MAJOR ARTICLE

Relative Efficacy of AS03-Adjuvanted Pandemic Influenza A(H1N1) Vaccine in Children: Results of a Controlled, Randomized Efficacy Trial

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Background. The vaccine efficacy (VE) of 1 or 2 doses of AS03-adjuvanted influenza A(H1N1) vaccine relative to that of 2 doses of nonadjuvanted influenza A(H1N1) vaccine in children 6 months to <10 years of age in a multinational study conducted during 2010–2011.

Methods. A total of 6145 children were randomly assigned at a ratio of 1:1:1 to receive 2 injections 21 days apart of A/California/7/2009(H1N1)-AS03 vaccine at dose 1 and saline placebo at dose 2, 2 doses 21 days apart of A/California/7/2009(H1N1)-AS03 vaccine (the Ad2 group), or 2 doses 21 days apart of nonadjuvanted A/California/7/2009(H1N1) vaccine (the NAd2 group). Active surveillance for influenza-like illnesses continued from days 14 to 385. Nose and throat samples obtained during influenza-like illnesses were tested for A/California/7/2009 (H1N1), using reverse-transcriptase polymerase chain reaction. Immunogenicity, reactogenicity, and safety were assessed.

Results. There were 23 cases of confirmed 2009 pandemic influenza A(H1N1) (A[H1N1]pdm09) infection for the primary relative VE analysis. The VE in the Ad2 group relative to that in the NAd2 group was 76.8% (95% confidence interval, 18.5%–93.4%). The benefit of the AS03 adjuvant was demonstrated in terms of the greater immunogenicity observed in the Ad2 group, compared with the NAd2 group.

Conclusion. The 4–8-fold antigen-sparing adjuvanted pandemic influenza vaccine demonstrated superior and clinically important prevention of A(H1N1)pdm09 infection, compared with nonadjuvanted vaccine, with no observed increase in medically attended or serious adverse events. These data support the use of adjuvanted influenza vaccines during influenza pandemics.

Clinical Trials Registration. NCT01051661.

Keywords. H1N1; pandemic influenza vaccine; influenza virus; children; efficacy.

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Children have high influenza attack rates and are key to viral transmission among households, daycare centers, and schools [1, 2]. During the 2009–2010 pandemic of influenza A(H1N1) (hereafter, A[H1N1]pdm09) infection, the highest attack rates were observed in children <18 years of age [3]. Inactivated influenza vaccines historically have shown only moderate efficacy in children, particularly among those <2 years of age [4], although improved efficacy of an oil-in-water–adjuvanted (MF59) trivalent seasonal influenza vaccine was recently reported in young children [5]. Moreover, an antigen-sparing AS03-adjuvanted inactivated pandemic influenza vaccine against A(H1N1)pdm09 offered children superior immunogenicity relative to conventional unadjuvanted formulations, with an acceptable safety profile [6]. Therefore, in a large cohort of children, we used real-time polymerase chain reaction (PCR)–based confirmation of A(H1N1)pdm09 infection to evaluate the efficacy of a split virion A/California/7/2009(H1N1)v-like AS03-adjuvanted vaccine (H1N1-AS03; Arepanrix [GlaxoSmithKline Vaccines]) relative to that of a nonadjuvanted H1N1 vaccine made with the same virus strain but formulated at a conventional dose in preventing A(H1N1)pdm09 infection. Antibody persistence was monitored for either 6 months or 1 year after vaccination. Our objective was to assess whether the vaccine candidate's superior immunogenicity translated into improved disease prevention.

METHODS

Study Design and Subjects

This randomized, prospective phase 3 observer-blinded study was conducted in 17 centers in Australia, Brazil, Colombia, Costa Rica, Mexico, the Philippines, Singapore, and Thailand between 15 February 2010 and 19 August 2011. The study was approved by an institutional review board at each participating center. Written informed consent was obtained from parents or guardians of participating healthy children 6 months to <10 years (6 month to <10) of age before the children received the first vaccine dose.

Randomization

Participants were randomly assigned at a ratio of 1:1:1 to 1 of 3 treatment groups to receive 2 intramuscular injections 21 days apart. Group Ad1 received H1N1-AS03 at dose 1 and placebo (saline) at dose 2; group Ad2 received 2 doses of H1N1-AS03; group NAd2 received 2 doses of nonadjuvanted A/California/7/2009(H1N1) vaccine. The randomization procedure used a minimization algorithm accounting for center, age stratum (6 to <36 months or 3 to <10 years), and prior seasonal influenza vaccination status. These factors had equal weight in the minimization algorithm; that is, a participant's vaccine group allocation was based on the balance of the combination of all the minimization factors. Each age stratum was capped such that it could contribute no more than 75% to the total population.

Vaccines

Each dose of H1N1-AS03 vaccine contained 1.9 μg of hemagglutinin (HA) mixed with $AS03_B$ in a total delivery volume of 0.25 mL. $AS03_B$ is an oil-in-water emulsion containing squalene and DL-α-tocopherol (5.93 mg) in an aqueous phase [7].

The nonadjuvanted vaccine contained 30 μg of HA in 1 mL, and the volume (ie, HA dose) administered varied with age: children <3 years of age in the NAd2 group received two 0.25-mL doses (ie, 7.5 μg of HA), and children 3 to <10 years of age received two 0.5-mL doses (ie, 15 μg of HA). Placebo was 0.5 mL of saline. Single lots of the adjuvanted antigen, nonadjuvanted antigen, and adjuvant were used. Vaccines were administered into the deltoid (or anterior thigh, if the child was <12 months of age), using a needle length suitable for intramuscular administration.

Study Objectives

The primary objective was to evaluate the efficacy of 2 doses of H1N1-AS03 relative to that of 2 doses of nonadjuvanted vaccine beginning 14 days after dose 1 and continuing until study conclusion on day 385. Noninferiority in terms of relative vaccine efficacy (VE) was concluded if the lower limit of the 95% confidence interval (CI) for relative VE against real-time PCR–confirmed A(H1N1)pdm09 infection (Ad2 vs NAd2) was ≥33%. Superiority was concluded if the lower limit of the 95% CI for relative VE was >0.

Secondary study objectives with predefined criteria (and no type 1 error adjustment) included (1) VE for Ad1 relative to that of NAd2, using the same criteria specified for the primary objective; (2) relative VE for any pneumonia, any pneumonia in individuals within 6 weeks of real-time PCR–positive A(H1N1)pdm09 infection, and any influenza-like illness (ILI); and (3) assessment of specific antibody titers at day 42.

The adjuvant effect on immunogenicity was assessed by comparing A/California/7/09 hemagglutination-inhibiting (HI) antibody responses at day 42 in terms of geometric mean titer (GMT) ratios and differences in seroconversion rate (SCR) by group. Adjuvant effect was demonstrated if the lower limit of the 95% CI for GMT ratio was >1.0 and the lower limit of the 95% CI for the group difference in the seroconversion rate was >0.

Reactogenicity and safety of the study vaccines and antibody persistence at days 182 and 385 were summarized descriptively as part of the evaluation of secondary objectives.

Evaluation of Influenza Outcomes

Passive surveillance began on day 0 and active surveillance via telephone contacts began for all subjects approximately 2 weeks after dose 1 and continued every 1–2 weeks until day 385. Parents and legal guardians notified the investigator if the child

developed an ILI (defined as a temperature ≥38.0°C by any route and at least 1 of the following: new or worsening cough, sore throat, nasal congestion, and/or rhinorrhea). Nasal and throat swab specimens were collected within 7 days after symptom onset. A 7-day symptom-free period was required between ILI episodes to consider the subsequent episode distinct from the initial episode. Detection of influenza A(H1N1) pdm09, using real-time PCR (and of all influenza virus strains, using multiplex PCR), is described in the Supplementary Materials. Only real-time PCR–positive samples underwent viral culture [8].

Immunogenicity Assessment

Blood samples were collected before vaccination and 21 days after receipt of dose 2 (ie, on day 42) from all subjects. HI antibody levels were measured in a random subset of approximately 60 children in each treatment group from each participating country. The HI assay used chicken erythrocytes [9–11], and the lowest dilution tested was 1:10. The titration end point was the highest dilution step that showed complete (ie, 100%) inhibition of hemagglutination. A further blood sample was collected at either day 182 or day 385 from consenting subjects.

Safety and Reactogenicity Assessment

Injection site and systemic symptoms were recorded on diary cards for 7 days after each dose. Solicited symptoms were based on the ability to report by age and were therefore different in younger children (age, <6 years) versus older children (age, ≥6 years). All other adverse events (AEs) were recorded from the first dose until day 42. Medically attended AEs, serious AEs (SAEs), and potential immune-mediated diseases were recorded until day 385. All solicited injection site reactions were considered causally related to vaccination. Potentially causal relationships between vaccination and all other AEs were assessed by the site investigator.

Statistical Methods

The analysis was performed by an externally contracted statistical analyst. A second statistician from the same company performed an independent quality validation.

Vaccine Efficacy

Efficacy was assessed in the according-to-protocol (ATP) efficacy cohort, which included all evaluable subjects who received 2 doses, who were successfully contacted at least once after the first vaccination, and who complied with protocol-defined procedures. The relative VE was calculated as 1 − relative risk (RR), where the RR is defined as the risk of real-time PCR–confirmed cases among subjects receiving H1N1-AS03 versus the risk of real-time PCR–confirmed cases among subjects receiving NAd2. RR was estimated via a Cox proportional hazard regression model (time to first event), with vaccine group as a fixed variable, and was adjusted by covariates of age, seasonal influenza vaccine history, and country. Subjects meeting censoring criteria (ie, receipt of protocol-forbidden vaccines or

Table 1. Number of Cases of Confirmed A(H1N1) Disease, Pneumonia, and Influenza-Like Illness During the Study Period (Days 14 to 385)

	Age, Study Group								
	All Subjects			6 mo to <3 y			3 y to <10 y		
Event	Ad2	Ad1	NAd2	Ad2	Ad1	NAd2	Ad2	Ad1	NAd2
ATP efficacy cohort followed up, subjects, no.[a]	1903	1913	1897	569	566	561	1334	1347	1336
Influenza-like illness	1390	1321	1330	616	626	609	774	695	721
Pneumonia	17	13	18	9	11	12	8	2	6
Real-time PCR–confirmed influenza[b]	3	6	11	1	0	2	2	6	9
Culture-confirmed influenza	2	5	6	0	0	0	2	5	6
Real-time PCR–confirmed influenza with pneumonia	0	0	0	0	0	0	0	0	0
Total vaccinated cohort followed up, subjects, no.	2048	2048	2049	610	612	613	1438	1436	1436
Influenza-like illness	1491	1441	1433	657	685	659	834	756	774
Pneumonia	18	14	22	9	11	15	9	3	7
Real-time PCR–confirmed influenza[b]	3	7	15	1	1	4	2	6	11
Culture-confirmed influenza	2	6	9	0	1	2	2	5	7
Real-time PCR–confirmed influenza with pneumonia	0	0	0	0	0	0	0	0	0

Data are no. of cases, unless otherwise indicated.

Abbreviations: Ad1, A/California/7/2009(H1N1)-AS03 vaccine at dose 1 and saline placebo at dose 2; Ad2, 2 doses of A/California/7/2009(H1N1)-AS03 vaccine; ATP, according to protocol; NAd2, 2 doses of nonadjuvanted A/California/7/2009(H1N1) vaccine; PCR, polymerase chain reaction.

[a] Subjects with protocol deviation or violation were excluded from the ATP cohort. However, subjects who received concomitant medication or vaccine were included in the time-to-event efficacy analysis and were censored at the time when the concomitant medication or vaccine was received.

[b] Three additional cases occurred before day 14.

medication or receipt of nonprotocol vaccines containing A/California/7/09-like H1N1 antigen) were included in the analysis until the date of censoring or were excluded if the censoring criteria were met before the disease end point occurred. The relative VE (with 95% CI) was calculated for the 14–385-day (primary end point) and 0–385-day surveillance periods. Secondary analyses were done on the total vaccinated cohort, which included all children who received at least 1 vaccine dose.

During study preparation the future behavior of the pandemic was uncertain, but we projected a substantive third wave in 2010. On the basis of 1800 evaluable subjects per group, an assumed attack rate of 20% among unvaccinated subjects, and an assumed VE for nonadjuvanted H1N1 vaccine of 40%, if 360 real-time PCR–confirmed influenza cases were identified during the surveillance period, a lower limit of ≥33% for the 95% CI for the relative VE could be demonstrated with >99.9% power, if the VE in the H1N1-AS03 group was assumed to be 60% relative to that of a notional placebo. Type 1 error adjustment was not made for secondary objective evaluations.

Immunogenicity End Points

The following parameters were calculated (with 95% CIs) based on A/California/7/09 HI titers: GMT; seroconversion rate, defined as the percentage of initially seronegative subjects (titer, <1:10) with a postvaccination titer of ≥1:40 or the percentage of initially seropositive vaccinees (titer, ≥1:10) with a ≥4-fold increase in the postvaccination titer; seroprotection rate, defined as the percentage of subjects with titers of ≥1:40 [12, 13]; and seroconversion factor, defined as the ratio of the postvaccination titer to the prevaccination titer.

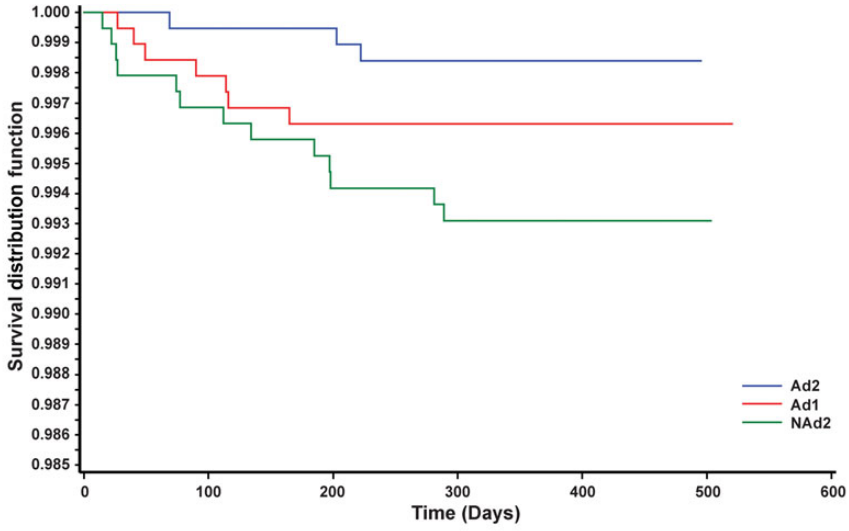
Reactogenicity End Points

Reactogenicity data were summarized by vaccine group and age stratum (from 6 months to <6 years and from 6 to <10 years) because a different AE intensity scale was used for children of different ages.

RESULTS

Study Subjects

Each study center contributed between 105 (1.7%) and 886 (14.4%) of the total 6145 enrolled and vaccinated subjects. Of these, 5900 (96%) completed the study to day 42, and 5851 (95%) completed the study to day 385. Two children in the Ad1 group withdrew before day 42 because of an AE or SAE: 1 child died of asthma and pneumonia 20 days after dose 1, and 1 child had a nonserious upper respiratory tract infection. Two children in Ad2 were withdrawn before day 385 because of SAEs: 1 child drowned, and 1 died from an intestinal



	Group	Day 14	Day 50	Day 100	Day 150	Day 200	Day 250	Day 300	Day 350	Day 400
No. at risk[a]	Ad2	1940	1907	1866	1875	1866	1855	1841	1930	173
	Ad1	1933	1900	1882	1873	1859	1851	1843	1837	189
	NAd2	1930	1895	1878	1861	1850	1835	1822	1813	166
Cumulative no. of events	Ad2	0	0	1	1	1	3	3	3	3
	Ad1	0	3	4	6	7	7	7	7	7
	NAd2[b]	0	4	6	8	11	11	13	13	13

Figure 1. Kaplan–Meier time-to-event curve for real-time polymerase chain reaction–confirmed 2009 pandemic influenza A(H1N1) infection reported from 14 days after vaccination through the end of influenza-like illness surveillance. [a]Data are for the time-to-event analysis of the according-to-protocol cohort; [b]Data for 1 subject were censored before the event and are thus not included. Abbreviations: Ad1, A/California/7/2009(H1N1)-AS03 vaccine at dose 1 and saline placebo at dose 2; Ad2, 2 doses of A/California/7/2009(H1N1)-AS03 vaccine; NAd2, 2 doses of nonadjuvanted A/California/7/2009(H1N1) vaccine.

Table 2. Time-to-Event Analysis of the Relative Vaccine Efficacy (VE) for Primary and Secondary Outcomes in the According-to-Protocol Efficacy Cohort

	Relative VE,[a] % (95% CI), by Age and Study Groups					
	All Subjects		6 to <36 mo		3 to <10 y	
Outcome, Surveillance Period	Ad2 vs NAd2	Ad1 vs NAd2	Ad2 vs NAd2	Ad1 vs NAd2	Ad2 vs NAd2	Ad1 vs NAd2
Real-time PCR–confirmed influenza						
Days 14–385	76.8 (18.5 to 93.4)[a,b,c]	46.4 (–34.4 to 78.6)	75.5 (–119.2 to 97.3)	74.6 (–127.3 to 97.2)	77.5 (–4.0 to 95.2)[a]	33.1 (–88.1 to 76.2)
Days 0–385	69.2 (5.4 to 89.9)[a,b]	31.0 (–61.4 to 70.5)	75.5 (–119.2 to 97.3)	49.4 (–176.1 to 90.7)	66.4 (–24.0 to 90.9)[a]	21.9 (–109.9 to 70.9)
Culture-confirmed influenza						
Days 14–385	74.9 (–18.2 to 94.7)[a]	25.5 (–114.9 to 74.1)	100	48.8 (–465.0 to 95.4)	66.2 (–67.3 to 93.2)	16.4 (–173.9 to 74.5)
Days 0–385	62.5 (–41.3 to 90.1)	13.0 (–140.0 to 68.5)	100	–1.7 (–622.2 to 85.7)	49.7 (–101.3 to 87.4)	16.4 (–173.9 to 74.5)
Any pneumonia						
Days 14–385	21.0 (–52.5 to 59.1)	34.9 (–31.0 to 67.6)	44.3 (–32.9 to 76.6)	20.6 (–75.0 to 64.0)	–33.6 (–285.2 to 53.6)	67.2 (–62.6 to 93.4)
Days 0–385	28.4 (–36.4 to 62.4)	31.5 (–32.2 to 64.4)	51.4 (–13.5 to 79.2)	24.0 (–60.7 to 64.1)	–33.6 (–285.2 to 53.6)	50.7 (–97.0 to 87.7)
Influenza-like illness						
Days 14–385	–2.5 (–12.6 to 6.8)	1.9 (–8.0 to 10.9)	–0.3 (–16.5 to 13.7)	–5.1 (–22.2 to 9.6)	–3.7 (–17.2 to 8.3)	6.4 (–5.9 to 17.4)
Days 0–385	–4.5 (–14.7 to 4.7)	–0.3 (–10.2 to 8.7)	–4.8 (–21.3 to 9.5)	–7.3 (–24.3 to 7.5)	–4.2 (–17.5 to 7.6)	4.2 (–8.2 to 15.1)

Findings were obtained by a Cox regression model with adjustment for covariate(s) of country, age, and seasonal influenza vaccine history.

Abbreviations: Ad1, one dose of A/California/7/2009(H1N1)-AS03 vaccine at dose 1 and saline placebo at dose 2; Ad2, 2 doses of A/California/7/2009(H1N1)-AS03 vaccine; CI, confidence interval; NAd2, 2 doses of nonadjuvanted A/California/7/2009(H1N1) vaccine; PCR, polymerase chain reaction; VE, Vaccine efficacy.

[a] Noninferiority criteria met.

[b] Superiority criteria met.

[c] Primary end point: noninferiority was concluded if the lower limit of the 95% CI for the relative VE in subjects with real-time PCR–confirmed A(H1N1) influenza (Ad2 divided by NAd2 group) was ≥33%. Superiority was achieved if the lower limit of the 95% CI for the relative VE in subjects with real-time PCR–confirmed A(H1N1) influenza (Ad2 divided by NAd2 group) was >0. For all other comparisons, the type 1 error was not controlled.

Table 3. Humoral Immune Response to A/California/7/2009(H1N1)v-like Strain After Vaccination in All Subjects and Across Age Strata in According-to-Protocol Cohorts Evaluated for Immunogenicity and Antibody Persistence

			SCR[c]				SPR[h]		
Age, Study Group	Time Point[a]	No.[b]	No.[d]	%[e] (95% CI[f])	SCF[g] (95% CI[f])	No.[b]	No.[d]	%[e] (95% CI[f])	GMT (95% CI[f])
All ages									
Ad2	Before	. . .	. . .	. . .	. . .	395	127	32.2 (27.6, 37.0)	14.1 (12.3, 16.3)
	Day 42	395	394	99.7 (98.6, 100)	110.9 (96.4, 127.6)	400	400	100 (99.1, 100)	1562.3 (1466.9, 1663.8)
	Day 182	110	104	94.5 (88.5, 98.0)	21.8 (17.8, 26.7)	564	558	98.9 (97.7, 99.6)	253.4 (231.7, 277.2)
	Day 365	95	88	92.6 (85.4, 97.0)	21.1 (16.9, 26.4)	522	508	97.3 (95.5, 98.5)	211.9 (193.8, 231.7)
Ad1	Before	. . .	. . .	. . .	. . .	381	100	26.2 (21.9, 31.0)	12.3 (10.7, 14.1)
	Day 42	381	373	97.9 (95.9, 99.1)	22.1 (20.4, 23.9)	388	383	98.7 (97.0, 99.6)	266.5 (235.5, 301.5)
	Day 182	126	87	69.0 (60.2, 77.0)	7.1 (6.2, 8.2)	566	408	72.1 (68.2, 75.7)	97.3 (86.4, 109.6)
	Day 365	83	52	62.7 (51.3, 73.0)	7.6 (6.1, 9.4)	502	394	78.5 (74.6, 82.0)	106.4 (94.7, 119.6)
NAd2	Before	. . .	. . .	. . .	. . .	381	108	28.3 (23.9, 33.2)	13.3 (11.5, 15.4)
	Day 42	381	342	89.8 (86.3, 92.6)	20.5 (18.3, 23.1)	387	359	92.8 (89.7, 95.1)	271.3 (235.4, 312.7)
	Day 182	110	66	60.0 (50.2, 69.2)	6.2 (5.1, 7.6)	563	454	80.6 (77.1, 83.8)	116.9 (104.1, 131.3)
	Day 365	89	36	40.4 (30.2, 51.4)	4.4 (3.6, 5.5)	502	341	67.9 (63.6, 72.0)	78.4 (68.9, 89.2)
6 mo to <3 y									
Ad2	Before	. . .	. . .	. . .	. . .	229	61	26.6 (21.0, 32.9)	12.4 (10.2, 15.1)
	Day 42	229	228	99.6 (97.6, 100)	140.8 (116.6, 170.0)	233	233	100 (98.4, 100)	1738.9 (1598.7, 1891.4)
	Day 182	70	66	94.3 (86.0, 98.4)	24.0 (18.5, 31.1)	186	184	98.9 (96.2, 99.9)	287.2 (246.8, 334.3)
	Day 365	62	58	93.5 (84.3, 98.2)	26.6 (20.0, 35.3)	173	169	97.7 (94.2, 99.4)	265.6 (228.9, 308.2)
Ad1	Before	. . .	. . .	. . .	. . .	228	55	24.1 (18.7, 30.2)	11.2 (9.3, 13.4)
	Day 42	228	224	98.2 (95.6, 99.5)	23.4 (21.2, 25.7)	232	229	98.7 (96.3, 99.7)	258.0 (219.9, 302.8)
	Day 182	75	54	72.0 (60.4, 81.8)	8.0 (6.8, 9.3)	177	120	67.8 (60.4, 74.6)	76.5 (62.0, 94.5)
	Day 365	51	35	68.6 (54.1, 80.9)	8.5 (6.5, 11.0)	163	128	78.5 (71.4, 84.6)	112.6 (91.3, 138.8)
NAd2	Before	. . .	. . .	. . .	. . .	220	55	25.0 (19.4, 31.3)	12.3 (10.0, 15.1)
	Day 42	220	187	85.0 (79.6, 89.4)	15.6 (13.5, 18.0)	223	199	89.2 (84.4, 93.0)	188.9 (156.1, 228.5)
	Day 182	61	32	52.5 (39.3, 65.4)	4.8 (3.7, 6.3)	184	117	63.6 (56.2, 70.5)	74.9 (59.1, 95.1)
	Day 365	55	18	32.7 (20.7, 46.7)	3.7 (2.8, 4.7)	158	82	51.9 (43.8, 59.9)	54.9 (42.2, 71.5)
3 y to <10 y									
Ad2	Before	. . .	. . .	. . .	. . .	166	66	39.8 (32.3, 47.6	16.9 (13.8, 20.8)
	Day 42	166	166	100 (97.8, 100)	79.9 (65.3, 97.6)	167	167	100 (97.8, 100)	1345.4 (1228.3, 1473.6)
	Day 182	40	38	95.0 (83.1, 99.4)	18.4 (13.1, 25.8)	378	374	98.9 (97.3, 99.7)	238.3 (213.3, 266.2)
	Day 365	33	30	90.9 (75.7, 98.1)	13.7 (9.9, 19.0)	349	339	97.1 (94.8, 98.6)	189.4 (169.6, 211.6)
Ad1	Before	. . .	. . .	. . .	. . .	153	45	29.4 (22.3, 37.3)	14.2 (11.5, 17.5)
	Day 42	153	149	97.4 (93.4, 99.3)	20.3 (17.7, 23.3)	156	154	98.7 (95.4, 99.8)	279.5 (229.6, 340.31)
	Day 182	51	33	64.7 (50.1, 77.6)	6.1 (4.7, 7.9)	389	288	74.0 (69.4, 78.3)	76.5 (62.0, 94.5)
	Day 365	32	17	53.1 (34.7, 70.9)		339	266	78.5 (73.7, 82.7)	103.6 (89.9, 119.3)

Table 3 continued.

Age, Study Group	Time Point[a]	No.[b]	SCR[c] No.[d]	SCR[c] % [e] (95% CI[f])	SCF[g] (95% CI[f])	No.[b]	SPR[h] No.[d]	SPR[h] % [e] (95% CI[f])	GMT (95% CI[f])
NAd2	Before	. . .	. . .	. . .	. . .	161	53	32.9 (25.7, 40.8)	14.8 (12.0, 18.1)
	Day 42	161	155	96.3 (92.1, 98.6)	29.9 (25.1, 35.7)	164	160	97.6 (93.9, 99.3)	444.0 (367.0, 537.0)
	Day 182	49	34	69.4 (54.6, 81.7)	8.5 (6.4, 11.4)	379	337	88.9 (85.3, 91.9)	145.1 (128.3, 164.0)
	Day 365	34	18	52.9 (35.1, 70.2)	6.1 (4.2, 8.7)	344	259	75.3 (70.4, 79.8)	92.3 (80.2, 106.3)

Abbreviations: Ad1, A/California/7/2009(H1N1)-AS03 vaccine at dose 1 and saline placebo at dose 2; Ad2, 2 doses of A/California/7/2009(H1N1)-AS03 vaccine; CI, confidence interval; GMT, geometric mean titer; NAd2, 2 doses of nonadjuvanted A/California/7/2009(H1N1) vaccine; SCR, seroconversion rate; SPR, seroprotection rate; SCF, seroconversion factor.

[a] Before vaccination and days 42, 182, and 365 days after receipt of dose 2.

[b] Data are no. of subjects with available results.

[c] Defined as an antibody titer ≥1:40 after vaccination for subjects who were initially seronegative (ie, those with a hemagglutinin-inhibiting antibody titer of <1:10) and as a postvaccination titer of ≥4 times the prevaccination titer for subjects who were initially seropositive.

[d] Data are no. of subjects with pre- and postvaccination results available.

[e] Data are percentage of subjects who reached the indicated end point.

[f] Center for Biologics Evaluation and Research criteria were fulfilled; the lower limit of the 95% CI for SCR was >40%, and the lower limit of the 95% CI for the percentage with a titer of ≥1:40 was >70%. Committee for Medicinal Products for Human Use criteria were fulfilled; the point estimate for SCR was >40%, the postvaccination point estimate for the percentage with a titer of ≥1:40 was >70%, and the point estimate for SCF was >2.5.

[g] Defined as the geometric mean ratio, calculated as mean[$\log_{10}$(postvaccination GMT/prevaccination GMT)].

[h] Defined as the percentage with an antibody titer of ≥1:40 after vaccination.

obstruction associated with parasitic gastroenteritis and aspiration pneumonia (95 days after dose 2). No event leading to withdrawal was considered related to vaccination. Subject flow through the study and reasons for withdrawal and elimination from ATP cohorts are given in Supplementary Figure 1.

The mean age (±SD) of all children at enrollment was 4.3 ± 2.64 years (range, 0.5–9 years); 49.8% (3058/6145) were female. The study groups in each analysis cohort were comparable in terms of demographic characteristics (Supplementary Table 1).

Vaccine Efficacy

There were 3731 nasopharyngeal swab specimens collected from 4653 ILI episodes (81%). Multiplex PCR–confirmed influenza virus infection (any strain from days 14 to 385) occurred in 9.7% (95% CI, 8.4%–11.1%) of children in the Ad2 group, 8.4% (95% CI, 7.2%–9.8%) in the Ad1 group, and 9.3% (95% CI, 8.1%–10.7%) in the NAd2 group.

During the entire study follow-up period, 28 children had real-time PCR–confirmed A(H1N1)pdm09 infection (Table 1): 11 were in the Philippines, 7 were in Thailand, 5 were in Australia, 3 were in Mexico, and 1 each was in Singapore and Costa Rica. Three children developed influenza before day 14. One child (in the NAd2 group) received the second vaccination 4 days after the protocol-specified visit window. One subject (in the NAd2 group) was censored upon receiving seasonal trivalent vaccine 8 months after dose 1 and 3 months before onset of A(H1N1)pdm09 disease. Therefore, among 5803 children included in ATP time-to-event efficacy analysis (days 14–385), 23 had real-time PCR-confirmed A(H1N1)pdm09 infection, giving an attack rate in each group of 0.15% (in the Ad2 group), 0.34% (in the Ad1 group), and 0.68% (in the NAd2 group) in the ATP cohort (Figure 1).

The efficacy of Ad2 over NAd2 for prevention of real-time PCR-confirmed A(H1N1)pdm09 infection from days 14 to 385 was 76.8% (95% CI, 18.5%–93.4%). Both noninferiority and superiority of Ad2 versus NAd2 were thus demonstrated according to the prespecified statistical criteria (Table 2). The relative VE without adjustment for covariates of age, seasonal influenza vaccine history, and country was 77.1% (95% CI, 19.6%–93.5%).

Efficacy, noninferiority, and superiority of the 2-dose adjuvanted regimen were confirmed in the analysis of the total vaccinated cohort: the efficacy estimate for Ad2 over NAd2 was 78.5% (95% CI, 25.1%–93.8%; days 14–385).

Secondary analyses showed that Ad2 was noninferior to NAd2 for efficacy in preventing culture-confirmed influenza (days 14–385) and in preventing real-time PCR–confirmed A(H1N1)pdm09 infection in the subgroup aged 3 to <10 years (Table 1). Noninferiority was also observed for these end points in the total vaccinated cohort analysis (data not shown).

The noninferiority criteria were not met for Ad1 versus NAd2 for the ATP population or at any analysis interval (days 14–385 or

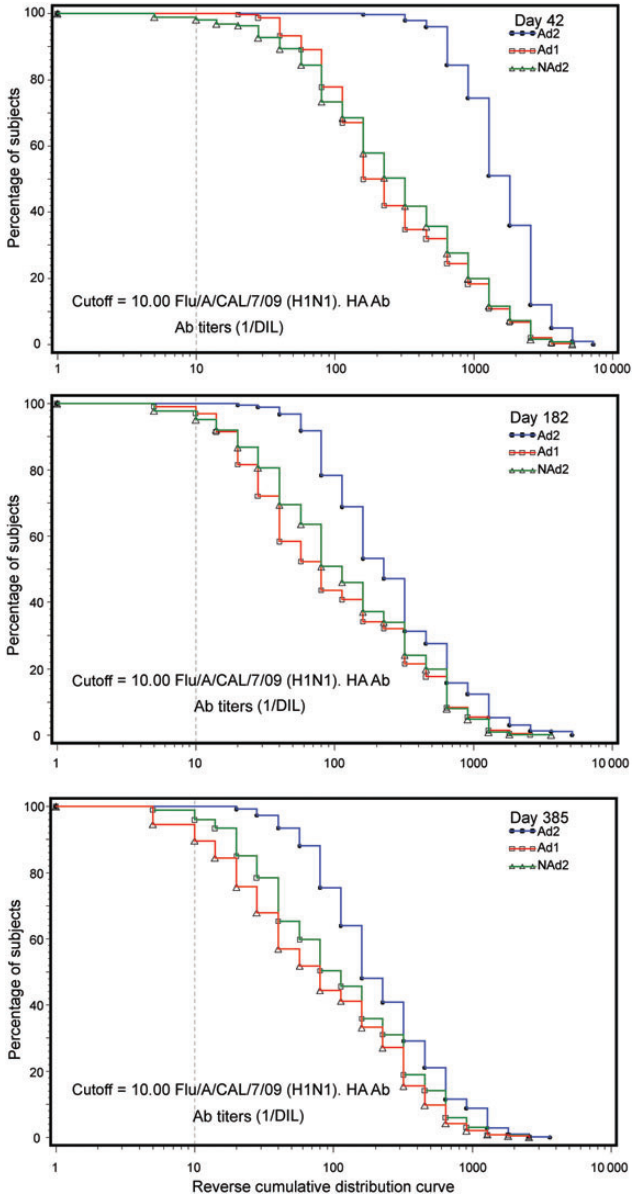


Figure 2. Reverse cumulative curves for hemagglutination-inhibiting (HI) antibody (Ab) titers at day 42, day 182, and day 385 in all children (ie, the according-to-protocol immunogenicity cohort). Abbreviations: Ad1, A/California/7/2009(H1N1)-AS03 vaccine at dose 1 and saline placebo at dose 2; Ad2, 2 doses of A/California/7/2009(H1N1)-AS03 vaccine; HA, hemagglutinin; NAd2, 2 doses of nonadjuvanted A/California/7/2009(H1N1) vaccine.

0–385) but were met for real-time PCR–confirmed A(H1N1) pdm09 infection (relative VE, 50.1% [95% CI, −23.5% to 79.9%]) in the total vaccinated cohort (days 14–385).

Immunogenicity

The ATP immunogenicity subset included 1175 children at day 42, 1693 at day 182, and 1526 at day 385. At day 42, the seroprotection rate was 100% for Ad2, 98.7% for Ad1, and 92.8% for NAd2 (Table 3). Center for Biologics Evaluation and Research and Committee for Medicinal Products for Human Use criteria (defined in Table 3) were fulfilled by all treatment groups (overall and by each age stratum) at day 42.

Adjuvant effect (Ad2 vs NAd2 groups) was demonstrated in terms of seroconversion rate (lower limit of the 95% CI for the difference between groups, 7.2%) and GMT ratios (lower limit for 95% CI, 4.9). Adjuvant effect was also demonstrated in both age strata (lower limit for 95% CI for the difference in seroconversion rate between groups, 10.3% for ages 6 to <36 months and 1.4% for ages 3 to <10 years; lower limit for the 95% CI of the GMT ratio, 7.7 for ages 6 to <36 months and 2.4 for ages 3 to <10 years; Figure 2).

Adjuvant effect was also observed for Ad1 versus NAd2 in terms of seroconversion rate overall (lower limit of the 95% CI for the difference between groups, 0.9%) but not for GMT ratios. Adjuvant effect was observed for children aged 6 to <36 months in terms of seroconversion rate (lower limit of the 95% CI for the difference between groups, 8.6%) and GMT ratios (lower limit for the 95% CI on the GMT ratio, 1.3).

At day 385, the seroprotection rate was 97.3% for Ad2, 78.5% for Ad1, and 67.9% for NAd2 (Table 3). HI GMTs were highest in the Ad2 group at all postvaccination time points (Table 3).

Reactogenicity and Safety

Pain at the injection site was the most frequently reported symptom in all groups after each dose (Figure 3) and was more frequent for Ad2 than NAd2. Grade 3 injection site reactions were reported by no more than 4.3% of children after either dose.

The percentage of children in each age stratum reporting systemic solicited symptoms was similar among treatment groups and after doses 1 and 2 (Figure 3), with the exception of fever (oral or axillary temperature, ≥38.0°C) in the Ad2 group, which increased from 10.2% (95% CI, 8.6%–11.9%) after dose 1 to 19.0% (95% CI, 16.9%–21.2%) after dose 2 in subjects aged 6 months to <6 years (an increase was observed in the subgroups aged 6 to <36 months and 3 to <6 years; Supplementary Table 2) and from 4.8% (95% CI, 3.3%–6.6%) to 8.7% (95% CI, 6.7%–11.1%), respectively, in subjects aged 6 to <10 years. In the Ad2 group, grade 3 fever (oral or axillary temperature of ≥39.0°C) was reported for 1.9% (95% CI, 1.2%–2.8%) of children aged 6 months to <6 years after dose 1 and for 3.1% (95% CI, 2.2%–4.2%) after dose 2. Fever (defined as a temperature of >40.0°C) was only reported after dose 1: 2 episodes occurred in children in the Ad2 group, and 3 episodes occurred in each of the other groups (Supplementary Table 2).

There were 15 children who experienced febrile convulsions through day 385: 4 were in the Ad2 group, 6 were in the Ad1 group, and 5 were in the NAd2 group. Seven febrile convulsions were reported as SAEs (Table 4). None occurred within 42 days after vaccination, and none were considered vaccine related. One so-called convulsion (fever was absent) was reported through day

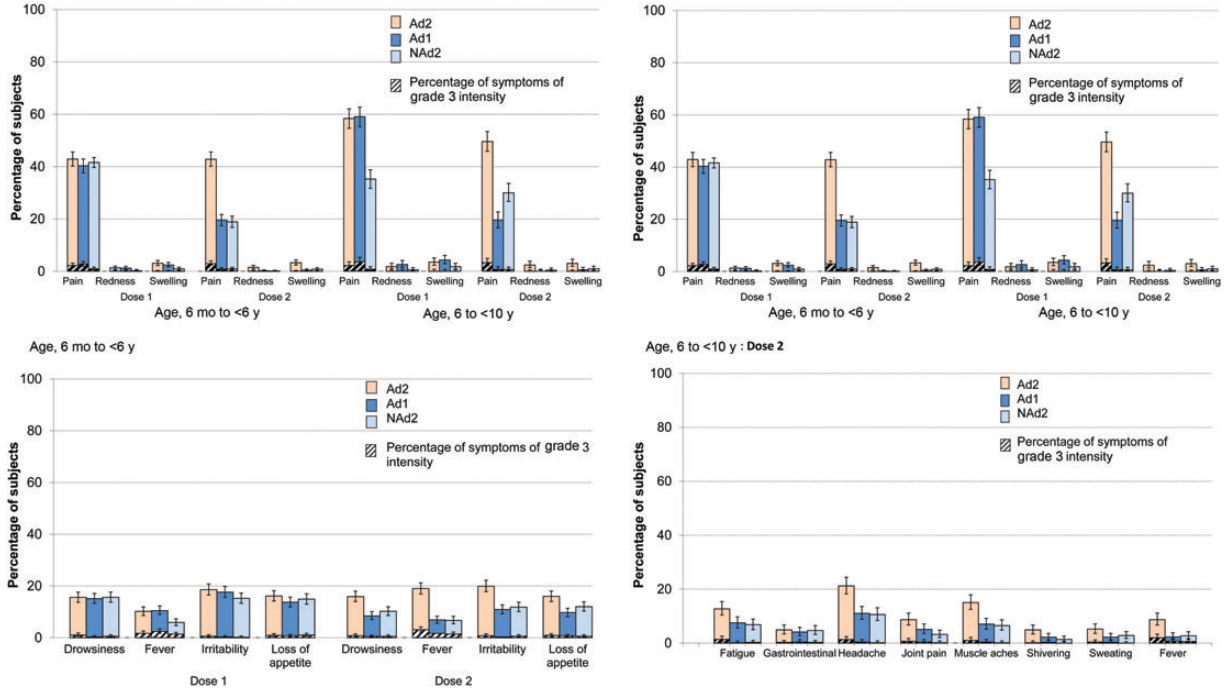


Figure 3. Percentage of subjects reporting injection site reactions and general symptoms 0–6 days after each vaccine dose. Vertical lines indicate 95% confidence intervals. In children <6 years of age, grade 3 severity was defined as pain (ie, crying when limb is moved or with spontaneous sensation of pain), redness or swelling in an area >100 mm in diameter, fever (ie, an oral or axillary temperature of ≥39.0°C), irritability (ie, crying that cannot be comforted or that prevents normal activity), drowsiness (ie, prevention of normal activity), and loss of appetite (not eating at all). In children 6 to <10 years of age, grade 3 severity was defined as pain that prevents normal activity, redness or swelling in an area >100 mm in diameter, fever (ie, an oral/axillary temperature of ≥39.0°C), and, for all other symptoms, prevention of normal activity.

42 after vaccination (onset, 7 days after vaccination in a subject from the Ad2 group who had preexisting epilepsy). The event lasted 6 days and was not considered to be vaccine related.

Percentages of children with any AE within 42 days of vaccination requiring a medically attended visit were similar among groups (23.4% for the Ad2 group, 22.9% for the Ad1 group, and 22.8% for the NAd2 group; Table 4).

During the 42-day follow-up period, 0.5% of subjects in the Ad2 group, 0.9% in the Ad1 group, and 1.2% in the NAd2 group reported at least 1 grade 3 AE. None of the grade 3 symptoms reported in the Ad2 group were considered to be vaccine related. One case of grade 3 headache in the Ad1 group and 1 case each of grade 3 vomiting and gastroenteritis in the NAd2 group were assessed as potentially vaccine related.

Percentages of children experiencing at least 1 SAE from days 0 to 385 were 3.7% in the Ad2 group, 3.2% in the Ad1 group, and 3.3% in the NAd2 group. The 10 most frequently reported SAEs were similar across groups (Table 4). One SAE (which involved a subject in theAd1 group, who required an emergency department visit for gastroenteritis, with onset on the day of dose 2) was considered to be vaccine related. There were 3 fatal events (all in the Philippines), and none were considered vaccine related: 1 involved a 10-year-old child (in the Ad2 group), who drowned; 1 involved a 20-month-old child (in the Ad2 group), who died from intestinal obstruction, parasitic gastroenteritis, and aspiration pneumonia; and 1 involved a 6-month-old child (in the Ad1 group), who had a history of pneumonia and asthma and died 20 days after vaccination from community-acquired pneumonia and asthma (real-time PCR negative). No autopsy was done.

During the study period, 1 potential immune-mediated disease was reported for the Ad2 group (0.05% of subjects), and 3 and 4 for the Ad1 (0.15%) and NAd2 (0.2%) groups, respectively (Table 4).

DISCUSSION

This is the first prospective efficacy study commencing during an influenza pandemic and the first to assess an AS03-adjuvanted inactivated influenza vaccine in children. We found a clinically important and statistically significant improvement in the efficacy of AS03-adjuvanted vaccine, compared with efficacy of the nonadjuvanted influenza vaccine. Moreover, improved efficacy was shown using one-fourth to one-eighth of the standard dose of HA, and noninferiority was shown for a single dose of adjuvanted vaccine (one-eighth to one-sixteenth

Table 4. Safety Outcomes and Common Adverse Event Presentations From Days 0 to 385 After Vaccination in the Total Vaccinated Cohort

	Ad2 Group (n = 2048)		Ad1 Group (n = 2048)		NAd2 Group (n = 2049)	
Outcome	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
Days 0–42 follow-up period						
Any unsolicited AE	913	44.6 (42.4, 46.8)	904	44.1 (42.0, 46.3)	895	43.7 (41.5, 45.9)
Any unsolicited AE with a medically attended visit	479	23.4 (21.6, 25.3)	468	22.9 (21.0, 24.7)	467	22.8 (21.0, 24.7)
Any related AE	60	2.9 (2.2, 3.8)	57	2.8 (2.1, 3.6)	52	2.5 (1.9, 3.3)
Any grade 3 AE	11	0.5 (0.3, 1.0)	18	0.9 (0.5, 1.4)	24	1.2 (0.8, 1.7)
Any related grade 3 AE	0	. . .	1 (vomiting)	<0.001	2 (headache)	<0.001 (vomiting, gastroenteritis)
Any SAE	8	0.4 (0.2, .8)	8	0.4 (0.2, .8)	9	0.4 (0.2, .8)
Any related SAE	0		1 (gastroenteritis)		0	
Days 0–385 follow-up period						
Any unsolicited AE	1189	58.1 (55.9, 60.2)	1173	57.3 (55.1, 59.4)	1190	58.1 (55.9, 60.2)
Any SAE	76	3.7 (2.9, 4.6)	66	3.2 (2.5, 4.1)	68	3.3 (2.6, 4.2)
10 most frequent SAEs						
Gastroenteritis	10	0.5	11	0.5	17	0.8
Pneumonia	9	0.4	9	0.4	12	0.6
Appendicitis	5	0.2	2	0.1	2	0.1
Bronchitis	2	0.1	5	0.2	2	0.1
Asthma	3	0.1	4	0.2	1	0.0
Asthmatic crisis	4	0.2	2	0.1	2	0.1
Viral infection	3	0.1	4	0.2	1	0.0
Febrile convulsion	1	0.0	4	0.2	2	0.1
Urinary tract infection	4	0.2	1	0.0	2	0.1
Upper respiratory tract infection	2	0.1	2	0.1	2	0.1
Any related SAE	0		1 (gastroenteritis)		0	
Any pIMD	1		3		4	
Alopecia areata	1 (onset day 67 after dose 2[a])		1 (onset day 81 after dose 2)		0	
Glomerulonephritis	0		1 (onset day 94 after dose 2)		1 (onset day 4 after dose 1)	
Acute glomerulonephritis	0		0		1 (onset day 67 after dose 2)	
ITP	0		1 (onset day 268 after dose 2)		0	
Guillain–Barré syndrome	0		0		1 (onset day 166 after dose 2[b])	
Erythema multiforme	0		0		1 (onset day 347 after dose 2[c])	

Abbreviations: Ad1, A/California/7/2009(H1N1)-AS03 vaccine at dose 1 and saline placebo at dose 2; Ad2, 2 doses of A/California/7/2009(H1N1)-AS03 vaccine; AE, adverse event; CI, confidence interval; ITP, idiopathic thrombocytopenic purpura; NAd2, 2 doses of nonadjuvanted A/California/7/2009(H1N1) vaccine; pIMD, potential immune-mediated disease; SAE, serious adverse event.

[a] Considered to be vaccine related.

[b] Subject had an unspecified infection in the month before onset.

[c] Diagnosis was changed by the investigator to nonspecific viral exanthema after unblinding.

of the standard HA dose), although only in the total vaccinated cohort (exploratory analysis).

We observed lower than expected numbers of real-time PCR–confirmed cases of A(H1N1)pdm09 infection, most likely because of limited A(H1N1)pdm09 exposure during 2010, owing to the absence of the anticipated third pandemic wave during 2010 in the participating countries [14]. Furthermore, all study subjects received active influenza vaccination. We believe our active surveillance was adequate, as many cases of ILI were evaluated, and subsequent testing revealed a substantial incidence of infection with respiratory syncytial virus and seasonal influenza virus types A and B. Potential effects of variability between countries in active surveillance performance were minimized by the randomization process. Overall influenza incidence rates in each group had overlapping 95% CIs, which does not support the proposition that the adjuvant groups experienced an unqualified general reduction in risk. Nevertheless, despite the low number of influenza cases, the relative

VE of the adjuvanted vaccine after 2 doses was higher than estimated and was sufficient overall to reach a definitive conclusion. However, the low case numbers led to wide CIs for relative VE estimates and increased the uncertainty regarding our exploration of secondary end points and subanalyses by age, although point estimates for relative VE were the same for subjects younger and those older than 3 years.

This study commenced during the 2009–2010 influenza A(H1N1) pandemic in expectation of a third pandemic wave, and it was not considered ethically acceptable to include a placebo group. Even though the study could not provide absolute VE values, these can be estimated using other sources from the literature. If an absolute VE of 40% for 2 doses of nonadjuvanted H1N1 vaccine is assumed (from the study by Vesikari et al [5], against mainly influenza A[H3N2]), the estimated absolute VE for Ad2 in our study is 86%; if an absolute VE for plain H1N1 antigen of 59% is assumed (efficacy estimate from the same influenza A[H1N1] antigen in a quadrivalent formulation) [15], the estimated absolute VE for Ad2 is 90%, which is consistent with short-term vaccine effectiveness estimates (86%–100%) reported in case-control studies involving children and adults who received 1 dose of H1N1-AS03 [16–18]. Adjuvant benefit in terms of immunogenicity, with higher and more persistent immune responses, was also demonstrated for 2 H1N1-AS03 doses over 2 nonadjuvanted vaccine doses, consistent with findings from previous clinical trials [6, 19].

Evidence from multiple clinical trials indicates transient increase in injection site reactions following AS03-adjuvanted versus nonadjuvanted influenza vaccines, but there has been no evidence of an increased incidence of medically attended events or SAEs associated with AS03 use [7]. Consistent with these studies, we observed higher rates of injection site pain in H1N1-AS03 recipients than in nonadjuvanted vaccine recipients. However, grade 3 pain was reported for no more than 3.6% of children. Reported potential immune-mediated diseases were evenly distributed across groups. The incidence of mild fever increased after the second H1N1-AS03 dose, as observed in other studies of this vaccine in healthy children [20]. There was no clustering of febrile convulsion cases in temporal relationship to vaccination.

After commencement of our study, several retrospective studies suggested an association between vaccination with another A(H1N1)pdm09 vaccine (*Pandemrix*™) and the subsequent onset of narcolepsy in individuals <21 years of age and in adults [21–31]. These retrospective observational studies alone are insufficient to ascribe the risk solely to the vaccine, as other factors may play a role. The recent identification of an epitope in the H1N1 HA protein that mimics an epitope present within hypocretin [32] suggests that exposure of individuals with the HLA DQ0602 allele to H1N1 can result in an autoimmune response involving $CD4^+$ T cells that could lead to the onset of narcolepsy. No narcolepsy cases were reported in the current study, which was too small to detect rare events such as narcolepsy.

Our results provide evidence of the potential benefit of AS03-adjuvanted pandemic influenza vaccines for control of future pandemics with respect to prevention of disease in a particularly vulnerable age segment. Similarly, the availability of effective adjuvanted influenza vaccines for children with reduced antigen content could offer opportunities for improved control of seasonal influenza. However, in either the seasonal or pandemic setting, use of AS03-adjuvanted influenza vaccines associated with injection site symptoms, fever, and a theoretical risk of rarer events will need to be balanced against the risk of death or severe illness caused by the seasonal or pandemic influenza viral strains.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. Travel assistance was provided to all investigators by GlaxoSmithKline (GSK) Biologicals to participate at the investigator meetings. T. N.'s institution has received research grants from GSK, Novartis, CSL, Pfizer, and Sanofi Pasteur. Prior to Oct 2012, he received a small payment for his role as a member of the independent data and safety monitoring board for GSK Vaccines' human papillomavirus vaccine. M. M. has received grants and honorarium from GSK and has received travel funds for presentations from GSK, Merck Sharp and Dohme, Novartis, Sanofi Pasteur, and United Laboratories, as well as payments for development of educational presentations from different organizations in the Philippines. L. W. has received grants from GSK and has been on the advisory boards for GSK, Merck, and Novartis. She has received travel and accommodation allowances from GSK, Abbott, and Novartis. R. U. G. served as an invited speaker for Sanofi Pasteur, GSK, Wyeth, Pfizer, and Merck. He has been on the advisory boards for Sanofi Pasteur, GSK, and Wyeth. He has received travel expenses from Sanofi Pasteur, GSK, Wyeth, and Pfizer to participate in conferences and meetings. M. A. P. S. has received investigator and institutional fees from GSK. He has received a consultancy fee from Novartis and was paid for an expert testimony by GSK. He has served as a member of advisory boards for GSK, Novartis, Sanofi, and Merck. He has received travel grants for presentations from GSK, Novartis, Merck, and Sanofi Pasteur. L. F. S. received an institutional grant to conduct the study. He has received travel grants from GSK to present study data at conferences. M. A. R. W. and S. L. have received consultancy fees and honoraria from GSK. M. H. M. has received travel grants to participate at congresses. P. Lopez's institution received a grant from GSK toward performing this study. C. B. T. has received grants from GSK, Intercell, Sanofi Pasteur, and Novartis and travel grants from Pediatrica. S. R. G., P. Li, S. D., L. F., G. D., T. B., B. L. I., and D. W. V. are or were employees of the GSK group of companies. G. D. received royalties for patents from Wyeth (the last payment was received in 2009). S. R. G., P. Li, L. F., G. D., T. B., B. L. I., and D. W. V. received restricted shares/stock options of the GSK group of companies. L. F. is now an employee of Novavax and reports owning Novavax stock.

Trademark Statement. *Pandemrix* is a trademark of the GlaxoSmithKline group of companies.

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

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