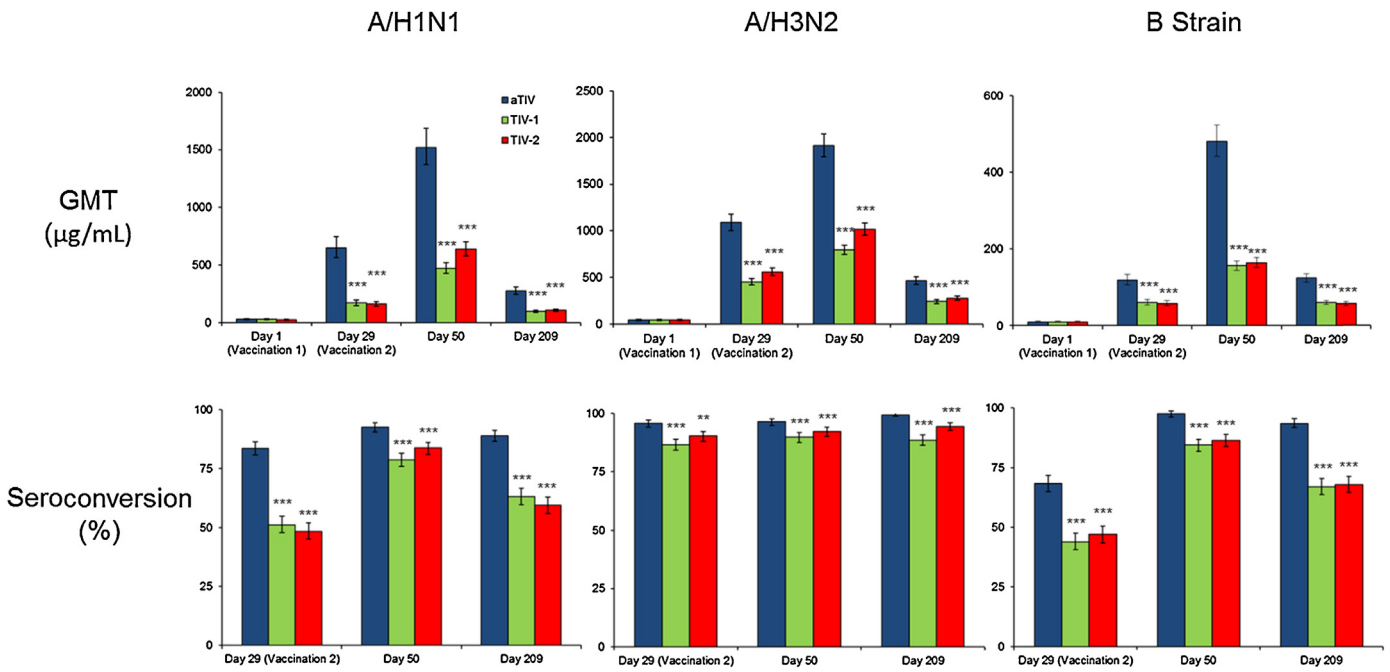

Fig. 2. HI antibody responses against each homologous strain in terms of geometric mean titer (GMT) and seroconversion rates at baseline (Day 1), after first (Day 29) and second (Day 50) vaccinations, and six months after vaccination (Day 209) in children aged 6 to <72 months (95% CI). Data are presented for the homologous FAS persistence set. $^{**}p < 0.01$, $^{***}p < 0.001$.

3.4. Safety

Overall, fewer children experienced unsolicited AEs in the aTIV group (49%) than in the TIV-1 (55%) or TIV-2 (58%) groups. No individual AE was reported as at least possibly related to study vaccination by >1% of children in any vaccine group. The most frequent AEs reported are all commonly seen in pediatric populations (Table 7).

There were eight deaths in the study: one in the aTIV group, four in the TIV-1 group, and three in the TIV-2 group. None was considered to be related to study vaccination. Two other children (both TIV-2 group) experienced AEs leading to study withdrawal; neither was considered related to study vaccination. SAEs were reported by 4% of children in the aTIV and TIV-2 groups and 5% of children in the TIV-1 group. A total of 27 children across vaccine groups experienced febrile convulsion during the study. One event was considered possibly related to vaccination: this occurred in a child in the aTIV group on Day 2, lasted less than a day, and was of mild severity.

4. Discussion

Currently available non-adjuvanted seasonal influenza vaccines have only modest efficacy in young children [8]. Addition of the MF59 adjuvant has been shown to enhance protection against influenza in another high-risk group, the elderly [18,27,28]. As young children are the second largest group at risk of complications from seasonal influenza [20], this study compared the

Table 2
Comparison of antibody responses in aTIV group versus TIV-1 and TIV-2 groups using ratios of GMTs (95% CI) and differences in seroconversion rates (95% CI) for children aged 6 to <72 months.

Strain		Ratios between GMTs		Difference in seroconversion rates	
		aTIV:TIV-1	aTIV:TIV-2	aTIV - TIV-1	aTIV - TIV-2
A/H1N1	Day 1	1.07 (0.87–1.30)	1.18 (0.97–1.44)	–	–
	Day 29	**3.82 (3.14–4.64)**	**4.07 (3.34–4.95)**	**28.4 (24.2–32.9)**	**30.9 (26.6–35.1)**
	Day 50	**3.21 (2.79–3.71)***	**2.38 (2.07–2.75)***	**14.4 (11.0–17.7)***	**9.3 (6.3–12.4)**
	Day 209	**2.58 (2.19–3.04)**	**2.84 (2.41–3.34)**	**19.2 (15.4–23.0)**	**21.7 (18.0–25.4)**
A/H3N2	Day 1	0.95 (0.78–1.16)	0.97 (0.80–1.19)	–	–
	Day 29	**2.40 (2.15–2.68)**	**1.95 (1.74–2.18)**	**9.1 (6.4–11.9)**	**5.6 (3.1–8.1)**
	Day 50	**2.40 (2.19–2.62)***	**1.88 (1.72–2.06)***	**6.8 (4.5–9.1)**	**3.9 (1.8–5.9)**
	Day 209	**1.91 (1.69–2.16)**	**1.67 (1.48–1.89)**	**3.8 (2.4–5.3)**	**1.8 (0.6–3.1)**
B strain	Day 1	0.99 (0.88–1.11)	0.97 (0.87–1.09)	–	–
	Day 29	**1.95 (1.65–2.29)**	**2.03 (1.73–2.39)**	**18.6 (13.9–23.4)**	**15.4 (10.7–20.1)**
	Day 50	**3.08 (2.73–3.47)***	**2.93 (2.60–3.30)***	**10.9 (8.3–13.4)**	**10.7 (8.1–13.3)**
	Day 209	**2.05 (1.81–2.32)**	**2.17 (1.92–2.46)**	**26.1 (22.5–29.6)**	**25.5 (21.9–29.0)**

Values are presented for the homologous full analysis set (FAS) persistence subset for Days 29 and 209. Data presented for Days 1 and 50 are presented for the FAS as superiority was tested on this data set. Significance testing was performed on each value from the relevant data set (Days 29 and 209 = homologous FAS persistence set, Days 1 and 50 = FAS). **Bold values** indicate significant higher antibody response of aTIV group.

* Indicates value significantly higher than corresponding superiority margin.

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Table 3
Geometric mean titers (GMTs) (95% CI) and seroconversion rates (95% CI) against homologous strains at baseline (Day 1) and three weeks after second vaccination (Day 50), for children aged 6 to <24 months. Data are presented for the full analysis set.

		Vaccine groups			Ratios between GMTs		Difference in seroconversion rates	
		aTIV (n = 266)	TIV-1 (n = 387)	TIV-2 (n = 389)	aTIV:TIV-1	aTIV:TIV-2	aTIV-TIV-1	aTIV-TIV-2
A/H1N1	Day 1 GMT	12 (9.52–14)	12 (9.95–14)	9.87 (8.36–12)	0.99 (0.76–1.28)	1.18 (0.91–1.53)	–	–
	Day 50 GMT	1080 (899–1297)	205 (176–238)	298 (256–347)	**5.28* (4.16–6.7)**	**3.63* (2.86–4.6)**	–	–
	Day 50 seroconversion (%)	95.5 (92.3–97.7)	77.8 (73.3–81.8)	85.1 (81.2–88.5)	–	–	**17.7* (12.9–22.6)**	**10.4 (6.1–14.7)**
A/H3N2	Day 1 GMT	15 (12–19)	17 (14–21)	15 (12–18)	0.88 (0.65–1.2)	1.01 (0.74–1.36)	–	–
	Day 50 GMT	1709 (1536–1903)	552 (505–603)	758 (694–828)	**3.10* (2.69–3.56)**	**2.25* (1.96–2.59)**	–	–
	Day 50 seroconversion (%)	98.1 (95.7–99.4)	92.5 (89.4–94.9)	95.6 (93.1–97.4)	–	–	**5.6 (2.5–8.7)**	2.5 (−0.12–5.1)
B	Day 1 GMT	7.75 (6.83–8.78)	7.92 (7.14–8.79)	8.18 (7.37–9.07)	0.98 (0.83–1.15)	0.95 (0.8–1.12)	–	–
	Day 50 GMT	616 (534–711)	112 (100–127)	133 (118–149)	**5.48* (4.55–6.6)**	**4.64* (3.86–5.59)**	–	–
	Day 50 seroconversion (%)	98.1 (95.7–99.4)	79.3 (75–83.3)	85.4 (81.4–88.7)	–	–	**18.8* (14.4–23.1)**	**12.8 (8.9–16.7)**

Bold values indicate significant higher antibody response of aTIV group.
* Indicates value significantly higher than corresponding superiority margin.

immunogenicity and safety of an MF59-adjuvanted influenza vaccine (aTIV) with two non-adjuvanted vaccines in young children. While a prior study has demonstrated the efficacy of aTIV in young children [29], here we present the first large scale phase III study evaluating the immunogenicity and safety of aTIV versus US licensed comparator vaccines in children 6 to <72 months of age, including a one year safety follow-up.

In this study, aTIV elicited significantly higher GMTs and seroconversion rates by Day 50 than either TIV vaccine, for all vaccine strains. This substantially enhanced immunogenicity mirrors results from previous studies comparing aTIV with non-adjuvanted inactivated influenza vaccines in young children, indicating an immunogenicity benefit of adjuvanted vaccines for this age group, when assessed at the conventional threshold antibody titer (≥40) [23,30].

As demonstrated by Black et al. [26] an HI titer ≥40 in children aged 6 to <72 months was associated with a population clinical protection level (against the A/H3N2 strain) of 22%, whereas a higher threshold HI titer of ≥110 was associated with the conventional 50% clinical protection rate against infection and titers of 215, 330, and 629 predicted protection rates of 70%, 80%, and 90%, respectively [31,32]. In our study, percentages of children with HI titers ≥215, ≥330 and ≥629 were significantly higher in the aTIV group than in either TIV group. The magnitude of the differences increased at higher titers, especially against the B strain, where a large difference between aTIV and TIVs was seen at titers of ≥110 and above. Thus the enhanced immunogenicity of aTIV was more pronounced at titers that may represent more meaningful correlates of clinical protection from influenza for this age group.

The increased antibody response to aTIV was evident after a single dose (measured on Day 29). As many influenza vaccine-naïve children do not complete the recommended two-dose vaccination schedule, the increased immunogenicity of aTIV would potentially increase protection against seasonal influenza in this age group [33]. In addition, aTIV elicited a persistent antibody response, echoing results from Vesikari et al., who found that the proportion of children with seroprotective antibody titers remained significantly higher for the adjuvanted vaccine six months after initial vaccination [23]. Although our study did not evaluate vaccine efficacy, both the significantly higher antibody response after one vaccine dose, together with the sustained response to Day 209, following the complete two-vaccination schedule, suggest a potential enhanced efficacy of aTIV in young children. Although non-adjuvanted vaccines have a long track record of use in children, they do not appear to induce satisfactory protective antibodies in unprimed children, especially the very young, and against influenza B strains [34,35]. Furthermore, influenza B frequently occurs in late season outbreaks, so the persistence of higher HI titers, and the maintenance of seroprotection until the end of the influenza season suggests more prolonged protection against this strain.

As the influenza virus evolves very rapidly, often there is antigenic mismatch between circulating strains and those included in vaccines [36]. Therefore, a seasonal vaccine needs to be able to induce a broad cross-reactive antibody response against antigenically distinct influenza strains to increase the likelihood of protection against current strains. aTIV induced significantly higher antibody responses than either TIV, against all three heterologous strains, with the largest differences after a single vaccine dose (on Day 29) being seen against the heterologous A/H3N2 strain. The heterologous strains evaluated in this study, when tested against ferret anti-sera, had a >64-fold difference in HI from the vaccine strains for the A/H1N1 and A/H3N2 strains and a >16-fold difference for the B strains: it is possible that the magnitude of cross-reactive response may differ against a more distantly related strain.

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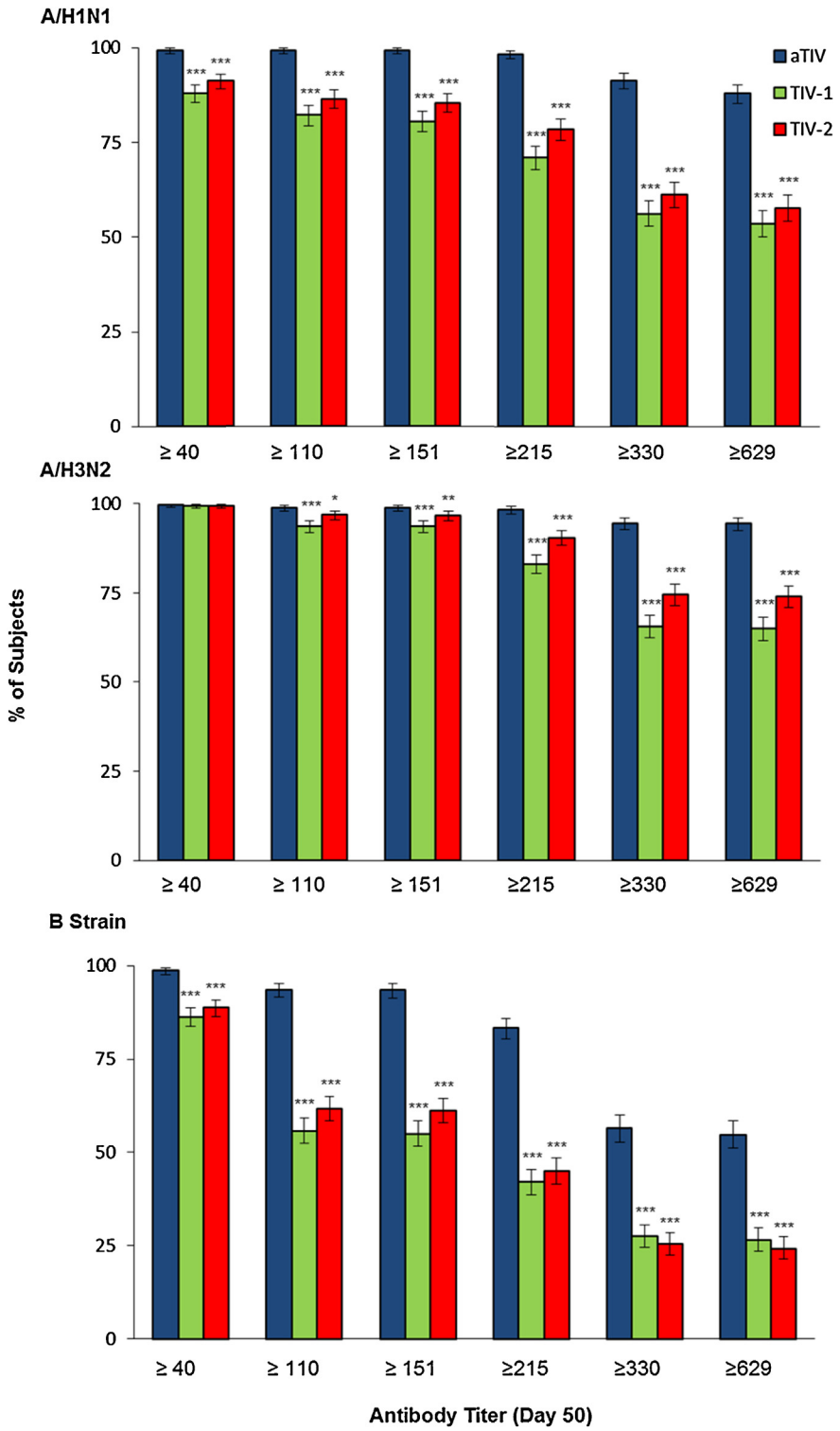

Fig. 3. Percentage of children (95% CI) with HI titers against homologous strains above increasing seroprotection thresholds (≥40, ≥110, ≥151, ≥215, ≥330, ≥629) at Day 50 in children aged 6 to <72 months. $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Similarly, a previous study in 6 to <36 month old children showed aTIV induced significantly higher GMTs against heterologous strains than the comparator TIV, with the highest antibody titers for the A/H3N2 strain [29]. A/H3N2 is known to have greater antigenic variability than the other two strains included in seasonal influenza vaccines, therefore an effective cross-reactive antibody response to this strain could provide increased efficacy against future circulating strains [12,37].

Solicited adverse reaction rates were highest in the aTIV group, which is in agreement with other studies on aTIVs in this age group [20,23,29]. In addition, rates of mild fever were similar to those found by Vesikari et al., who also reported higher rates of

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Table 4
Antibody responses for children (6 to <36 months) for TIV-1 compared with TIV-2 for ratios between vaccine group geometric mean titers (GMT) and differences in seroconversion rates (97.4% CI). Data are presented for the per-protocol set. Bold = non-inferiority of TIV-1 to TIV-2 met (lower bound of multiplicity-adjusted two-sided CI (97.4%) exceeded 0.667 for the GMTs and -10% for seroconversion).

Strain		Ratio of GMTs	Difference in seroconversion (%)
A/H1N1	Day 1	1.17 (0.92–1.48)	–
	Day 50	0.76 (0.62–0.93)	−5.3 (−10.13 to −0.47)
A/H3N2	Day 1	1.02 (0.79–1.32)	–
	Day 50	**0.77 (0.68–0.86)**	**−2.84 (−6.16–0.48)**
B	Day 1	1.00 (0.87–1.15)	–
strain	Day 50	**0.94 (0.80–1.11)**	**−2.49 (−7.01–2.03)**

fever in 36 to <72 month old recipients of aTIV [23,29]. Although some adjuvanted influenza vaccines have been linked to narcolepsy [38], there is no evidence to date of an association between MF59-adjuvanted vaccine receipt and narcolepsy [39,40]. In addition, no case of narcolepsy was reported in this study. Overall, despite the higher reactogenicity, the number of children completing the study was similar for all vaccine groups, and the enhanced immunogenicity of aTIV can be seen to offset the increase in mild to moderate transient reactions experienced from the use of this vaccine.

In summary, this study demonstrates that aTIV, a trivalent MF59-adjuvanted seasonal subunit influenza vaccine, induced significantly higher antibody titers in young children than non-adjuvanted inactivated influenza vaccines, even after the first dose. The superior antibody responses after two doses of aTIV remained significantly higher than responses to the TIVs for six months after vaccination, against both homologous and heterologous A and B strains. This higher response was more pronounced when tested at increasing threshold antibody titers, which may be more appropriate for this age group. Thus, despite the increased reactogenicity, aTIV may therefore provide enhanced protection in children aged 6 to <72 months against both homologous and heterologous influenza strains.

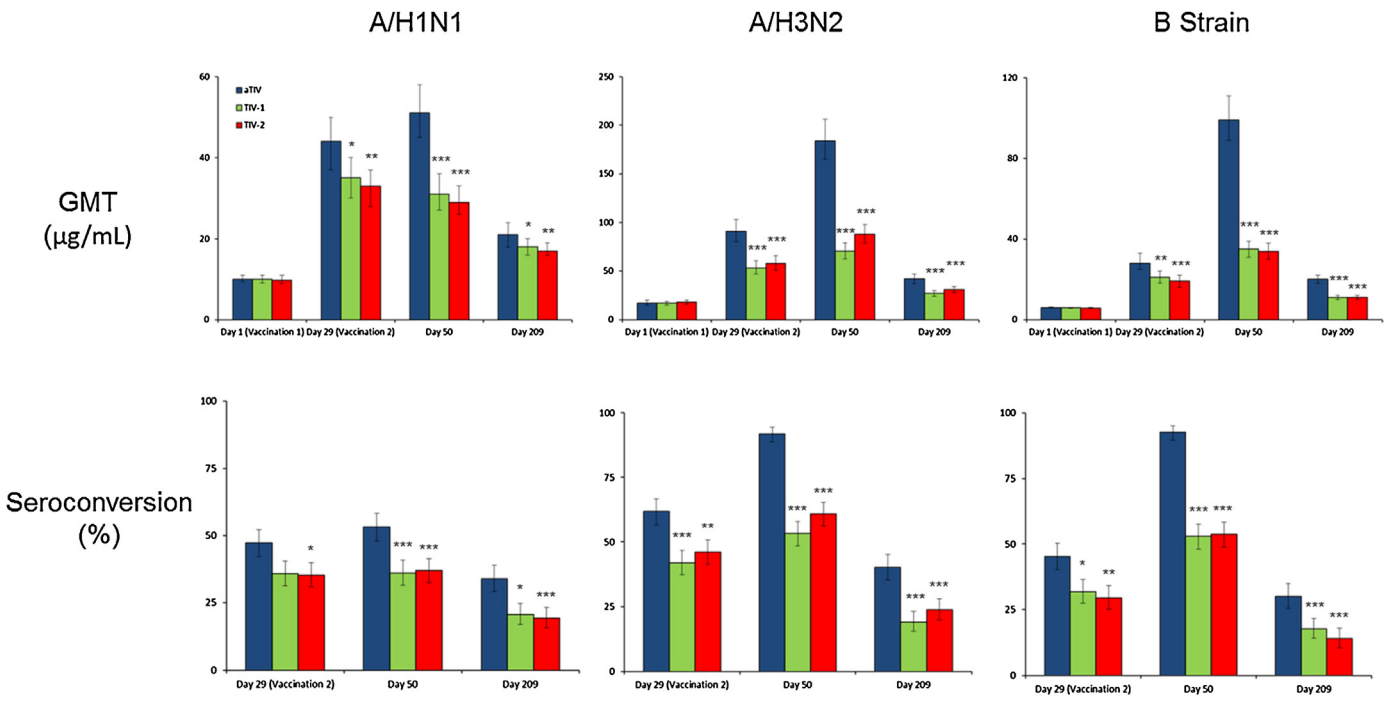

Fig. 4. HI antibody responses against heterologous strains in terms of geometric mean titer (GMT) and seroconversion rates at baseline (Day 1), after first (Day 29) and second (Day 50) vaccinations, and six months after vaccination (Day 209) in children aged 6 to <72 months (95% CI). Data are presented for the heterologous FAS persistence set. Heterologous strains were A/New Jersey/8/1976 (H1N1), A/Uruguay/716/2007 (H3N2) and B/Malaysia/2506/2004. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 5
Percentages of children experiencing mild to moderate (open figures) and severe (in brackets) solicited local adverse reactions from 6 h to ≤7 days after each vaccination.

	6 to <24 months			6 to <36 months			36 to <72 months			All ages (6 to <72 months)		
	aTIV	TIV-1	TIV-2	aTIV	TIV-1	TIV-2	aTIV	TIV-1	TIV-2	aTIV	TIV-1	TIV-2
After 1st vaccination	*n* = 832	*n* = 557	*n* = 562	*n* = 1494	*n* = 1025	*n* = 1010	*n* = 1499	*n* = 405	*n* = 415	*n* = 2991	*n* = 1430	*n* = 1422
Ecchymosis (%)	2 (0)	3 (0)	3 (0)	3 (0)	3 (0)	4 (0)	5 (<1)	4 (0)	4 (0)	4 (<1)	3 (0)	4 (0)
Erythema (%)	5 (0)	5 (0)	6 (0)	5 (0)	5 (0)	5 (0)	8 (<1)	7 (0)	6 (0)	6 (<1)	5 (0)	5 (0)
Induration (%)	2 (0)	2 (0)	2 (0)	3 (0)	3 (0)	2 (0)	7 (<1)	3 (0)	5 (0)	5 (<1)	3 (0)	3 (0)
Swelling (%)	1 (0)	1 (0)	1 (0)	2 (0)	1 (0)	1 (0)	4 (<1)	2 (0)	3 (0)	3 (<1)	2 (0)	2 (0)
Tenderness (%)	6 (0)	3 (0)	5 (0)	7 (0)	5 (0)	6 (0)	–	–	–	7 (0)	5 (0)	6 (0)
Pain at injection site (%)	–	–	–	–	–	–	33 (<1)	17 (<1)	20 (<1)	33 (<1)	17 (<1)	20 (<1)
After 2nd vaccination	*n* = 816	*n* = 547	*n* = 546	*n* = 1465	*n* = 1008	*n* = 985	*n* = 1553	*n* = 418	*n* = 423	*n* = 3018	*n* = 1426	*n* = 1408
Ecchymosis (%)	2 (0)	1 (0)	3 (0)	3 (0)	1 (0)	2 (0)	4 (<1)	4 (0)	4 (0)	3 (<1)	2 (0)	3 (0)
Erythema (%)	6 (<1)	3 (0)	3 (0)	5 (<1)	3 (0)	2 (0)	7 (1)	5 (<1)	5 (0)	6 (<1)	4 (<1)	3 (0)
Induration (%)	3 (<1)	1 (0)	2 (0)	2 (<1)	1 (0)	2 (0)	7 (<1)	4 (0)	3 (0)	5 (<1)	2 (0)	2 (0)
Swelling (%)	2 (0)	<1 (0)	1 (0)	2 (0)	<1 (0)	1 (0)	8 (<1)	2 (0)	2 (0)	5 (<1)	1 (0)	1 (0)
Tenderness (%)	5 (0)	2 (0)	2 (0)	5 (<1)	3 (0)	3 (0)	–	–	–	5 (<1)	3 (0)	3 (0)
Pain at injection site (%)	–	–	–	–	–	-	27 (1)	15 (<1)	16 (0)	27 (1)	15 (<1)	16 (0)

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Table 6
Percentages of children experiencing mild to moderate (open figures) and severe (in brackets) solicited systemic adverse effects 6 h to ≤7 days after each vaccination.

	6 to <24 months			6 to <36 months			36 to <72 months			All ages (6 to <72 months)		
	aTIV	TIV-1	TIV-2	aTIV	TIV-1	TIV-2	aTIV	TIV-1	TIV-2	aTIV	TIV-1	TIV-2
After 1st vaccination	*n* = 834	*n* = 558	*n* = 563	*n* = 1496	*n* = 1026	*n* = 1011	*n* = 1578	*n* = 425	*n* = 432	*n* = 3074	*n* = 1451	*n* = 1443
Chills (%)	–	–	–	–	–	–	7 (<1)	2 (0)	2 (0)	7 (<1)	2 (0)	2 (0)
Myalgia (%)	–	–	–	–	–	–	10 (<1)	5 (0)	4 (0)	10 (<1)	5 (0)	4 (0)
Arthralgia (%)	–	–	–	–	–	–	5 (0)	2 (0)	2 (0)	5 (0)	2 (0)	2 (0)
Headache (%)	–	–	–	–	–	–	13 (0)	6 (1)	6 (0)	13 (0)	5 (1)	6 (0)
Fatigue (%)	–	–	–	–	–	–	10 (<1)	7 (<1)	5 (0)	10 (<1)	7 (<1)	5 (0)
Change in eating habits (%)	12 (<1)	11 (0)	11 (0)	11 (<1)	10 (<1)	11(<1)	11 (<1)	7 (1)	7 (<1)	11 (<1)	9 (<1)	10 (<1)
Diarrhea (%)	14 (1)	14 (<1)	15 (<1)	13 (<1)	12 (<1)	14 (<1)	7 (<1)	8 (<1)	5 (0)	10 (<1)	11 (<1)	11 (<1)
Irritability (%)	17(<1)	14 (0)	15 (<1)	14 (<1)	12 (<1)	13 (<1)	–	–	–	14 (<1)	12 (<1)	13 (<1)
Crying (%)	11 (<1)	9 (<1)	10 (<1)	10 (<1)	7 (<1)	9 (<1)	–	–	–	10 (<1)	7 (<1)	9 (<1)
Sleepiness (%)	14 (0)	13 (<1)	13 (0)	12 (0)	11 (<1)	12 (0)	–	–	–	12 (0)	11 (<1)	12 (0)
Vomiting (%)	7 (<1)	5 (0)	5 (1)	6 (<1)	4 (0)	6 (<1)	5 (<1)	5 (<1)	3 (0)	6 (<1)	4 (<1)	5 (<1)
Fever (%)	13 (<1)	11 (<1)	10 (<1)	13 (<1)	9 (<1)	10 (<1)	17 (<1)	6 (<1)	5 (0)	15 (<1)	8 (<1)	9 (<1)
After 2nd vaccination	*n* = 816	*n* = 548	*n* = 546	*n* = 1466	*n* = 1009	*n* = 985	*n* = 1553	*n* = 418	*n* = 423	*n* = 3016	*n* = 1427	*n* = 1407
Chills (%)	–	–	–	–	–	–	5 (<1)	4 (0)	2 (0)	5 (<1)	4 (0)	2 (0)
Myalgia (%)	–	–	–	–	–	–	7 (<1)	6 (0)	4(<1)	7 (<1)	6 (0)	4 (<1)
Arthralgia (%)	–	–	–	–	–	–	4 (<1)	3 (0)	3 (0)	4 (<1)	3 (0)	3 (0)
Headache (%)	–	–	–	–	–	–	8 (0)	6 (<1)	5 (<1)	8 (0)	6 (<1)	5 (<1)
Fatigue (%)	–	–	–	–	–	–	6 (<1)	5 (0)	4 (0)	6 (<1)	5 (0)	4 (0)
Change in eating habits (%)	7 (<1)	5 (0)	8 (<1)	6 (<1)	6 (0)	7 (<1)	6 (<1)	7 (0)	7 (0)	6 (<1)	6 (0)	7 (<1)
Diarrhea (%)	11 (<1)	11 (<1)	9 (<1)	9 (<1)	10 (<1)	8 (<1)	4 (0)	4 (0)	4 (<1)	6 (<1)	8 (<1)	7 (<1)
Irritability (%)	11 (<1)	8 (<1)	10 (<1)	9 (<1)	7 (0)	8 (<1)	–	–	–	9 (<1)	7 (<1)	8 (<1)
Crying (%)	6 (0)	5 (0)	7 (0)	5 (<1)	5 (<1)	6 (0)	–	–	–	5 (<1)	5 (0)	6 (<1)
Sleepiness (%)	7 (0)	5 (0)	6 (1)	6 (<1)	6 (0)	6 (<1)	–	–	–	6 (<1)	6 (0)	6 (<1)
Vomiting (%)	4 (0)	3 (1)	3 (<1)	3 (<1)	3 (<1)	3 (<1)	2 (0)	3 (0)	3 (0)	3 (<1)	3 (<1)	3 (<1)
Fever (%)	14(0)	11 (<1)	11(<1)	13 (0)	9 (<1)	9 (<1)	14 (0)	8 (0)	6 (0)	14 (0)	9 (<1)	8 (<1)

Table 7
Percentage of subjects reporting the most frequent unsolicited adverse events from Day 1 to Day 50.

	6 to <24 Months			6 to <36 Months			36 to <72 Months			TOTAL 6 to <72 Months		
	aTIV (*n* = 846)	TIV-1 (*n* = 570)	TIV-2 (*n* = 573)	aTIV (*n* = 1522)	TIV-1 (*n* = 1043)	TIV-2 (*n* = 1033)	aTIV (*n* = 1601)	TIV-1 (*n* = 434)	TIV-2 (*n* = 441)	aTIV (*n* = 3123)	TIV-1 (*n* = 1477)	TIV-2 (*n* = 1474)
Upper respiratory tract infection (%)	22	20	21	20	19	20	9	9	10	14	16	17
Nasopharyngitis (%)	13	13	14	11	12	13	7	6	9	9	10	12
Gastroenteritis (%)	7	7	7	6	6	7	1	3	2	4	5	6
Viral infection (%)	5	6	5	4	5	4	2	1	2	3	4	3
Pyrexia (%)	3	4	5	3	4	4	2	2	2	2	3	4
Rhinitis (%)	4	4	3	3	4	3	2	3	2	3	4	3
Bronchitis (%)	3	2	4	3	2	3	2	3	2	2	3	3

Author contributions

All authors participated in the conception, design and implementation of this trial. All authors were involved in the interpretation of analysed data and the decision to submit for publication.

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Conflict of Interest statement

SP, SB, HB, NNB, EF, GDC and VN are permanent employees of Novartis Vaccines. TN's institution (MCRI), LB's institute (Philippine General Hospital) and BQ's institute (Research Institute for Tropical Medicine) received funding from Novartis to carry out this study, and for other studies of influenza, typhoid fever and/or meningococcal vaccines. All other authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.08.068.

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