

A phase 1, open-label safety and immunogenicity study of an AS03-adjuvanted trivalent inactivated influenza vaccine in children aged 6 to 35 months

Alfonso Carmona Martinez¹, Ignacio Salamanca de la Cueva¹, Philippe Boutet², Carline Vanden Abeele², Igor Smolenov³, and Jeanne-Marie Devaster^{2,*}

¹Instituto Hispalense de Pediatría; Sevilla, Spain; ²GlaxoSmithKline Vaccines; Rixensart, Belgium; ³GlaxoSmithKline Vaccines; Rixensart, Belgium (Current: Novartis Vaccines and Diagnostics; Amsterdam, The Netherlands)

Keywords: AS03, seasonal influenza vaccine, children

Background: There is a need for better vaccines and vaccine strategies to reduce the burden of influenza in very young children.

Methods: This phase 1, open-label study assessed the reactogenicity, safety, and immunogenicity of an inactivated trivalent influenza vaccine (TIV) containing low doses of hemagglutinin antigen (7.5 µg each strain), adjuvanted with a tocopherol-based oil-in-water emulsion Adjuvant System (AS03). Influenza vaccine-naïve children aged 6–35 months were sequentially enrolled to receive TIV-AS03_D (1.48 mg tocopherol) or TIV-AS03_C (2.97 mg tocopherol), then a 6-month booster of conventional TIV. The primary endpoint was the incidence of fever (axillary temperature >38 °C) for 7 days post-vaccination. Immune responses were assessed by hemagglutination-inhibition (HI) assay.

Results: Forty children were sequentially enrolled into the TIV-AS03_D or the TIV-AS03_C group. Fever >38.0 °C was reported in 5/20 (25.0%) and 7/20 (35.0%) children after the first and second doses of TIV-AS03_D, respectively, and in 7/20 (35.0%) children after 1 dose of TIV-AS03_C; the latter fulfilled the holding rule for safety, and the second dose of TIV-AS03_C was cancelled. HI immune responses exceeded adult European licensure criteria for the immunogenicity, and all children had HI antibody titers ≥ 1:40 after 1 dose of TIV booster against booster strains.

Conclusions: One dose of primary vaccine containing a low dose of antigen and AS03 may be a possible influenza vaccination strategy for young children. The relatively high frequency of fever warrants further investigation, although the generalizability of the findings are uncertain given that many of the children had antibody evidence suggesting recent infection with A(H1N1)pdm09.

Introduction

The global burden of influenza in children is high, and infants and toddlers aged <3 y are particularly vulnerable to influenza infection and severe respiratory complications; influenza-related childhood mortality in the US is reported to be highest in children aged <6 mo, followed by those aged 6 to 23 mo.^{1–4} Inactivated trivalent influenza vaccines (TIVs) are indicated for the active immunization of children from 6 mo of age, but in vaccine-naïve children less than 9 y of age, 2 doses given at least 28 d apart are needed to provide adequate immunogenicity.⁵

The oil-in-water adjuvants AS03 and MF59 have been shown to enhance the immunogenicity of influenza vaccines in children,^{6–9} and in children aged 6 to <36 mo, the efficacy of 2 doses of MF59-adjuvanted TIV against PCR-confirmed seasonal influenza was 79% compared with 40% for conventional TIV.⁷ Experience with AS03-adjuvanted vaccines against

pandemic influenza show that this Adjuvant System containing α-tocopherol and squalene in an o/w emulsion improves immunogenicity compared with non-adjuvanted vaccine in various populations.^{10,11}

An AS03-adjuvanted vaccine against A(H1N1)pdm09 was recommended for use in children, with the pediatric formulation containing a 1.9 µg dose of A(H1N1)pdm09 hemagglutinin antigen (HA) and adjuvanted with AS03_B containing half the amount of tocopherol as the adult AS03_A formulation. During the pandemic vaccination campaign, 2 doses of this vaccine given 21 d apart was shown to be more immunogenic than non-adjuvanted whole virion vaccine in children aged 6 mo to 12 y, and seroconversion rates were >90% in children aged 6 mo to 3 y.⁹ However, an increase in injection site and general adverse events (AEs) after the second compared with the first dose of AS03_B-adjuvanted A(H1N1)pdm09 vaccine was observed, including an increase in the rate of fever (≥38 °C).^{12,13}

*Correspondence to: Jeanne-Marie Devaster; Email: jeanne-marie.devaster@gsk.com

Submitted: 01/30/2014; Revised: 03/21/2014; Accepted: 04/02/2014; Published Online: 04/30/2014
<http://dx.doi.org/10.4161/hv.28743>

Table 1. Demographic details in the total vaccinated cohort

		TIV-AS03 _D n = 20	TIV-AS03 _C n = 20
Age, months	Mean	23.2	18.6
	SD	9.82	7.46
	Median	27.0	20.5
	Minimum	8	8
	Maximum	35	35
Sex, n (%)	Female	11 (55.0)	7 (35.0)
	Male	9 (45.0)	13 (65.0)
Geographic ancestry, n (%)	White-Caucasian or European heritage	20 (100)	20 (100)

TIV, trivalent inactivated influenza vaccine; AS03, oil-in-water emulsion Adjuvant System containing 1.48 mg (AS03_D) or 2.97 mg (AS03_C) of α -tocopherol; SD, standard deviation.

This experience highlights the need to empirically establish an antigen and adjuvant formulation that balances immunogenicity and reactogenicity for use in young children.

In this phase 1, open-label study of influenza vaccine-naïve children aged 6 to 35 mo, we assessed the reactogenicity, safety, and immunogenicity of a seasonal TIV containing 7.5 μ g HA of each strain, adjuvanted with various doses of AS03, followed by a 6-mo booster dose of conventional TIV (non-adjuvanted containing 15 μ g HA of each strain). The rationale for assessing different doses of AS03 was to ascertain if lowering the dose could reduce vaccine reactogenicity without compromising immunogenicity, and the rationale for the booster dose was to assess the quality of the immunological memory created by primary vaccination.

Results

Twenty children received 2 doses of TIV-AS03_D and 20 received 1 dose of TIV-AS03_C. The mean age was 23.2 mo in the TIV-AS03_D group and 18.6 mo in the TIV-AS03_C group (Table 1). There were no study discontinuations for an adverse event/serious adverse event (AE/SAE). The first child was enrolled on 30 March 2010 and the last study visit was on 15 June 2010.

From the 40 children in the total vaccinated cohort, the per-protocol immunogenicity cohort included 14 and 17 children in the TIV-AS03_D and TIV-AS03_C groups, respectively; in the TIV-AS03_D group, 5 children were excluded due to receiving a forbidden vaccine, and 1 did not comply with the blood sampling schedule; in the TIV-AS03_C group, 3 children were excluded, including 1 each for receiving a forbidden vaccine, non-compliance with the blood sampling schedule, and because essential serology data were missing.

Reactogenicity

During the 7-d post-vaccination period, fever >38.0 °C was reported in 5 (25.0%; 95% confidence interval [CI]: 8.7, 49.1) and in 7 (35.0%; 95% CI: 15.4, 59.2) children after the first and second TIV-AS03_D vaccination, respectively, including 3 (15.0%;

95% CI: 3.2, 37.9) and 5 (25.0%; 95% CI: 8.7, 49.1) reports, respectively, that were considered to be related to vaccination. After vaccination with TIV-AS03_C, there were 7 (35.0%; 95% CI: 15.4, 59.2) reports of fever >38.0 °C including 5 (25.0%; 95% CI: 8.7, 49.1) that were considered to be related to vaccination. The incidence of fever >38.0 °C during 3 d after the first dose of TIV-AS03_C fulfilled the pre-defined holding-rule.

All fever (≥ 37.5 °C) was reported in 7 (35.0%; 95% CI: 15.4, 59.2) and 9 (45.0%; 95% CI: 23.1, 68.5) children, after the first and second TIV-AS03_D vaccination, respectively, and in 12 (60.0%; 95% CI: 36.1, 80.9) children after the TIV-AS03_C vaccination (Table 2). The majority of fever episodes lasted for up to 1 d: TIV-AS03_D 4 and 6 episodes, post dose-1 and dose-2, respectively; TIV-AS03_C, 11 episodes. There were 2 episodes of fever (any grade) that lasted for 2 d after the second dose of TIV-AS03_D, and 5 episodes that lasted ≥ 3 d: TIV-AS03_D 3 and 1 episodes, post dose-1 and dose-2, respectively; TIV-AS03_C, 1 episode.

The most frequent solicited general events (any grade) after the first dose of TIV-AS03_D were loss of appetite (9; 45.0%; 95% CI: 23.1, 68.5) and any grade fever (7; 35.0%; 95% CI: 15.4, 59.2), and after the second dose this was any grade fever (9; 45.0%; 95% CI: 23.1, 68.5) (Table 2). The most frequent general events (any grade) after TIV-AS03_C were fever (12; 60.0%; 95% CI: 36.1, 80.9) and irritability (9; 45.0%; 95% CI: 23.1, 68.5). The incidence of grade 3 general events was low (Table 2).

Injection site pain was the most frequent solicited injection site event in both vaccine groups (Fig. 1). Pain (any grade) was reported in 6 (30.0%; 95% CI: 11.9, 54.3) children in each vaccine group after the first vaccination, and in 7 (35.0%; 95% CI: 15.4, 59.2) children after the second TIV-AS03_D vaccination. There were no reports of grade 3 pain. The mean duration of pain overall was between 1 and 3.5 d. There were 2 (10.0%; 95% CI: 1.2, 31.7) reports of redness in each vaccine group after the first vaccination, and 6 (30.0%; 95% CI: 11.9, 54.3) reports after the second TIV-AS03_D vaccination, of which 5 (25.0%; 95% CI: 8.7, 49.1) were grade 2.

Reactogenicity during the 7-d post-vaccination period after the TIV booster dose is shown in Table 3. The rate of all fever (≥ 37.5 °C) after the TIV booster was the same in groups primed with TIV-AS03_D (n = 4; 20.0%; 95% CI: 5.7, 43.7) and with TIV-AS03_C (n = 4; 21.1%; 95% CI: 6.1, 45.6). Most of the children (6/8) developed fever on the day of vaccination or the next day with fever of maximum 38.0 °C. For the 2 others, 1 had an episode of fever starting at day 3 with a maximum of 38.5 °C in the TIV-AS03_D group and 1 an event starting at day 5 with a maximum >40 °C and was considered not related to vaccination with a diagnosed tonsillitis in the TIV-AS03_C group.

Safety

From day 0 to day 42, at least 1 unsolicited AE(s) classified by MedDRA (Medical Dictionary for Regulatory Activities) Preferred Term was reported by 14 (70.0%) children with TIV-AS03_D (2 doses) and 12 (60.0%) children with TIV-AS03_C (1 dose) (Table S1). The most frequent unsolicited AEs after 2 doses of TIV-AS03_D were upper respiratory tract infection and pyrexia

Table 2. Incidence of solicited general events during the 7-d post-vaccination period

		TIV-AS03 _D n = 20	TIV-AS03 _C n = 20
Dose 1		n; % (95% CI)	n; % (95% CI)
Drowsiness	All	4; 20.0 (5.7, 43.7)	7; 35.0 (15.4, 59.2)
	Grade 3	0; 0.0 (0.0, 16.8)	1; 5.0 (0.1, 24.9)
Irritability	All	4; 20.0 (5.7, 43.7)	9; 45.0 (23.1, 68.5)
	Grade 3	1; 5.0 (0.1, 24.9)	1; 5.0 (0.1, 24.9)
Loss of appetite	All	9; 45.0 (23.1, 68.5)	5; 25.0 (8.7, 49.1)
	Grade 3	0; 0.0 (0.0, 16.8)	1; 5.0 (0.1, 24.9)
Vomiting	All	5; 25.0 (8.7, 49.1)	4; 20.0 (5.7, 43.7)
	Grade 3	2; 10.0 (1.2, 31.7)	1; 5.0 (0.1, 24.9)
Temperature (axillary, °C)	All (≥37.5)	7; 35.0 (15.4, 59.2)	12; 60.0 (36.1, 80.9)
	>38.0	5; 25.0 (8.7, 49.1)	7; 35.0 (15.4, 59.2)
	>39.0	0; 0.0 (0.0, 16.8)	1; 5.0 (0.1, 24.9)
	>40.0	0; 0.0 (0.0, 16.8)	1; 5.0 (0.1, 24.9)
Dose 2			
Drowsiness	All	4; 20.0 (5.7, 43.7)	
	Grade 3	0; 0.0 (0.0, 16.8)	
Irritability	All	4; 20.0 (5.7, 43.7)	
	Grade 3	0; 0.0 (0.0, 16.8)	
Loss of appetite	All	5; 25.0 (8.7, 49.1)	
	Grade 3	0; 0.0 (0.0, 16.8)	
Vomiting	All	3; 15.0 (3.2, 37.9)	
	Grade 3	0; 0.0 (0.0, 16.8)	
Temperature (axillary, °C)	All (≥ 37.5)	9; 45.0 (23.1, 68.5)	
	>38.0	7; 35.0 (15.4, 59.2)	
	>39.0	2; 10.0 (1.2, 31.7)	
	>40.0	0; 0.0 (0.0, 16.8)	

TIV, trivalent inactivated influenza vaccine; AS03, oil-in-water emulsion Adjuvant System containing 1.4mg (AS03_D) or 2.97 mg (AS03_C) of α -tocopherol; SD: standard deviation; CI, confidence interval.

with 5 reports each (25.0% each), and after 1 dose of TIV-AS03_C there were 7 (35.0%) reports of upper respiratory tract infection (Table S1). From day 0 to month 6 + 21 d, 18 (90.0%) children in the TIV-AS03_D group and all children in the TIV-AS03_C group experienced a medically-attended adverse event (MAE); in both groups, MAEs were most frequently upper respiratory tract infection (Table S2).

There were 3 SAEs reported from day 0 to month 6 + 21 d, all of which were in the TIV-AS03_C group, including one case each of tonsillitis, vasovagal syncope, and bronchopneumonia. There were no potential immune-mediated diseases (pIMDs). None of the unsolicited AEs/SAEs were considered to be associated with the study vaccines. No child was withdrawn from the study due to an AE/SAE.

Immunogenicity

Both vaccines elicited strong immune responses. The hemagglutination-inhibition (HI) Geometric Mean Titer (GMTs) at day 42 after 2 doses of TIV-AS03_D were 905.2

(A(H1N1)pdm09), 722.5 (A/H3N2), and 460.5 (B-strain), and after 1 dose of TIV-AS03_C were 617.1 (A(H1N1)pdm09), 160.0 (A/H3N2) and 63.1 (B-strain). HI seroprotection rates (SPRs; proportion with titer of $\geq 1:40$), seroconversion rates (SCRs; proportion of seronegative at baseline with post-vaccination titer of $\geq 1:40$, or pre-vaccination titer of $\geq 1:10$ and ≥ 4 -fold increase post-vaccination), and Geometric Mean Fold Rises (GMFRs; fold increase in GMT for post- vs. pre-vaccination) exceeded adult licensure criteria for influenza vaccines against all vaccine strains at day 42 (Fig. 2). Apart from the B-strain in the TIV-AS03_C group, the SPRs and SCRs were 100% for all vaccine strains (Fig. 2). Antibody titers 21 d after the second dose of TIV-AS03_D were stronger than antibody titers 42 d after 1 dose of TIV-AS03_C.

At month 6, all children in the TIV-AS03_D group remained seropositive ($\geq 1:10$) for the primary vaccine strains, and the month 6 seropositivity rates in the TIV-AS03_C group ranged from 57.9% to 89.5% (Table 4). At month 6 + 21 d, SPRs against the

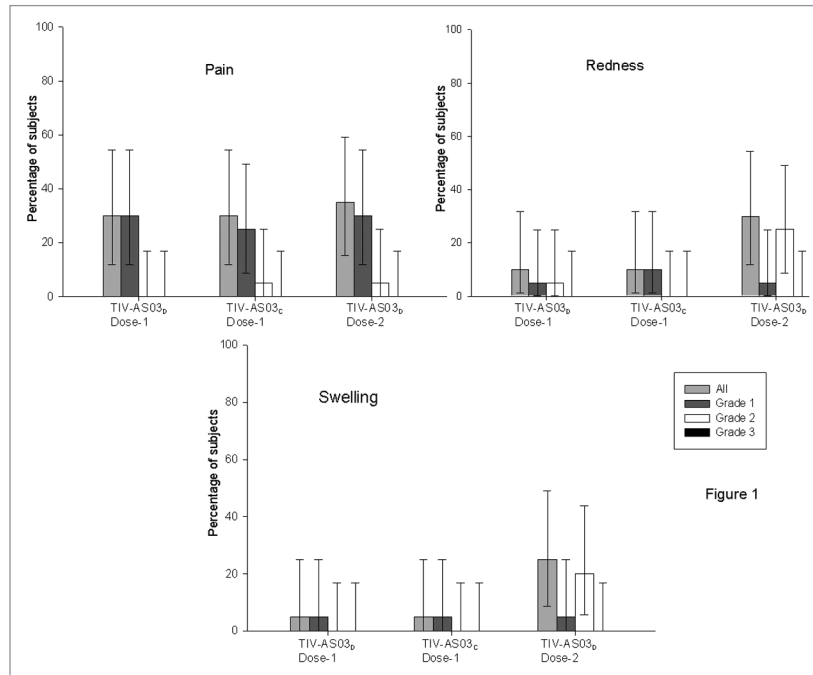


Figure 1. Incidence of solicited injection site events during the 7-d post-vaccination period. *Footnote:*TIV, inactivated trivalent influenza vaccine;AS03, oil-in-water emulsion Adjuvant System containing 1.48mg (AS03_D) or 2.97 mg (AS03_C) of α -tocopherol;SD, standard deviation;CI, confidence interval; Pain: Grade 1, minor reaction on touch; Grade 2, cries on touch; Grade 3, cries when limb is moved/spontaneously painful; Redness/swelling: Grade 1, >0 to \leq 20 mm; Grade 2, >20 to \leq 50 mm; Grade 3, >50 mm.

Table 3. Incidence of solicited injection site and general events during the 7-d post-vaccination period after vaccination with TIV booster

		TIV-AS03 _D n = 20	TIV-AS03 _C n = 19
Symptom	Type	n; % (95% CI)	n; % (95% CI)
Pain	All	10; 50.0 (27.2, 72.8)	4; 21.1 (6.1, 45.6)
	Grade 3	0; 0.0 (0.0, 16.8)	0; 0.0 (0.0, 17.6)
Redness	All	8; 40.0 (19.1, 63.9)	5; 26.3 (9.1, 51.2)
	Grade 3	1; 5.0 (0.1, 24.9)	0; 0.0 (0.0, 17.6)
Swelling	All	5; 25.0 (8.7, 49.1)	3; 15.8 (3.4, 39.6)
	Grade 3	1; 5.0 (0.1, 24.9)	0; 0.0 (0.0, 17.6)
Drowsiness	All	5; 25.0 (8.7, 49.1)	3; 15.8 (3.4, 39.6)
	Grade 3	1; 5.0 (0.1, 24.9)	0; 0.0 (0.0, 17.6)
Irritability	All	6; 30.0 (11.9, 54.3)	6; 31.6 (12.6, 56.6)
	Grade 3	0; 0.0 (0.0, 16.8)	0; 0.0 (0.0, 17.6)
Loss of appetite	All	4; 20.0 (5.7, 43.7)	2; 10.5 (1.3, 33.1)
	Grade 3	0; 0.0 (0.0, 16.8)	0; 0.0 (0.0, 17.6)
Vomiting	All	3; 15.0 (3.2, 37.9)	2; 10.5 (1.3, 33.1)
	Grade 3	0; 0.0 (0.0, 16.8)	0; 0.0 (0.0, 17.6)
Fever (axillary temp, °C)	All (\geq 37.5)	4; 20.0 (5.7, 43.7)	4; 21.1 (6.1, 45.6)
	>38.0	1; 5.0 (0.1, 24.9)	3; 15.8 (3.4, 39.6)
	>39.0	0; 0.0 (0.0, 16.8)	1; 5.3 (0.1, 26.0)
	>40.0	0; 0.0 (0.0, 16.8)	1; 5.3 (0.1, 26.0)

TIV, trivalent inactivated influenza vaccine; AS03, oil-in-water emulsion Adjuvant System containing 1.48 mg (AS03_D) or 2.97 mg (AS03_C) α -tocopherol; CI, confidence interval

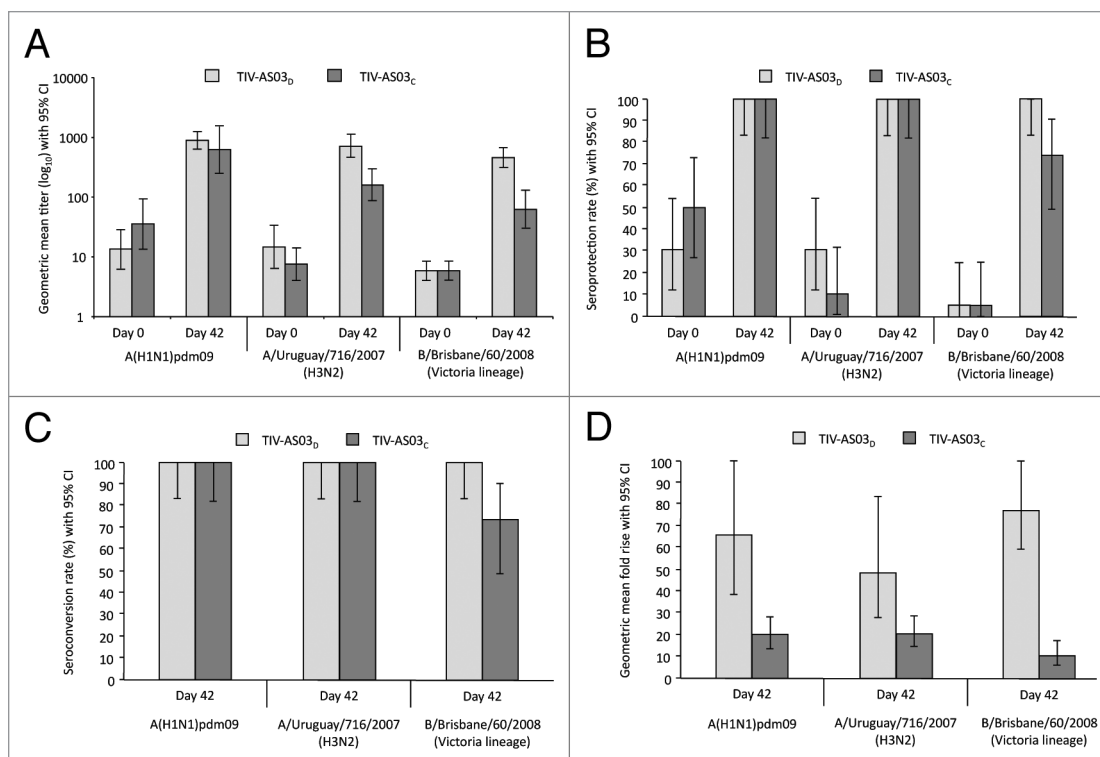


Figure 2. Hemagglutination-inhibition antibody titers at day 0 and day 42; Geometric mean titer (A); Seroprotection rate (B); Seroconversion rate (C); Geometric mean fold rise (D): children received 2 doses of TIV-AS03_D or 1 dose of TIV-AS03_C; *Footnote:* TIV, inactivated trivalent influenza vaccine; AS03, oil-in-water emulsion Adjuvant System containing 1.48 mg (AS03_D) or 2.97 mg (AS03_C) α -tocopherol; CI, Confidence Interval

TIV booster strains were 100% in both vaccine groups. HI SPRs, SCRs, and GMFRs exceeded adult influenza vaccine licensure criteria for the TIV booster vaccine strains that were homologous (A(H1N1)pdm09 and B-strain) and heterologous (A/H3N2) to the primary vaccine strains (Table 5).

Incidence of fever by baseline serostatus

All children who were seropositive for A(H1N1)pdm09 pre-vaccination developed post-vaccination fever (≥ 37.5 °C) after 1 dose of vaccine (Fig. 3). After the first dose of vaccine in both vaccine groups, fever >38 °C was more common in children who were seropositive for A(H1N1)pdm09 pre-vaccination than in those who were seronegative. Overall, 10/16 children who were seropositive for A(H1N1)pdm09 at baseline developed fever >38 °C after the first vaccination, compared with 2/24 seronegative children. No relationship was observed for the incidence of fever and A/H3N2 or B-strain baseline serostatus (Fig. 4).

Discussion

This phase 1, open-label study assessed the reactogenicity, safety, and immunogenicity, of a seasonal TIV containing a low dose of antigen, and adjuvanted with AS03_D (1.48 mg tocopherol) or AS03_C (2.97 mg tocopherol) in influenza vaccine-naïve children aged 6 to 35 mo. At 21 d after 2 doses of TIV-AS03_D, the seroconversion rate was 100% against each vaccine strain. TIV-AS03_C was also highly immunogenic, but the incidence of

fever >38.0 °C during 3 d after the first dose was 35.0% (7/20) which fulfilled the pre-defined holding-rule for safety; the GlaxoSmithKline Vaccine Safety Monitoring Board cancelled the second dose of TIV-AS03_C and the sequential enrolment of children to receive TIV-AS03_D (5.93 mg tocopherol).

In view of the considerable burden of influenza infection and the relatively poor antibody responses to trivalent influenza vaccines in children aged <2 y, there is a need for seasonal influenza vaccines with improved immunogenicity profiles for use in unprimed infants. The formulation of TIVs with an oil-in-water adjuvant was recently shown to be a feasible strategy for enhancing immunogenicity, and improving efficacy of seasonal influenza vaccine in young children; the large, randomized field study showed that TIV formulated with MF59 provided better efficacy against PCR-confirmed influenza than conventional TIV when given as a 2 dose schedule over consecutive seasons.⁷ In addition, the use of adjuvanted TIV for out of season priming, followed by a booster dose of conventional TIV is a possible vaccination strategy for very young children. For example, in a study of previously unprimed children aged 15 to 20 mo, 1 dose of TIV containing the A(H1N1)pdm09 strain elicited antibody responses of $\geq 1:40$ against A(H1N1)pdm09 in children who had received monovalent AS03-adjuvanted A(H1N1)pdm09 vaccine 1 yr before, but 2 doses of TIV were needed to provide adequate immune responses to the A/H3N2 and B-strains.¹⁴

In our study, 21 d after 2 doses of TIV-AS03_D or 42 d after 1 dose of TIV-AS03_C, HI SPRs, SCRs, and GMFRs exceeded adult

Table 4. Hemagglutination-inhibition based seropositivity rates, SPRs, and GMTs against the AS03-TIV vaccine strains at day 42 and month 6 after 2 doses of TIV-AS03_D or 1 dose of TIV-AS03_C and 21 d after the TIV booster dose (month 6 + 21 d)

Antibody	Group	Timing	N	Titer ≥ 1:10	Titer ≥ 1:40	GMT	
				n; % (95% CI)	n; % (95% CI)	value (95% CI)	min, max
A(H1N1)pdm09	TIV-AS03 _D	day 0	20	6; 30.0 (11.9, 54.3)	6; 30.0 (11.9, 54.3)	13.7 (6.5, 28.9)	<10.0, 320.0
		day 42	20	20; 100 (83.2, 100)	20; 100 (83.2, 100)	905.2 (649.0, 1262.6)	320.0, 2560.0
		M6	20	20; 100 (83.2, 100)	20; 100 (83.2, 100)	146.7 (100.4, 214.3)	40.0, 640.0
		M6+21	20	20; 100 (83.2, 100)	20; 100 (83.2, 100)	697.9 (502.6, 968.9)	160.0, 2560.0
	TIV-AS03 _C	day 0	20	10; 50.0 (27.2, 72.8)	10; 50.0 (27.2, 72.8)	35.4 (13.6, 92.6)	<10.0, 640.0
		day 42	19	19; 100 (82.4, 100)	19; 100 (82.4, 100)	617.1 (251.0, 1516.8)	40.0, 5120.0
		M6	19	17; 89.5 (66.9, 98.7)	13; 68.4 (43.4, 87.4)	111.1 (47.3, 260.6)	<10.0, 1280.0
		M6+21	19	19; 100 (82.4, 100)	19; 100 (82.4, 100)	921.8 (721.1, 1178.5)	453.0, 2560.0
A/Uru/716/07 (H3N2)	TIV-AS03 _D	day 0	20	6; 30.0 (11.9, 54.3)	6; 30.0 (11.9, 54.3)	14.9 (6.6, 33.5)	<10.0, 320.0
		day 42	20	20; 100 (83.2, 100)	20; 100 (83.2, 100)	722.5 (473.8, 1101.7)	160.0, 3620.0
		M6	19	19; 100 (82.4, 100)	19; 100 (82.4, 100)	97.8 (57.8, 165.5)	40.0, 1280.0
		M6+21	20	20; 100 (83.2, 100)	20; 100 (83.2, 100)	607.6 (366.5, 1007.3)	80.0, 2560.0
	TIV-AS03 _C	day 0	20	2; 10.0 (1.2, 31.7)	2; 10.0 (1.2, 31.7)	7.6 (4.1, 13.9)	<10.0, 640.0
		day 42	19	19; 100 (82.4, 100)	19; 100 (82.4, 100)	160.0 (86.3, 296.7)	40.0, 5120.0
		M6	19	14; 73.7 (48.8, 90.9)	5; 26.3 (9.1, 51.2)	21.5 (10.2, 45.4)	<10.0, 1280.0
		M6+21	19	19; 100 (82.4, 100)	19; 100 (82.4, 100)	595.0 (375.2, 943.5)	80.0, 3620.0
B/Bri/60/08	TIV-AS03 _D	day 0	20	1; 5.0 (0.1, 24.9)	1; 5.0 (0.1, 24.9)	5.9 (4.1, 8.5)	<10.0, 160.0
		day 42	20	20; 100 (83.2, 100)	20; 100 (83.2, 100)	460.5 (320.4, 661.8)	113.0, 5120.0
		M6	20	20; 100 (83.2, 100)	18; 90.0 (68.3, 98.8)	60.6 (43.0, 85.5)	20.0, 640.0
		M6+21	20	20; 100 (83.2, 100)	20; 100 (83.2, 100)	273.9 (169.4, 442.9)	57.0, 1280.0
	TIV-AS03 _C	day 0	20	1; 5.0 (0.1, 24.9)	1; 5.0 (0.1, 24.9)	5.9 (4.1, 8.5)	<10.0, 160.0
		day 42	19	19; 100 (82.4, 100)	14; 73.7 (48.8, 90.9)	63.1 (30.9, 128.8)	10.0, 5120.0
		M6	19	11; 57.9 (33.5, 79.7)	6; 31.6 (12.6, 56.6)	17.0 (8.7, 33.1)	<10.0, 1280.0
		M6+21	19	19; 100 (82.4, 100)	19; 100 (82.4, 100)	325.9 (222.0, 478.3)	160.0, 1810.0

TIV, trivalent inactivated influenza vaccine; AS03, oil-in-water emulsion Adjuvant System containing 1.48 mg (AS03_D) or 2.97 mg (AS03_C) α-tocopherol

EU licensure criteria for the immunogenicity of influenza vaccine against all vaccine strains, and apart from the B-strain in the TIV-AS03_C group, the SPRs and SCRs were 100%. At 6 mo, SPRs against primary vaccine strains were 100% for A(H1N1)pdm09 and A/H3N2 (A/Uruguay) and 90.0% against the B-strain in the TIV-AS03_D group (2 doses), and 68.4%, 26.3%, and 31.6% against A(H1N1)pdm09, A/H3N2 (A/Uruguay), and the B-strain, respectively in the TIV-AS03_C group (1 dose). Despite the differences in persistence against the primary vaccination strains at 6 mo, the booster dose of TIV induced HI SPRs of 100% against all booster vaccine strains in both primary vaccine groups. Furthermore, the A/H3N2 strain in the TIV booster (A/Victoria) was a different strain to that in the primary vaccines (A/Uruguay), suggesting that heterologous priming is feasible even with 1 dose of AS03-adjuvanted priming vaccine. Although based on a small number of children, our results show that 1 dose of TIV-AS03_C followed by a 6-mo homologous or heterologous booster dose of TIV is a possible strategy for influenza vaccine-naïve children.

AS03-adjuvanted pandemic influenza vaccines are reported to be more reactogenic than non-adjuvanted vaccines, although increased reactogenicity is considered acceptable in a pandemic setting.^{6,9,12,15} Notably, previous studies in children show that AS03_D-adjuvanted A(H1N1)pdm09 vaccine vs. non-adjuvanted A(H1N1)pdm09 vaccine is associated with higher rates of injection site pain and general symptoms such as irritability and loss of appetite.⁹ In our study, we assessed vaccine formulated with AS03_C and AS03_D to evaluate whether reducing the dosage of adjuvant could improve the tolerability profile of the vaccine. We showed that the most frequent solicited injection site event was pain, which was mostly mild in intensity. Injection site pain was reported by 30.0% of children in both vaccine groups after the first dose and by 35.0% of children after the second dose of TIV-AS03_D. Consistent with the known reactogenicity profile of AS03-adjuvanted pandemic vaccines in younger children, the most frequent solicited general events in the TIV-AS03_D group during each 7-d post vaccination period were fever (≥37.5 °C), loss of appetite, and vomiting. The incidence of fever >38 °C

Table 5. Hemagglutination-inhibition assay based immunogenicity against the TIV booster strains at month 6 and month 6 + 21 d (according-to-protocol cohort): TIV-AS03_D (2 dose primary vaccination) and TIV-AS03_C (1 dose primary vaccination)

	A(H1N1)pdm09				A/Victoria/210/2009 (H3N2)				B/Brisbane/60/2008			
	TIV-AS03 _D n = 14		TIV-AS03 _C n = 17		TIV-AS03 _D n = 14		TIV-AS03 _C n = 17		TIV-AS03 _D n = 14		TIV-AS03 _C n = 17	
	M6	M6+D21	M6	M6+D21	M6	M6+D21	M6	M6+D21	M6	M6+D21	M6	M6+D21
Titer ≥ 1:10% (95% CI)	100 (76.8, 100)	100 (76.8, 100)	94.1 (71.3, 99.9)	100 (80.5, 100)	42.9 (17.7, 71.1)	100 (76.8, 100)	17.6 (3.8, 43.4)	100 (80.5, 100)	100 (76.8, 100)	100 (76.8, 100)	64.7 (38.3, 85.8)	100 (80.5, 100)
GMT Value (95% CI)	160.0 (99.9, 256.2)	689.3 (480.3, 989.2)	120.2 (51.5, 280.9)	869.0 (670.9, 1125.7)	25.0 (8.1, 77.4)	353.3 (215.8, 578.4)	8.2 (4.2, 15.7)	226.2 (143.6, 356.3)	56.6 (43.1, 74.3)	262.5 (152.6, 451.4)	19.6 (9.5, 40.2)	294.9 (203.7, 427.1)
SPR % (95% CI)	100 (76.8, 100)	100 (76.8, 100)	70.6 (44.0, 89.7)	100 (80.5, 100)	42.9 (17.7, 71.1)	100 (76.8, 100)	11.8 (1.5, 36.4)	100 (80.5, 100)	92.9 (66.1, 99.8)	100 (76.8, 100)	35.3 (14.2, 61.7)	100 (80.5, 100)
SCR % (95% CI)	-	57.1 (28.9, 82.3)	-	58.8 (32.9, 81.6)	-	71.4 (41.9, 91.6)	-	100 (80.5, 100)	-	71.4 (41.9, 91.6)	-	94.1 (71.3, 99.9)
GMFR Value (95% CI)	-	4.3 (2.6, 7.2)	-	7.2 (3.3, 16.0)	-	14.1 (5.0, 40.0)	-	27.7 (16.6, 46.5)	-	4.6 (2.8, 7.8)	-	15.1 (8.0, 28.4)

TIV, trivalent inactivated influenza vaccine; AS03, oil-in-water emulsion Adjuvant System containing 1.48 mg (AS03_D) or 2.97 mg (AS03_C) α-tocopherol; D, day; CI, confidence interval; GMT, geometric mean titer; SPR, seroprotection rate; SCR, seroconversion rate; GMFR, geometric fold rise; children received 2 doses of TIV-AS03_D or 1 dose of TIV-AS03_C.

increased from 25.0% to 35.0% after the first and second doses of TIV-AS03_D, respectively, and there were 7 reports (35.0%) of fever >38 °C after TIV-AS03_C, which resulted in the cancellation of the second dose of TIV-AS03_C.

An exploratory analysis of fever after 1 dose of each vaccine according to baseline serostatus for the vaccine strains was conducted. Although very few children overall were seropositive for the A/H3N2 strain and the B-strain pre-vaccination (8 and 2 children, respectively), 16 children were seropositive for the A(H1N1)pdm09 strain. The analysis showed that after 1 dose of vaccine, 10/16 children who were seropositive for the A(H1N1)pdm09 strain pre-vaccination developed fever >38 °C, compared with 2/24 seronegative children. Since the study was conducted out of the classical influenza season period as from April 2010 just after the pandemic period, recent exposure of children to the A(H1N1)pdm09 strain was likely to be high. The subjects who were primed with natural pandemic influenza strain showed relatively high rates of fever after the first dose of the candidate vaccine formulation. Similar observations were made in previous studies post-dose 2 in pandemic influenza naive children.¹⁶⁻¹⁹ The higher fever rates observed might have been associated with a stronger increase of the humoral response after dose 1 in already primed children. However this would require further investigation as the number of vaccinees is low and the study design limited the blood sampling to the post dose 2 time point.

However, the rate of fever >38 °C after the TIV booster dose was n = 1 (5.0%) and n = 3 (15.8%) in children who were primed with TIV-AS03_D or TIV-AS03_C, respectively, suggesting that although fever was increased during the primary vaccinations,

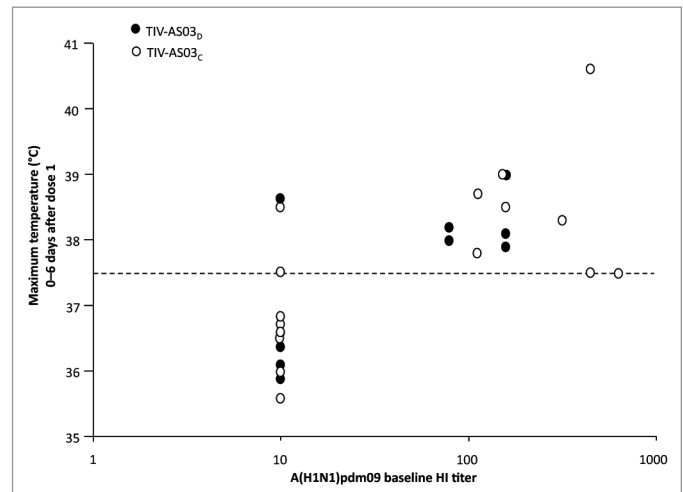


Figure 3. Maximum temperature during the 7-d post-vaccination period (dose 1) by baseline hemagglutination-inhibition antibody titers against A(H1N1)pdm09. TIV, inactivated trivalent influenza vaccine; HI, hemagglutination inhibition; AS03, oil-in-water emulsion Adjuvant System containing 1.48 mg (AS03_D) or 2.97 mg (AS03_C) α-tocopherol; Children with titers of ≥ 10 were considered to be seropositive.

this did not impact reactogenicity following booster doses of classical TIV. No SAEs were considered vaccine related and no febrile convulsion occurred in the days following vaccination.

In summary, this study showed that a TIV containing a low dose of antigen and adjuvanted with a low dose of AS03 (AS03_D or AS03_C) induced strong immune responses to the vaccine strains in unprimed children aged 6 to 35 mo, which persisted at 6 mo post-vaccination. The use of 1 single dose

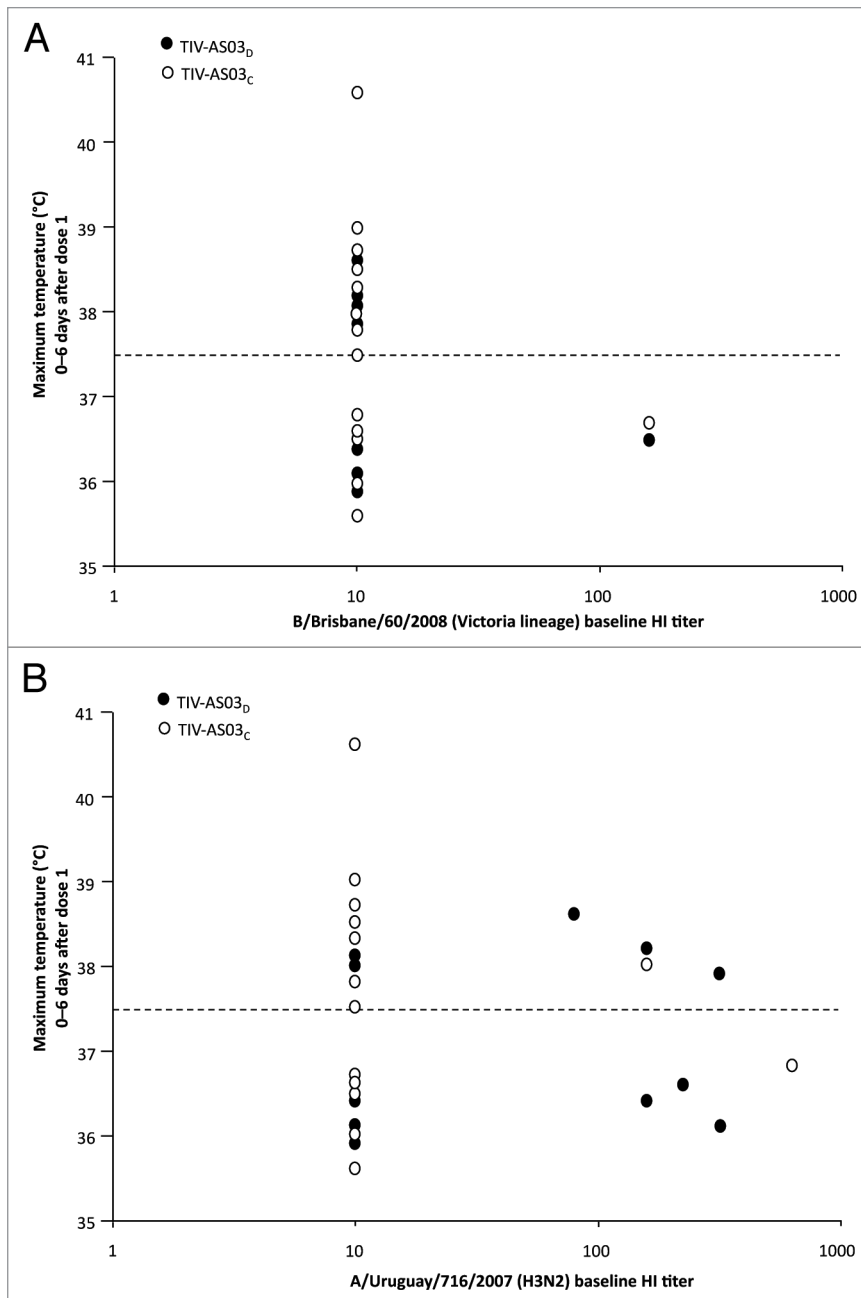


Figure 4. Maximum temperature during the 7-d post-vaccination period (dose 1) by baseline hemagglutination-inhibition antibody titers against influenza B (A) and A/H3N2 (B). AS03, oil-in-water emulsion Adjuvant System containing 1.48 mg (AS03_D) or 2.97 mg (AS03_C) α -tocopherol; Children with titers of ≥ 10 were considered to be seropositive.

of a conventional TIV at 6 mo post-priming provided 100% seroprotection (antibody titer $\geq 1:40$) in all children for each booster strain after 2-dose priming with AS03_D and single dose priming with AS03_C. There was a relatively high frequency of fever, yet apart from fever, the tolerability and safety profile of TIV-AS03_D and TIV-AS03_C was consistent with that previously reported with AS03-adjuvanted pandemic vaccine in very young children. The generalizability of the findings however is uncertain given that the study was not controlled and the vaccine was administered “out of season”. Pre-screening was

not done so as to reduce the number of blood draws and since it would not have ruled out any intercurrent infection. Therefore many of the children had antibody evidence suggesting recent infection with A(H1N1)pdm09. Based on these pilot data, a more complete dose range study in which both antigen and AS03 dose are varied would be needed to confirm that indeed 1 dose of primary vaccine containing a low dose of antigen and AS03 may be a possible influenza vaccination strategy for young children.

Methods

Participants

This phase 1, open-label study assessed the reactogenicity, safety, and immunogenicity of 2 doses of AS03-adjuvanted TIV followed by a 6-mo booster dose of non-adjuvanted TIV in children aged between 6 and 35 mo. The study was conducted at the Instituto Hispalense de Pediatría, Seville, Spain.

Eligible children were healthy, were influenza vaccine-naïve, and had received other appropriate scheduled childhood vaccination administered 30 d before or 30 d after the study vaccine. Parents/guardians were deemed to be able to comply with the protocol and provided informed written consent. The exclusion criteria list included the receipt of any seasonal or pandemic influenza vaccine before or during the study, or any investigational vaccine within the preceding 30 d or during the study.

The study protocol was approved by Independent Ethics Committees or local or central Institutional Review Boards, and was conducted in accordance with Good Clinical Practice, the principles of the Declaration of Helsinki, and all regulatory requirements. ClinicalTrials.gov, number NCT01096056.

Vaccines and design

Children were scheduled to receive TIV containing 7.5 μ g HA of each strain based on the World Health Organisation’s (WHO) recommended A/H3N2 and B-strain, plus the A(H1N1)pdm09 strain: A(H1N1)pdm09, A/Uruguay/716/2007 (H3N2), and B/Brisbane/60/2008 (B-strain; Victoria lineage). The vaccine was adjuvanted with an oil-in-water emulsion based Adjuvant System containing the following differing amounts of α -tocopherol and squalene, respectively: 1.48 mg and 1.337 mg (AS03_D), 2.97 mg and 2.675 mg (AS03_C), and 5.93 mg, and 5.35 mg (AS03_B). The TIV and the Adjuvant System were manufactured by GlaxoSmithKline Vaccines.

Three sequential phases comprising TIV-AS03_D, TIV-AS03_C, and TIV-AS03_B were planned, each including 20 children scheduled to receive 1 dose of vaccine in the first instance; the administration of a second dose 21 d after the first, and the sequential enrollment of the next vaccine with the higher dose of AS03 was conditional on the outcome of an internal safety assessment based on pre-defined holding rules. A review of reactogenicity data was performed for 3 d after the first dose of TIV-AS03_D and the study proceeded to day 21; children received a second dose of TIV-AS03_D and the next sequential group received the first dose of TIV-AS03_C. The incidence of fever during the 3-d post-vaccination period for TIV-AS03_C fulfilled the pre-defined holding-rule and, therefore, no further dosing of the investigational vaccine was performed in the study (i.e., the second dose of TIV-AS03_C and TIV-AS03_B were not administered).

The 6-mo booster vaccine was TIV (*Fluarix*TM; GlaxoSmithKline Vaccines) containing 15 µg HA of each strain: A(H1N1)pdm09 strain, A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (B strain; Victoria lineage), i.e., homologous booster for A(H1N1)pdm09 strain and B-strain, heterologous for A/H3N2 strain. Vaccines were administered intramuscularly into the anterolateral region of the thigh in children aged <12 mo and in the deltoid of children aged ≥12 mo.

Endpoints

The primary endpoint was the incidence of axillary temperature >38 °C during the 7-d post-vaccination period following each dose of adjuvanted vaccine. Secondary objectives were to assess the incidence of solicited AEs during the 7-d post-vaccination periods and unsolicited AEs during the 21-d post-vaccination periods after adjuvanted vaccine. MAEs, SAEs, and pIMDs were assessed from the day of vaccination (day 0) until 21 d after the TIV booster vaccination (month 6 + 21 d).

Secondary immunogenicity endpoints were HI assay-based antibody titers against the vaccine strains pre-vaccination (day 0) and at day 42 (21 d after the second dose of TIV-AS03_D and 42 d after the first dose of TIV-AS03_C).

Further assessments included: immunogenicity before the TIV booster vaccination at month 6 and 21 d after the TIV booster against the strains in the primary vaccine (primary vaccine strains) and booster vaccine strains; and the incidence of post-vaccination fever according to baseline seropositivity status for each primary vaccine strain.

Reactogenicity and safety

Solicited injection site and general AEs were assessed on the day of each vaccination and for 6 subsequent days (7-d follow-up). Solicited symptoms were recorded by parents/guardians and graded for intensity using diary cards. Solicited injection site events were pain (Grade 1 pain was defined as a minor reaction on touch; Grade 2 as cries on touch and Grade 3 as cries when limb is moved/spontaneously painful), redness, and swelling; general solicited events were drowsiness, fever, irritability/fussiness, appetite loss, and vomiting. Fever was defined as an axillary temperature: ≥37.5 °C; >38.0 °C; >39.0 °C; >40.0 °C. Temperature was recorded in the evening.

Unsolicited AEs, MAEs, SAEs and pIMDs were classified by MedDRA Preferred Terms. All injection site reactions were considered to be vaccine-related and investigators judged the relationship between vaccination and solicited general symptoms and unsolicited symptoms.

Immunogenicity

Blood was collected on day 0, day 42, month 6, and month 6 + 21 d for serological testing. Antibody titers against the vaccine strains were assessed using validated micro-titer HI assay as previously described.²⁰

HI antibody responses were described as the anti-log of the arithmetic mean of the log-10 transformed titers (GMTs), SCR, SPR, and GMFR. Children with titers of ≥1:10 were considered to be seropositive.

Analyses

A sample size of 20 children per group was based on the assumption that an incidence of fever >38.0 °C of 20% may be within clinical acceptance whereas an incidence of >50% raises clinical concern. Based on a sample of 20 children, an incidence of fever >38.0 °C of ≥35.0% was identified in the protocol as a holding rule for the study.

The incidences of solicited and unsolicited AEs, MAEs, and SAEs were calculated with a 2-sided 95% CI. Immunogenicity endpoints were provided with a 2-sided 95% CI. The HI antibody titers were assessed according to the European All Committee for Medicinal Products for Human Use (CHMP) recommendations for influenza vaccines in adults.²¹ The licensure criteria are fulfilled if the point estimate for SPR is >70%, SCR >40%, and GMFR >2.5.

Reactogenicity, safety, and immunogenicity at day 0 and day 42 were assessed in the total vaccinated cohort, including all children who received at least 1 dose of AS03 vaccine. Immunogenicity analyses at month 6 and month 6 + 21 d were performed on the according-to-protocol (ATP) cohort including vaccinated children without protocol violation and who complied with the vaccination and serological testing schedule.

Disclosure of Potential Conflicts of Interest

J.-M.D., P.B., C.V.A. declare that they are employees of GlaxoSmithKline group of companies. J.-M.D. and I.S. report ownership of GlaxoSmithKline stock options.

Funding

GlaxoSmithKline Biologicals SA was the funding source and was involved in all stages of the study conduct and analysis. GlaxoSmithKline Biologicals SA also took responsibility for all costs associated with the development and publishing of this manuscript. All authors had full access to the data and had final responsibility to submit for publication.

Acknowledgments

The authors are grateful to the participating children and their parents, clinicians, nurses, and laboratory technicians at the study site and the sponsor's project staff for their support and contributions throughout the study.

We are grateful to the teams of GlaxoSmithKline Vaccines for their contribution to this study: Nathalie Stassen for her contribution to the study supervision; Maria Del Pilar Garcia-Corbeira, Eduardo Fernandez-Ruiz as local study coordinators; Aurélie Olivier, Julie De Wever for preparation of the study protocol and report; Karl Walravens for clinical readout; Adriana Bastidas, clinical safety representative; Walther Dewé, project statistician; Paul Gillard, clinical representative; Vincent Dodeur, clinical data coordinator; Lidia Oostvogels and Bruce Innis for critical reading of the manuscript and helpful suggestions. We thank Annick Moon (Moon Medical Communications Ltd, UK, on behalf of GlaxoSmithKline Vaccines) for writing assistance, Preethi Govindarajan (GlaxoSmithKline Vaccines) for formatting support, Géraldine Drevon (GlaxoSmithKline

Vaccines), and Sarah Fico (Business and Decision Life Sciences, on behalf of GlaxoSmithKline Vaccines) for editorial assistance and manuscript coordination.

All authors participated in the implementation of the study including substantial contributions to conception and design, the gathering of the data, or analysis and interpretation of the data. All authors were involved in the drafting of the article or revising it critically for important intellectual content, and final approval of the manuscript.

Supplemental Materials

Supplemental materials may be found here:
www.landesbioscience.com/journals/vaccines/article/28743

References

- Nair H, Brooks WA, Katz M, Roca A, Berkley JA, Madhi SA, Simmerman JM, Gordon A, Sato M, Howie S, et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. *Lancet* 2011; 378:1917-30; PMID:22078723; [http://dx.doi.org/10.1016/S0140-6736\(11\)61051-9](http://dx.doi.org/10.1016/S0140-6736(11)61051-9)
- Thompson WW, Shay DK, Weintraub E, Brammer L, Bridges CB, Cox NJ, Fukuda K. Influenza-associated hospitalizations in the United States. *JAMA* 2004; 292:1333-40; PMID:15367555; <http://dx.doi.org/10.1001/jama.292.11.1333>
- Neuzil KM, Mellen BG, Wright PF, Mitchel EF Jr., Griffin MR. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. *N Engl J Med* 2000; 342:225-31; PMID:10648763; <http://dx.doi.org/10.1056/NEJM200001273420401>
- Bhat N, Wright JG, Broder KR, Murray EL, Greenberg ME, Glover MJ, Likos AM, Posey DL, Klimov A, Lindstrom SE, et al.; Influenza Special Investigations Team. Influenza-associated deaths among children in the United States, 2003-2004. *N Engl J Med* 2005; 353:2559-67; PMID:16354892; <http://dx.doi.org/10.1056/NEJMoa051721>
- Centers for Disease Control and Prevention. Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices—United States 2013-2014. *Morbidity and Mortality Weekly Report*. 2009; 62(RR-7)
- Díez-Domingo J, Garcés-Sánchez M, Baldo J-M, Planelles MV, Ubeda I, JuBert A, Marés J, Moris P, Garcia-Corbeira P, Dramé M, et al. Immunogenicity and Safety of H5N1 A/Vietnam/1194/2004 (Clade 1) AS03-adjuvanted prepandemic candidate influenza vaccines in children aged 3 to 9 years: a phase ii, randomized, open, controlled study. *Pediatr Infect Dis J* 2010; 29:e35-46; PMID:20375709
- Vesikari T, Knuf M, Wutzler P, Karvonen A, Kieninger-Baum D, Schmitt HJ, Baehner F, Borkowski A, Tsai TF, Clemens R. Oil-in-water emulsion adjuvant with influenza vaccine in young children. *N Engl J Med* 2011; 365:1406-16; PMID:21995388; <http://dx.doi.org/10.1056/NEJMoa1010331>
- Vesikari T, Pellegrini M, Karvonen A, Groth N, Borkowski A, O'Hagan DT, Podda A. Enhanced immunogenicity of seasonal influenza vaccines in young children using MF59 adjuvant. *Pediatr Infect Dis J* 2009; 28:563-71; PMID:19561422; <http://dx.doi.org/10.1097/INF.0b013e31819d6394>
- Waddington CS, Walker WT, Oeser C, Reiner A, John T, Wilkins S, Casey M, Eccleston PE, Allen RJ, Okike I, et al. Safety and immunogenicity of AS03B adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. *BMJ* 2010; 340:c2649; PMID:20508026; <http://dx.doi.org/10.1136/bmj.c2649>
- Carter NJ, Plosker GL. Prepandemic influenza vaccine H5N1 (split virion, inactivated, adjuvanted) [Prepandrix]: a review of its use as an active immunization against influenza A subtype H5N1 virus. *BioDrugs* 2008; 22:279-92; PMID:18778110; <http://dx.doi.org/10.2165/00063030-200822050-00001>
- Garçon N, Vaughn DW, Didierlaurent AM. Development and evaluation of AS03, an Adjuvant System containing α -tocopherol and squalene in an oil-in-water emulsion. *Expert Rev Vaccines* 2012; 11:349-66; PMID:22380826; <http://dx.doi.org/10.1586/erv.11.192>
- Carmona A, Omeñaca F, Tejedor JC, Merino JM, Vaman T, Dieussaert I, Gillard P, Aristegui J. Immunogenicity and safety of AS03-adjuvanted 2009 influenza A H1N1 vaccine in children 6-35 months. *Vaccine* 2010; 28:5837-44; PMID:20600478; <http://dx.doi.org/10.1016/j.vaccine.2010.06.065>
- García-Sicilia J, Gillard P, Carmona A, Tejedor JC, Aristegui J, Merino JM, Behre U, Caplanusi A, Vaman T, Dieussaert I. Immunogenicity and safety of AS03-adjuvanted H1N1 pandemic vaccines in children and adolescents. *Vaccine* 2011; 29:4353-61; PMID:21504774; <http://dx.doi.org/10.1016/j.vaccine.2011.04.011>
- Gilca V, De Serres G, Hamelin ME, Boivin G, Ouakki M, Boulianne N, Sauvageau C, Dionne M, Gilca R, Skowronski D. Antibody persistence and response to 2010-2011 trivalent influenza vaccine one year after a single dose of 2009 AS03-adjuvanted pandemic H1N1 vaccine in children. *Vaccine* 2011; 30:35-41; PMID:22063386; <http://dx.doi.org/10.1016/j.vaccine.2011.10.062>
- Rümke HC, Bayas JM, de Juanes JR, Caso C, Richardus JH, Campins M, Rombo L, Duval X, Romanenko V, Schwarz TF, et al. Safety and reactogenicity profile of an adjuvanted H5N1 pandemic candidate vaccine in adults within a phase III safety trial. *Vaccine* 2008; 26:2378-88; PMID:18407382; <http://dx.doi.org/10.1016/j.vaccine.2008.02.068>
- Carmona A, Omeñaca F, Tejedor JC, Merino JM, Vaman T, Dieussaert I, Gillard P, Aristegui J. Immunogenicity and safety of AS03-adjuvanted 2009 influenza A H1N1 vaccine in children 6-35 months. *Vaccine* 2010; 28:5837-44; PMID:20600478; <http://dx.doi.org/10.1016/j.vaccine.2010.06.065>
- García-Sicilia J, Gillard P, Carmona A, Tejedor JC, Aristegui J, Merino JM, Behre U, Caplanusi A, Vaman T, Dieussaert I. Immunogenicity and safety of AS03-adjuvanted H1N1 pandemic vaccines in children and adolescents. *Vaccine* 2011; 29:4353-61; PMID:21504774; <http://dx.doi.org/10.1016/j.vaccine.2011.04.011>
- Waddington CS, Walker WT, Oeser C, Reiner A, John T, Wilkins S, Casey M, Eccleston PE, Allen RJ, Okike I, et al. Safety and immunogenicity of AS03B adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. *BMJ* 2010; 340:c2649; PMID:20508026; <http://dx.doi.org/10.1136/bmj.c2649>
- Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. *N Engl J Med* 2006; 354:1343-51; PMID:16571878; <http://dx.doi.org/10.1056/NEJMoa055778>
- Hehme N, Künzel W, Petschke F, Türk G, Raderecht C, van Hoecke C, Sängler R. Ten years of experience with the trivalent split-influenza vaccine, Fluarix™. *Clin Drug Investig* 2002; 22:751-69; <http://dx.doi.org/10.2165/00044011-200222110-00004>
- Committee for Medicinal Products for Human Use. Note on guidance on harmonization of requirements for influenza vaccines CPMP/BWP/214/96. March 1997. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf