Efficacy, immunogenicity, and safety evaluation of an MF59-adjuvanted quadrivalent influenza virus vaccine compared with non-adjuvanted influenza vaccine in children: a multicentre, randomised controlled, observer-blinded, phase 3 trial

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Summary

Background Young children have immature immune systems and respond poorly to standard influenza vaccines. The oil-in-water emulsion adjuvant MF59 can increase antigen uptake, macrophage recruitment, lymph node migration, and avidity to influenza virus. Therefore, we aimed to assess the relative efficacy, immunogenicity, and safety of an MF59-adjuvanted, quadrivalent, inactivated (subunit) influenza vaccine (aIIV4) compared with a US-licensed non-adjuvanted influenza vaccine in children.

Methods We did a multicentre, randomised controlled, observer-blinded, phase 3 trial of 146 sites including hospitals, clinics, and clinician offices in nine countries over two influenza seasons. We included children of either sex aged 6 months through 5 years. We stratified eligible participants and randomly assigned them (1:1), using a block size of four, to receive either aIIV4 or non-adjuvanted inactivated influenza vaccine (ie, trivalent inactivated influenza vaccine [IIV3] or quadrivalent inactivated influenza vaccine [IIV4]). We masked participants, parents or guardians, and outcome assessors to the administered vaccine. Designated personnel who were not masked administered aIIV4 in both seasons, or IIV3 in season one and IIV4 in season two. All vaccinations were administered intramuscularly. Children aged 6 through 35 months received one or two 0·25 mL doses, whereas those aged 3 through 5 years received one or two doses of 0·5 mL. The number of doses was dependent on previous vaccination status: vaccine-naive participants received a total of two doses of study vaccine, the first on day 1 and the second on day 29, whereas non-naive participants received only one dose on day 1. The primary outcome was relative vaccine efficacy assessed by RT-PCR-confirmed influenza due to any influenza strain in the overall study population and in prespecified age and dose subgroups. Immunogenicity against homologous and heterologous strains of influenza and safety were also measured. This study is registered with ClinicalTrials.gov, number NCT01964989.

Findings Between Nov 3, 2013, and March 5, 2014 (season one), and between Sept 30, 2014, and March 29, 2015 (season two), 10 644 participants were enrolled in this study. Of these participants, 10 612 were vaccinated (n=5338 with aIIV4 and n=5274 with comparator). Relative vaccine efficacy was not different between aIIV4 and the comparator vaccines in the overall study population (relative vaccine efficacy –0·67, 95% CI –19·81 to 15·41). The relative vaccine efficacy in the 6 through 23-month subgroup was significantly greater for aIIV4 than for the comparator vaccine (relative vaccine efficacy 31·37, 95% CI 3·14–51·38). aIIV4 elicited superior immunogenic response compared with the comparator for all four vaccine strains (geometric mean titre ratios 1·91 [95% CI 1·8–2·0] for A/H1N1, 1·71 [1·6–1·8] for A/H3N2, 2·19 [2·0–2·4] for B/Yamagata, and 2·27 [2·0–2·6] for B/Victoria) and three heterologous strains (1·94 [1·6–2·3] for A/H3N2, 2·17 [1·8–2·6] for B/Yamagata, and 2·12 [1·6–2·7] for B/Victoria) in participants aged 6 months through 5 years. The highest geometric mean titre ratios were observed in participants aged 6 through 23 months. Safety profiles were similar but more frequent solicited adverse events were reported with aIIV4 than with the comparator (3748 [73%] of 5138 *vs* 3242 [64%] of 5056).

Interpretation Although there was no additional benefit of aIIV4 compared with the US-licensed non-adjuvanted influenza vaccines in the overall study population, in the youngest and most vulnerable population of children in this trial, aIIV4 provided greater protection against influenza than a non-adjuvanted vaccine when assessed in this prespecified age group of 6 through 23 months. Additional clinical benefit was also apparent early after first vaccination in vaccine-naive participants aged 6 months through 5 years. Finally, aIIV4 and comparator had similar efficacy and vaccine safety profiles in children aged 6 months through 5 years.

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Research in context

Evidence before this study

We searched PubMed on Sept 5, 2017, using the search terms "quadrivalent influenza vaccine" and "children" with no restrictions to language or dates. Our search revealed 104 articles published between 2006 and 2017. Of these articles, ten were classified as clinical trials assessing the efficacy, immunogenicity, or safety of a quadrivalent vaccine in a paediatric population. None of these studies examined adjuvanted vaccines. We did a second PubMed search using the terms "adjuvanted inactivated influenza vaccine" and "children", which identified 25 clinical trials published between 2004 and 2015. One of these studies examined the relative efficacy of MF59-adjuvanted vaccine versus a non-adjuvanted comparator in children. In that study, adjuvanted trivalent inactivated influenza vaccine (aIIV3) was significantly more efficacious than non-adjuvanted trivalent inactivated influenza vaccine (IIV3) against laboratory-confirmed influenza in children aged 6 months through 5 years and in a subgroup aged 6 through 23 months.

Added value of this study

This study was done mainly during a season in which there was a mismatch between the recommended vaccine A/H3N2 strain and the predominantly circulating A/H3N2 virus strain. Despite the mismatch, aIIV4 was significantly more efficacious than the non-adjuvanted vaccines in preventing influenza in children aged 6 through 23 months.

Implications of all the available evidence

Young children, particularly those younger than 2 years, have immature immune systems that respond poorly to standard influenza vaccines. In this study, aIIV4 offered an additional benefit over currently licensed influenza vaccines in this age group, which should be considered in public health recommendations. However, no significant difference in vaccine efficacy was shown for the overall study population aged 6 months through 5 years.

Introduction

Children are at increased risk for influenza-induced morbidity and mortality,[1,2] and they have a major role in the transmission of influenza during epidemics.[3,4] In an average year, influenza affects approximately 13% of children younger than 5 years, or roughly 90 million children worldwide.[5,6] Influenza also accounts for 10% of paediatric admissions to hospital for respiratory illnesses and 2–7% of deaths in children younger than 5 years annually, with the highest percentages among those younger than 2 years.[7,8]

Vaccinating children against influenza not only directly benefits children themselves but also helps prevent the spread of influenza.[9] Unfortunately, currently licensed trivalent inactivated influenza vaccines (IIV3) often do not elicit robust antibody responses in young children, particularly against B strains. Meanwhile, two separate B lineages (B/Victoria/2/87 and B/Yamagata/16/88) have co-circulated during most influenza seasons since the 1980s.[10,11] Quadrivalent inactivated influenza vaccines (IIV4) were developed containing both influenza B lineages and might offer a potential advantage over IIV3.[12,13]

The oil-in-water emulsion adjuvant MF59 (Seqirus, formerly the influenza business of Novartis Vaccines and Diagnostics) can increase antigen uptake, macrophage recruitment, and lymph node migration.[14] MF59 also increases the avidity of antibody binding to influenza virus.[15] MF59-adjuvanted IIV3 (Fluad) showed increased efficacy and antibody responses to homologous and heterologous strains compared with non-adjuvanted IIV3 in children.[16,17] An MF59-adjuvanted, quadrivalent, inactivated (subunit) influenza vaccine (aIIV4) containing strains from the two influenza A strains and both the B/Yamagata and B/Victoria lineages should similarly enhance vaccine efficacy in children. Therefore, in this phase 3 trial, we assessed the relative efficacy, immunogenicity, and safety of aIIV4 compared with a US-licensed non-adjuvanted influenza vaccine in children.

See Online for appendix

Methods

Study design and participants

We did a multicentre, randomised controlled, observer-blinded, phase 3 trial of 146 sites including hospitals, clinics, and clinician offices in Finland, the USA, Canada, Italy, Poland, Spain, Philippines, Thailand, and Taiwan. We did the study over two influenza seasons. In the northern hemisphere, the end of an influenza season was considered as June 30, whereas in the tropical nations (Philippines and Thailand) the season end was Oct 31.

The study was approved by either a central or local institutional review board or ethics committee, and was done in accordance with the Declaration of Helsinki and the International Council for Harmonisation Guideline for Good Clinical Practice.

We included children of either sex aged 6 months through 5 years who were healthy or at high risk of complications from influenza. We excluded children who had any medical condition meeting the definition of an adverse event of special interest (appendix), any seizure condition, or any fatal condition. The appendix lists all inclusion and exclusion criteria. We obtained written informed consent from the parents or legal guardians, or both, of all participants before enrolment.

Randomisation and masking

We stratified eligible participants according to site, vaccine dose, risk status for influenza complications

(see the appendix for these definitions), and historical influenza vaccination status. For historical vaccination status, only participants who reported less than two doses of seasonal influenza vaccine since July 1, 2010, were considered vaccine naive. Vaccination history was based on previous documentation (eg, a vaccination card) or through the parent's or legal guardian's verbal recall if no such documentation was available.

Additionally, we used a web-based interactive response technology system to randomly assign participants (1:1), using a block size of four, to receive either aIIV4 or non-adjuvanted inactivated influenza vaccine (ie, IIV3 or IIV4). Blocks were dynamically assigned to the site strata, from a central block pool, upon the first participant enrolment into the strata. Subsequent participants enrolled into the site strata were allocated to the next available treatment group in the randomisation block. Randomisation lists were generated by Oracle randomisation statisticians and verified by the sponsor's statisticians. Treatment lists were generated by Fisher Clinical Services and verified by the sponsor's statisticians and the Clinical supply department.

Site-specific blinding procedures were documented before the study start. Therefore, we masked all site personnel except those administering the vaccine (ie, the nurse or physician), for which they had no role in the assessment of the participants in the trial and were instructed not to reveal the identity of the study vaccines to the participants, parents or guardians, and investigators or study nurses involved in the monitoring or conduct of the study, except in an emergency. We also masked the participants and parents or guardians to the vaccine administered; for example, we requested them to look the other way upon vaccination or by shielding the syringe.

For the immunogenicity subgroup, we randomly selected participants in a one-to-one ratio from the two vaccine study groups, including both healthy participants and those at high risk of influenza complications. We also stratified the immunogenicity subgroup by country, dose cohort, and vaccine status.

Procedures

We used aIIV4 (Seqirus, Rosia, Italy) and non-adjuvanted inactivated influenza vaccine (ie, IIV3 or IIV4, both of which are split virion type licensed products [Fluzone, Sanofi Pasteur, Swiftwater, PA, USA]). Fluzone IIV3 was used as the comparator in season one because Fluzone IIV4 did not receive approval from the US Food and Drug Administration for use in children aged 6 months or older until June, 2013, and insufficient amounts of IIV4 were available the first season. Fluzone IIV4 was used as the comparator in season two. The aIIV4 used in seasons one and two, and IIV4 used in season two contained a total haemagglutinin (HA) concentration of 60 µg in the 0·5 mL dose and 30 µg in the 0·25 mL dose, which consisted of 15 µg or 7·5 µg (depending on dose) of HA from each influenza strain as recommended by WHO for the northern hemisphere 2013–14 and 2014–15 seasons: A/H1N1 (A/California/7/2009 pdm09-like virus), A/H3N2 (A/Texas/50/2012), B/Yamagata (B/Massachusetts/2/2012), and B/Victoria (B/Brisbane/60/2008). The IIV3 comparator used in season one contained 15 µg of HA in the 0·5 mL dose or 7·5 µg in the 0·25 mL dose from A/H1N1 and A/H3N2 as well as the B/Yamagata lineage, for a total of 45 µg in the 0·5 mL dose or 22·5 µg in the 0·25 mL dose of HA in the vaccine.

Children aged 6 through 35 months received one or two 0·25 mL doses, whereas those aged 3 through 5 years received one or two doses of 0·5 mL. The number of doses was dependent on previous vaccination status: vaccine-naive participants received a total of two doses of study vaccine, the first on day 1 and the second on day 29, whereas non-naive participants received only one dose on day 1. All vaccinations were administered intramuscularly in the deltoid, except when the child did not have sufficient deltoid mass in which case injections were administered in the anterolateral thigh.

We did active surveillance for influenza-like illness weekly via telephone contact or text message, or both, or during planned clinic visits for 180 days after last vaccination or until the end of the influenza season, whichever was longer. Parents or guardians of the study participants were instructed to report symptoms of influenza-like illness to the study site and schedule a clinic visit for nasopharyngeal swab collection, preferably within 3 days or at least within 6 days after a participant exhibited a body temperature of 37·8°C or more and at least one of the following respiratory symptoms: cough, sore throat, nasal congestion, or runny nose. RT-PCR was used to confirm influenza infection from nasopharyngeal swab samples, which were also cultured in parallel. The cultured virus was analysed for antigenic characterisation by the University of Rochester Vaccine Evaluation Unit (Rochester, NY, USA) and by Viroclinics Biosciences BV (Rotterdam, Netherlands). RT-PCR and culture tests were done at Focus Diagnostics Clinical Trials (San Juan Capistrano, CA, USA).

Strain antigenic typing was determined using haemagglutination inhibition or microneutralisation assays. For some A/H3N2 isolates, haemagglutination inhibition was done with ferret antisera raised against an exclusively cell-grown A/H3N2 and an egg-propagated A/H3N2 virus reference standard. For A/H3N2 isolates that did not bind red blood cells but could be grown, a standard microneutralisation assay was done using egg-propagated A/H3N2 to both raise ferret antisera and to serve as the reference standard.[18] Matched strains were those with less than eight-fold difference in titre and unmatched strains were those

with eight-fold difference or more in titre compared with the reference standard.

We assessed antibody responses with haemagglutination inhibition assays using serum samples collected before vaccination (day 1 for non-vaccine-naive participants and days 1 and 29 for vaccine-naive participants) and 21 days after the last vaccination (day 22 for non-vaccine-naive participants and day 50 for vaccine-naive participants). We also collected samples for haemagglutination inhibition titre measurement 6 months after the last vaccination (day 181 for non-vaccine-naive participants and day 209 for vaccine-naive participants). Strains selected for heterologous testing were A/Brisbane/59/2007 for A/H1N1, A/Hong Kong/4801/2014 for A/H3N2, B/Phuket/3073/2013 for B/Yamagata, and B/Malaysia/2506/2004 for B/Victoria.

From 6 h to day 7 after each vaccination, the participant's parent or guardian, or both, was instructed to keep a daily record of specific local and systemic adverse events. These solicited adverse events included tenderness, erythema, induration, and ecchymosis at the injection site, as well as irritability, sleepiness, changed eating habits, vomiting, diarrhoea, chills, fever, and the use of antipyretics or analgesics for prevention or treatment of pain or fever, or both. Other adverse events and associated medications were also tracked from the day of vaccination until 21 days after the last vaccination. Safety surveillance was done from the first vaccination and continued until the termination visit, which occurred on day 366 for non-vaccine-naive participants and on day 390 for vaccine-naive participants. Surveillance included adverse events of special interest, which specifically focused on potentially immune-mediated diseases (the appendix summarises the full list of medical concepts), new-onset chronic diseases, serious adverse events, and events leading to study withdrawal.

Outcomes

The primary outcome was the relative vaccine efficacy of aIIV4 versus non-adjuvanted vaccine (ie, IIV3 or IIV4) based on RT-PCR-confirmed influenza cases occurring between 21 days and 180 days (inclusive) after the last vaccination or until the end of the influenza season, whichever was longer, in participants aged 6 months through 5 years. Early vaccine efficacy was assessed as the relative vaccine efficacy at 7 days or more and 14 days or more after the first but before the second dose of vaccine in vaccine-naive participants as well as at 7 days or more and 21 days or less after the last vaccination, in all participants.

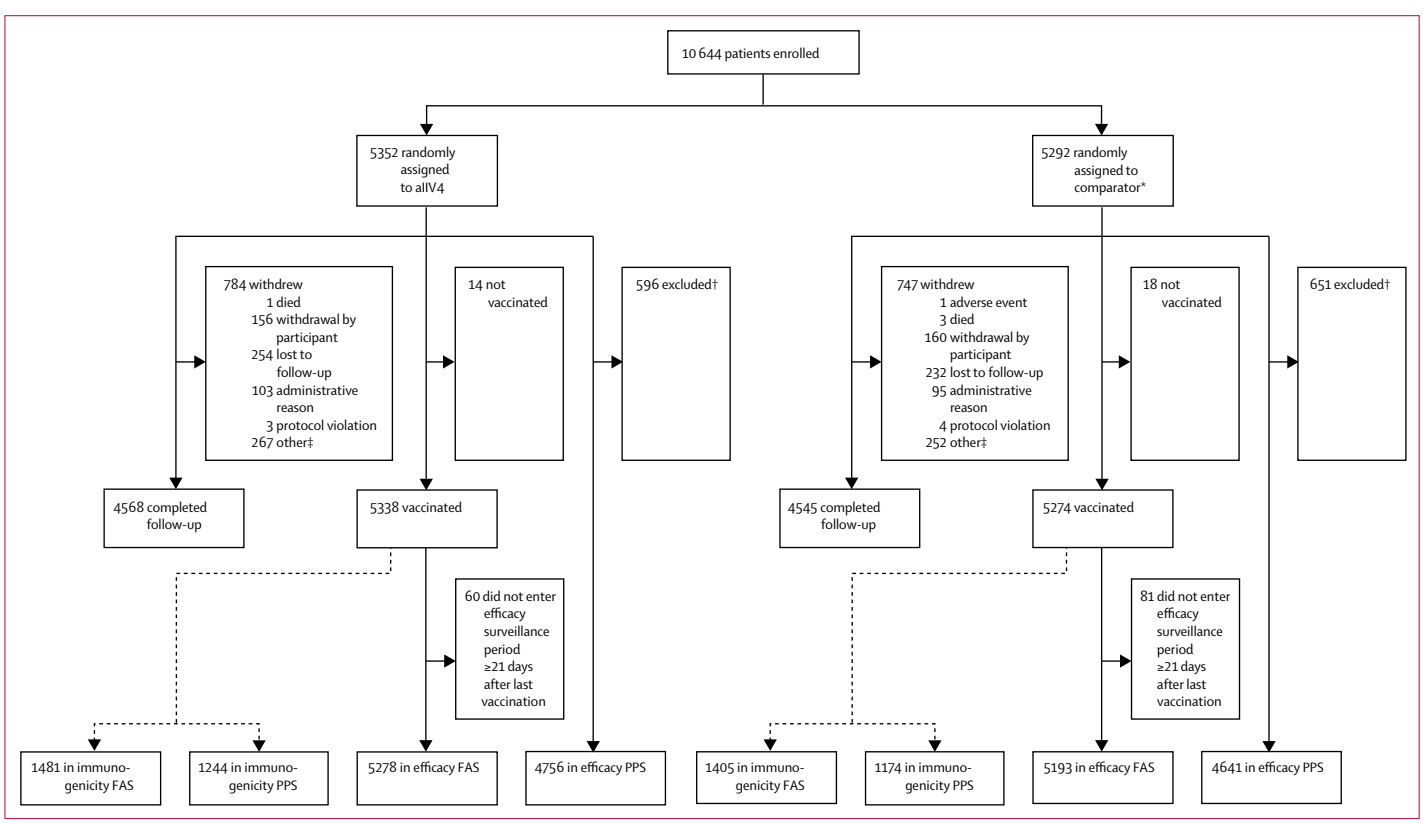

Figure 1: **Trial profile**

aIIV4=MF59-adjuvanted, quadrivalent, subunit inactivated influenza vaccine. IIV3=trivalent inactivated influenza vaccine. IIV4=quadrivalent inactivated influenza vaccine. FAS=full analysis set. PPS=per-protocol set. *The comparator was non-adjuvanted IIV3 in season one and non-adjuvanted IIV4 in season two. †The appendix shows the full list of those who were excluded from the efficacy PPS. ‡Included reasons such as non-compliance, participant withdrawn by investigator, and participant enrolled in an extension study.

Efficacy determinations in the first season were based on A/H1N1, A/H3N2, and B/Yamagata, the B lineage common to aIIV4 and the IIV3 comparator. Children infected with B/Victoria from season one were not included in the efficacy analyses because this strain was absent from the comparator vaccine in season one. Season two efficacy assessments were made using all four virus strains. We assessed all efficacy outcomes for all participants in the following prespecified age and dose cohorts: 6 through 23 months, 0·25 mL dose (6 through 35 months), and 0·5 mL dose (3 through 5 years). A post-hoc analysis assessed relative vaccine efficacy in participants aged 2 through 5 years, irrespective of dose. Secondary efficacy outcomes included culture-confirmed influenza regardless of antigenic match, and antigenically matched and unmatched strains in the same timeframe as the primary efficacy outcome.

Secondary immunogenicity outcomes included assessment of geometric mean titres (GMT) at baseline, before first vaccination, and 3 weeks and 6 months after final vaccination in all participants. In vaccine-naive participants who received two doses, immunogenicity was also assessed 4 weeks after first vaccination. The ratios of GMT values for aIIV4 versus non-adjuvanted vaccine were also determined at each timepoint. Data from 3 weeks after final vaccination in naive and non-naive participants were pooled. We pooled data from seasons one and two because the same strains were included in the vaccine. The vaccine-group difference in the percentage of those achieving seroconversion was assessed; seroconversion was defined as a post-vaccination haemagglutination inhibition titre of 1:40 for participants who were negative at baseline (haemagglutination inhibition <1:10) or a minimum four-fold increase in haemagglutination inhibition titre for those who were positive at baseline (≥1:10). Antibody responses were also evaluated according to the Center for Biologics Evaluation and Research criteria (appendix). The proportion of participants with haemagglutination inhibition titres of 1:110 or more, 1:151 or more, 1:215 or more, 1:330 or more, and 1:629 or more was also evaluated at the same timepoints outlined above.[19] Subgroup analyses were preplanned for the immunogenicity endpoints for the same age and dose cohorts as for efficacy. A post-hoc analysis was done for children aged 2 through 5 years.

Safety and tolerability were assessed in terms of the percentage of participants with adverse events. Any other outcome or subgroup analyses as specified in the protocol but not listed here were not included in this manuscript.

Statistical analysis

Sample size and power considerations for the primary aIIV4 efficacy objective were based on data extracted from a previous efficacy study of adjuvanted IIV3 (aIIV3) in paediatric participants.[20] This study was planned with a group-sequential design. Assuming a relative vaccine efficacy of 36% and an influenza event rate of 2·50% in the non-adjuvanted comparator group, the sample size of approximately 8124 evaluable participants aged 6 months through 5 years for both vaccine groups, with a number of 323 influenza events, predicted about 97·5% power for the adjusted 2·02% level one-sided log-rank test for equality of survival

	Efficacy full analysis set		Immunogenicity full analysis set	
	aIIV4 (n=5278)	Comparator* (n=5193)	aIIV4 (n=1481)	Comparator* (n=1405)
Mean age, months	38·4 (18·4)	38·0 (18·4)	35·9 (18·6)	35·3 (18·4)
Age groups				
6 through 23 months	1299 (25%)	1339 (26%)	428 (29%)	427 (30%)
2 through 5 years	3979 (75%)	3854 (74%)	1053 (71%)	978 (70%)
Dose groups				
0·25 mL	2484 (47%)	2471 (48%)	822 (56%)	798 (57%)
0·5 mL	2794 (53%)	2722 (52%)	659 (44%)	607 (43%)
Sex				
Boys	2669 (51%)	2652 (51%)	734 (50%)	708 (50%)
Girls	2609 (49%)	2541 (49%)	747 (50%)	697 (50%)
Vaccine-naive status				
Naive	3553 (67%)	3525 (68%)	922 (62%)	866 (62%)
Non-naive	1725 (33%)	1668 (32%)	559 (38%)	539 (38%)
Season				
Season one	757 (14%)	699 (13%)	684 (46%)	625 (44%)
Season two	4521 (86%)	4494 (87%)	797 (54%)	780 (56%)
Met protocol criteria				
No	54 (1%)	57 (1%)	22 (1%)	17 (1%)
Yes	5224 (99%)	5136 (99%)	1459 (99%)	1388 (99%)
HI titre less than 1:10†				
A/H1N1, naive	NA	NA	484 (52%)	473 (55%)
A/H1N1, non-naive	NA	NA	126 (23%)	122 (23%)
A/H3N2, naive	NA	NA	451 (49%)	443 (51%)
A/H3N2, non-naive	NA	NA	81 (14%)	71 (13%)
B/Yamagata, naive	NA	NA	593 (64%)	570 (66%)
B/Yamagata, non-naive	NA	NA	266 (48%)	261 (48%)
B/Victoria, naive	NA	NA	682 (74%)	351 (79%)‡
B/Victoria, non-naive	NA	NA	302 (54%)	183 (54%)‡
HI titre 1:40 or more				
A/H1N1, naive	NA	NA	366 (40%)	332 (38%)
A/H1N1, non-naive	NA	NA	371 (66%)	350 (65%)
A/H3N2, naive	NA	NA	410 (44%)	359 (41%)
A/H3N2, non-naive	NA	NA	418 (75%)	402 (75%)
B/Yamagata, naive	NA	NA	134 (15%)	120 (14%)
B/Yamagata, non-naive	NA	NA	134 (24%)	131 (24%)
B/Victoria, naive	NA	NA	127 (14%)	49 (11%)‡
B/Victoria, non-naive	NA	NA	138 (25%)	93 (28%)‡

Data are mean (SD) or n (%). aIIV4=MF59-adjuvanted, quadrivalent, subunit inactivated influenza vaccine. HI=haemagglutination inhibition. NA=not available. IIV3=trivalent inactivated influenza vaccine. IIV4=quadrivalent inactivated influenza vaccine. *The comparator was non-adjuvanted IIV3 in season one and non-adjuvanted IIV4 in season two. †Information about pre-vaccination titres is not available for all participants in the full analysis set efficacy population. ‡Only results from season two were included for the comparator.

Table 1: **Baseline demographic and clinical characteristics of study population**

curves to detect the difference between the two groups. Assuming a 15% dropout of participants, approximately 9558 participants were to be enrolled for each vaccine group. As per protocol, at a number of 323 RT-PCR-confirmed cases, a final analysis for efficacy was to be done. This number was reached at the interim analysis done by an independent data monitoring committee at the end of season two. Therefore, no further participants were enrolled in a subsequent season.

Relative vaccine efficacy was defined as $1-HR=1-\lambda_{aIIV4}(t)/\lambda_{Comparator}(t)$, in which HR is the hazard ratio and $\lambda(t)$ is the hazard rates of each vaccine group, which was further defined as $\lambda(t)=f(t)/S(t)$ for which $f(t)$ is the rate of first-occurrence RT-PCR-confirmed influenza events per unit time (t) and $S(t)$ is the survival function (ie, function of time without influenza events). The HRs and related 95% CIs for onset of RT-PCR-confirmed influenza were estimated by a Cox proportional hazards regression model with treatment effect as a main effect and stratifying covariates (season, vaccine naivety, dose group, high risk of influenza complications, and country) as random effects. Success for superiority was specified as relative vaccine efficacy of aIIV4 versus comparator with a lower bound of the 95% CI more than 0. An exploratory test for heterogeneity (Breslow-Day test) for the consistency of first-occurrence RT-PCR-confirmed relative vaccine efficacy across age subgroups (6 through 23 months and 2 through 5 years) was done post hoc.

For the immunogenicity outcomes, aIIV4 was considered superior if the lower boundary of the two-sided 95% CI for the ratio of GMT_{aIIV4} to $GMT_{Comparator}$ for haemagglutination inhibition antibody was more than one and the lower boundary of the two-sided 95% CI for the difference of percentages of participants seroconverted for haemagglutination inhibition antibody was more than 0%.

The efficacy full analysis set comprised all participants who received study vaccine and provided efficacy data. For the immunogenicity analyses, the full analysis set population included all participants who received study vaccine and who provided at least one evaluable serum sample both before (baseline) and after vaccination. The per-protocol set included all study participants and associated data from the full analysis set who were not excluded for reasons prespecified before unmasking. The safety analysis set included all participants who were exposed to the study vaccine and provided safety data. We did all statistical analyses using SAS (version 9.3). This study is registered with ClinicalTrials.gov, number NCT01964989.

	aIIV4 (n=5278)	Comparator† (n=5193)	Relative vaccine efficacy‡ (95% CI)
RT-PCR-confirmed influenza (primary endpoint)			
Any strain§	256 (5%)	252 (5%)	−0·67 (−19·81 to 15·41)
A/H1N1	7 (<1%)	17 (<1%)	59·39 (2·06 to 83·16)
A/H3N2	200 (4%)	196 (4%)	−1·33 (−23·41 to 16·79)
B/Yamagata	36 (1%)	36 (1%)	2·09 (−55·44 to 38·33)
B/Victoria¶	14 (<1%)	9 (<1%)	−54·47 (−256·90 to 33·14)
Culture-confirmed influenza (secondary endpoint)			
Any strain§	140 (3%)	146 (3%)	5·21 (−19·53 to 24·83)
A/H1N1	5 (<1%)	9 (<1%)	NA‖
A/H3N2	96 (2%)	104 (2%)	8·60 (−20·62 to 30·75)
B/Yamagata	29 (<1%)	27 (1%)	−5·56 (−78·32 to 37·52)
B/Victoria¶	10 (<1%)	8 (<1%)	NA‖
Matched**	74 (1%)	80 (2%)	8·44 (−25·61 to 33·26)
Unmatched**	65 (1%)	62 (1%)	−3·47 (−46·55 to 26·94)

Data are n (%), unless otherwise stated. aIIV4=MF59-adjuvanted, quadrivalent, subunit inactivated influenza vaccine. NA=not applicable. IIV3=trivalent inactivated influenza vaccine. IIV4=quadrivalent inactivated influenza vaccine. *Includes full analysis set, including all participants from seasons one and two who received at least one dose of study vaccination and provided efficacy endpoint data. †The comparator was non-adjuvanted IIV3 in season one and non-adjuvanted IIV4 in season two. ‡Result is based on the Cox proportional hazards model for time until onset of the first confirmed influenza, adjusting for vaccine naivety, risk factor, season, dose group, and country as random effect. §Any strain regardless of antigenic match. For any participant who had multiple confirmed influenza infections, only the first-occurrence confirmation was included under any strain. However, for all confirmed influenza infections, the first occurrence for each strain was counted separately under each respective individual confirmed strain. Therefore, the sum of cases under each strain can be higher compared with the total number of cases reported under any strain. ¶B/Victoria cases from season one (one RT-PCR confirmed case for aIIV4 and two RT-PCR confirmed cases for the comparator vaccine) have not been included in the analysis. ‖Per the statistical analysis plan, relative vaccine efficacy was not calculated if number of cases was less than 20. **Matched strains are those with a less than eight-fold difference in titre and unmatched strains are those with eight-fold difference or more in titre compared with the vaccine strain.

Table 2*: First-occurrence RT-PCR-confirmed and culture-confirmed influenza and relative vaccine efficacy in participants aged 6 months through 5 years

Role of the funding source

The study was funded by Novartis Vaccines and Diagnostics and Seqirus UK Ltd. Of note, Novartis' influenza vaccine business was acquired by the CSL-group on July 31, 2015, and currently operates as Seqirus. The funder of the study had primary responsibility for study design and study vaccines, protocol development, study monitoring, data analysis, data management, and writing of the report. BL, MER, LI, MdB, JO, and EH had full access to the raw data. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Season one enrolment occurred between Nov 3, 2013, and March 5, 2014, and season two enrolment between Sept 30, 2014, and March 29, 2015. A total of 10 644 participants were enrolled in this study (1486 in 2013–14 [season one] and 9158 in 2014–15 [season two]). Of these participants randomly assigned to the two groups, 5338 were vaccinated with aIIV4 and 5274 were vaccinated with the comparator vaccine across the two seasons (figure 1). 2686 (25·2%) of 10 644 were younger than 2 years. The immunogenicity subset included all participants enrolled in season one and 1770 from season two; in total, 1481 participants were assigned to receive aIIV4 and 1405 to receive the comparator vaccine. The number of enrolled participants varied by sites from one to 476, and most participants were

	Age 6 through 23 months			Age 2 through 5 years		
	aIIV4 (n=1299)	Comparator† (n=1339)	Relative vaccine efficacy‡ (95% CI)	aIIV4 (n=3979)	Comparator† (n=3854)	Relative vaccine efficacy‡ (95% CI)
RT-PCR-confirmed influenza						
Any strain§	55 (4%)	79 (6%)	31·37 (3·14 to 51·38)	201 (5%)	173 (4%)	−14·99 (−40·93 to 6·18)
A/H1N1	2 (<1%)	5 (<1%)	NA¶	5 (<1%)	12 (<1%)	NA¶
A/H3N2	44 (3%)	66 (5%)	34·50 (4·05 to 55·28)	156 (4%)	130 (3%)	−19·28 (−50·57 to 5·51)
B/Yamagata	5 (<1%)	9 (1%)	NA¶	31 (1%)	27 (1%)	−11·25 (−86·42 to 33·61)
B/Victoria‖	4 (<1%)	0	NA¶	10 (<1%)	9 (<1%)	NA¶
Culture-confirmed influenza						
Any strain§	31 (2%)	48 (4%)	35·96 (−0·63 to 59·25)	109 (3%)	98 (3%)	−9·18 (−43·44 to 16·90)
A/H1N1	1 (<1%)	4 (<1%)	NA¶	4 (<1%)	5 (<1%)	NA¶
A/H3N2	23 (2%)	38 (3%)	40·23 (−0·35 to 64·40)	73 (2%)	66 (2%)	−8·95 (−52·02 to 21·91)
B/Yamagata	5 (<1%)	7 (1%)	NA¶	24 (1%)	20 (1%)	−16·84 (−111·54 to 35·47)
B/Victoria‖	2 (<1%)	0	NA¶	8 (<1%)	8 (<1%)	NA¶
Matched**	19 (1%)	32 (2%)	40·92 (−4·27 to 66·52)	55 (1%)	48 (1%)	−12·16 (−65·19 to 23·85)
Unmatched**	12 (1%)	14 (1%)	13·51 (−87·08 to 60·01)	53 (1%)	48 (1%)	−8·26 (−60·01 to 26·75)

Data are n (%), unless otherwise stated. aIIV4=MF59-adjuvanted, quadrivalent, subunit inactivated influenza vaccine. NA=not applicable. IIV3=trivalent inactivated influenza vaccine. IIV4=quadrivalent inactivated influenza vaccine. *Includes participants from seasons one and two who received at least one dose of study vaccination and provided efficacy endpoint data. †The comparator was non-adjuvanted IIV3 in season one and non-adjuvanted IIV4 in season two. ‡Result is based on the Cox proportional hazards model for time until onset of the first confirmed influenza, adjusting for vaccine naivety, risk factor, season, dose group, and country as random effect. §Any strain regardless of antigenic match. For any participant who had multiple confirmed influenza infections, only the first-occurrence confirmation was included under any strain. However, for all confirmed influenza infections, the first occurrence for each strain was counted separately under each respective individual confirmed strain. Therefore, the sum of cases under each strain can be higher compared with the total number of cases reported under any strain. ¶Per the statistical analysis plan, relative vaccine efficacy was not calculated if number of cases was less than 20. ‖B/Victoria cases from season one have not been included in the analysis. **Matched strains are those with a less than eight-fold difference in titre and unmatched strains are those with eight-fold difference or more in titre compared with the vaccine strain.

Table 3: **First-occurrence RT-PCR-confirmed and culture-confirmed influenza and relative vaccine efficacy in participants aged 6 through 23 months and 2 through 5 years***

enrolled from the USA (n=4508), Philippines (n=2273), and Thailand (n=2040).

In both the efficacy and immunogenicity full analysis set, demographic and baseline characteristics were similar between vaccine groups, including the proportion of participants with baseline haemagglutination inhibition titres of less than 1:10 and 1:40 or more (table 1). The number of vaccine-naive participants with a baseline haemagglutination inhibition titre of less than 1:10 against H3N2 was 451 (49%) of 922 participants who received aIIV4 and 443 (51%) of 866 who received the comparator (table 1). By contrast, the number of participants aged 6 through 23 months who had a baseline haemagglutination inhibition titre of less than 1:10 against H3N2 was 282 (66%) of 428 for those who received aIIV4 versus 280 (66%) of 427 for those who received the comparator. In total, 2235 (84%) of 2673 participants aged 6 through 23 months received two doses of vaccine versus 4642 (58%) of 7939 aged 2 through 5 years.

A total of 508 cases of first-occurrence RT-PCR-confirmed influenza occurred during the study; ten during season one and 498 during season two. There was no difference between aIIV4 and the comparator in the prevention of RT-PCR-confirmed influenza in participants aged 6 months through 5 years (relative vaccine efficacy −0·67, 95% CI −19·81 to 15·41; table 2). Although there were differences in attack rate between the countries, there was no difference in relative vaccine efficacy by country (data not shown). Similar results were observed for culture-confirmed influenza regardless of antigenic match (table 2; appendix). Among participants with first-occurrence RT-PCR-confirmed influenza, 34 (0·8%) of 4521 participants from the aIIV4 group and 32 (0·7%) of 4494 from the comparator group had moderate-to-severe influenza, and the relative vaccine efficacy was −4·78 (95% CI −70·23 to 35·51; appendix). Using the antigenic typing assays, more than half of A/H3N2 strains from season two were determined to be antigenically distinct from egg-propagated A/Texas/50/2012, the H3N2 vaccine strain.

In participants aged 6 through 23 months, the relative vaccine efficacy of aIIV4 against RT-PCR-confirmed influenza was significantly more than the comparator vaccine (31·37, 95% CI 3·14–51·38; table 3). Relative vaccine efficacy for aIIV4 was higher in those aged 6 through 23-months against culture-confirmed influenza from any strain and culture-confirmed matched cases than for the comparator, although differences were not significant (table 3). The exploratory Breslow-Day test and a test for interaction for heterogeneity across age group strata suggested that age was an effect modifier (p=0·023). The appendix includes

	aIIV4	Comparator†	Relative vaccine efficacy‡ (95% CI)
Vaccine-naive participants			
Number of participants	3559	3535	..
Any strain‡, ≥7 days after first and up to second vaccination	16	35	54·66 (18·08–74·91)
≥14 days after first and up to second vaccination	8	27	70·56 (35·19–86·62)
All participants			
Number of participants	5286	5208	..
Any strain,§ ≥7 days and ≤21 days after last vaccination	4	15	NA¶

aIIV4=MF59-adjuvanted, quadrivalent, subunit inactivated influenza vaccine. NA=not applicable. IIV3=trivalent inactivated influenza vaccine. IIV4=quadrivalent inactivated influenza vaccine. *Includes full analysis set. †The comparator was non-adjuvanted IIV3 in season one and non-adjuvanted IIV4 in season two. ‡Result is based on the Cox proportional hazards model for time until onset of the first confirmed influenza, adjusting for vaccine naivety, risk factor, season, dose group, and country as random effect. §Any strain regardless of antigenic match. ¶Per the statistical analysis plan, relative vaccine efficacy was not calculated if number of cases was less than 20.

Table 4: **First-occurrence RT-PCR-confirmed influenza and relative vaccine efficacy in participants aged 6 months through 5 years during specified early efficacy time periods***

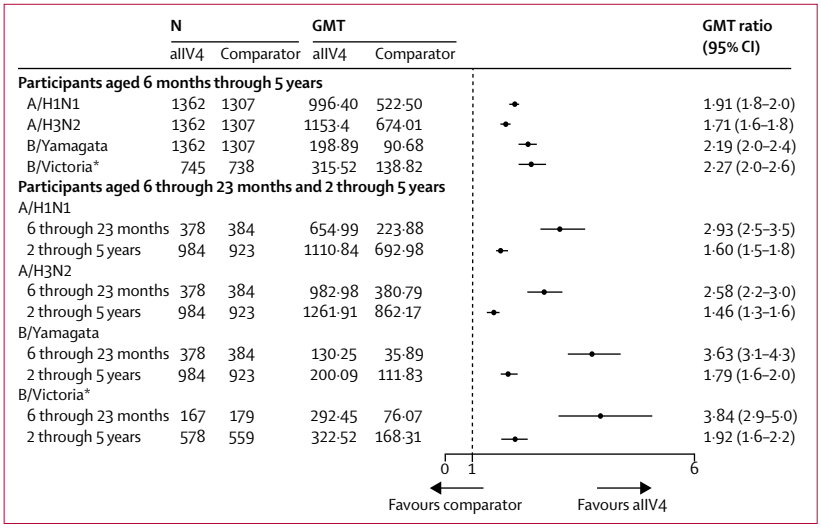

Figure 2: **GMTs and vaccine group ratios against homologous vaccine strains 21 days after last vaccination for the immunogenicity full analysis set**

Error bars are 95% CIs. Analysis of the immunogenicity full analysis set. Superiority is defined as a lower boundary of the 95% CI greater than one. The comparator was non-adjuvanted IIV3 in season one and non-adjuvanted IIV4 in season two. aIIV4=MF59-adjuvanted, quadrivalent, subunit inactivated influenza vaccine. IIV3=trivalent inactivated influenza vaccine. IIV4=quadrivalent inactivated influenza vaccine. GMT=geometric mean titre. *B/Victoria results from only season two are presented for both vaccine groups and used in the vaccine comparison analysis.

data for the relative vaccine efficacy of aIIV4 for the two dose groups (0·25 mL and 0·5 mL), and shows that for both subgroups the relative vaccine efficacy of aIIV4 is similar to the comparator vaccine. Kaplan-Meier curves for time until first-occurrence RT-PCR-confirmed influenza in participants aged 6 months through 5 years and those aged 6 through 23 months are shown in the appendix.

In vaccine-naive participants aged 6 months through 5 years, the relative vaccine efficacy was 5·80 (95% CI –16·91 to 24·10). In an assessment of early efficacy in these participants, the relative vaccine efficacy was 54·66 (95% CI 18·08–74·91) between 7 days after the first dose and the time of the second dose, and was 70·56 (35·19–86·62) 14 days or more after the first dose until the second vaccination (table 4). Additionally, regardless of previous vaccination status, fewer participants who received aIIV4 were diagnosed with influenza between 7 days and 21 days after the last vaccination compared with the comparator (four *vs* 15; table 4).

In the overall study population, aIIV4 elicited a robust post-vaccination immune response. As shown in figure 2, the lower limit of the confidence interval of GMT ratios for all homologous strains 21 days after last vaccination was 1·3 or more, demonstrating superiority, and the highest GMT ratios against all homologous strains were observed in participants aged 6 through 23 months compared with other age groups. In vaccine-naive participants, the GMTs for homologous A/H1N1 and A/H3N2 after a single dose of aIIV4 were similar to the GMTs after two doses of the comparator vaccine (appendix). Additionally, the GMT ratios after a single dose ranged from 2·00 (95% CI 1·8–2·3) for B/Yamagata to 2·80 (2·5–3·2) for A/H1N1 (appendix).

At 180 days after the last vaccination, the GMTs were higher in the aIIV4 group than in the comparator vaccine group for all homologous strains in all age groups. GMT ratios ranged from 1·57 (95% CI 1·4–1·7) for A/H3N2 to 1·86 (1·7–2·0) for A/H1N1 and B/Yamagata.

aIIV4 was superior to comparator vaccine in seroconversion for all homologous strains 21 days after last vaccination in all participants (appendix). The Center for Biologics Evaluation and Research criteria for seroconversion were met by all participants receiving aIIV4 and comparator across the overall groups as well as age and dose subgroups (appendix).

Consistent with greater relative vaccine efficacy in participants aged 6 through 23 months, 40·3% more of participants receiving aIIV4 than those receiving comparator achieved a haemagglutination inhibition titre of 1:629 or more against the homologous A/H3N2 strain; whereas the percentage difference was 13·0% for those aged 2 through 5 years. The same trends in differences in percentages of participants reaching the specified threshold titres were observed for the other vaccine strains (figure 3).

The GMTs for the heterologous strains for the A/H3N2 and the B strains were higher for aIIV4 than for the comparator vaccine group 21 days after the last vaccination (appendix). For the heterologous A/H3N2 and B strains, the percentages of participants with seroconversion and haemagglutination inhibition titre of 1:40 or more were higher in the aIIV4 group than in the comparator vaccine group (appendix). Most importantly, the immunogenicity of aIIV4 was significantly greater against the heterologous (egg-propagated) A/Hong Kong/4801/2014 (H3N2) strain than the comparator. Similar to the other efficacy and immunogenicity analyses, the highest GMT ratios were observed in

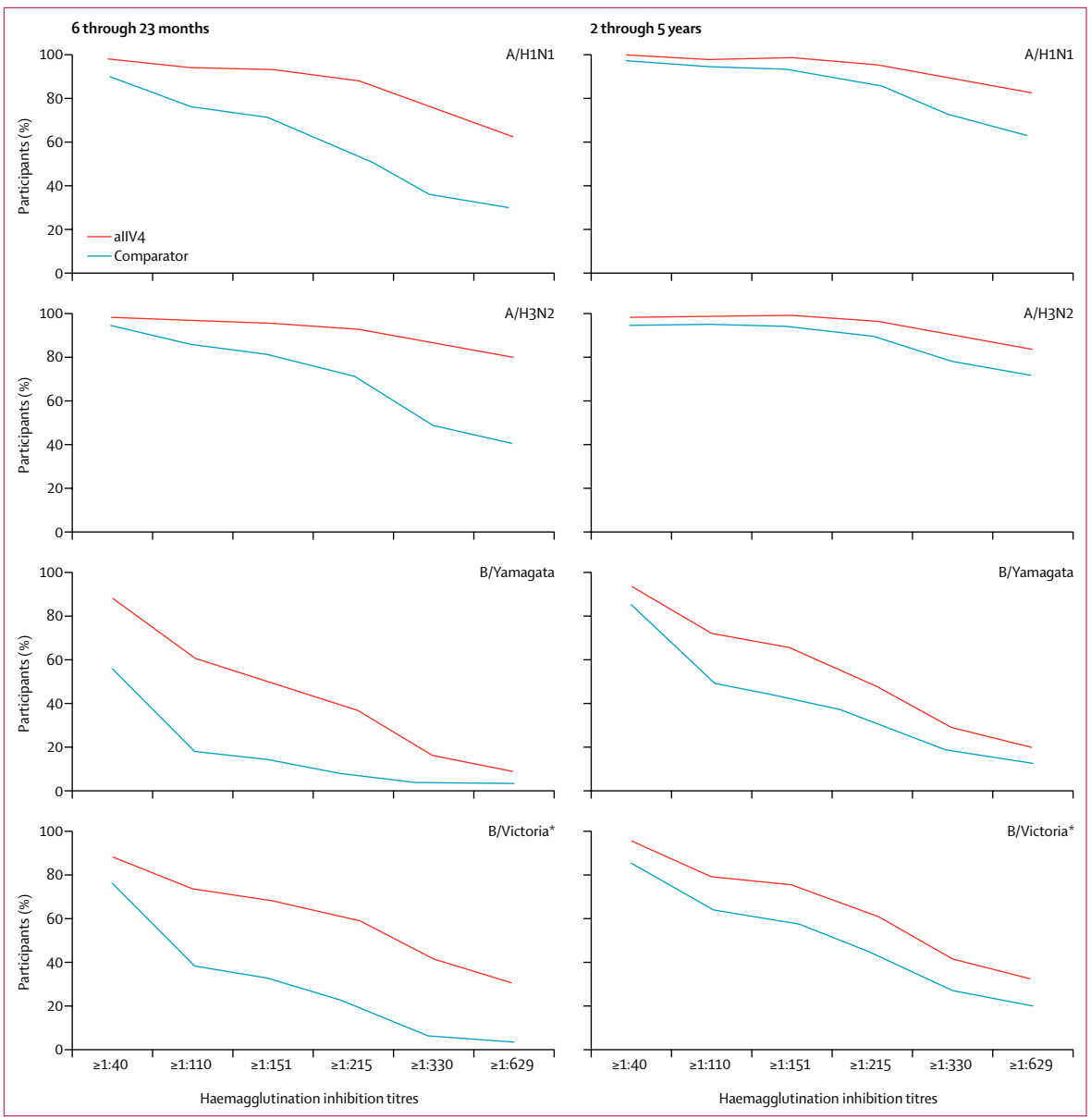

***Figure 3*: Proportion of participants aged 6 through 23 months and 2 through 5 years achieving threshold haemagglutination inhibition titres at either day 22 for non-naive participants or day 50 for vaccine-naive participants (data are combined)**

The comparator was non-adjuvanted IIV3 in season one and non-adjuvanted IIV4 in season two. aIIV4=MF59-adjuvanted, quadrivalent, subunit inactivated influenza vaccine. IIV3=trivalent inactivated influenza vaccine. IIV4=quadrivalent inactivated influenza vaccine. *B/Victoria results from only season two are presented for both vaccine groups.

participants aged 6 through 23 months for all influenza strains except heterologous A/H1N1 (appendix). The heterologous A/H1N1 strain, from the period before the 2009 pandemic, showed low responses with both aIIV4 and the comparator vaccine.

In the overall population, the incidence of any solicited adverse event was higher in participants who received aIIV4 than in those who received the comparator vaccine (3748 [73%] of 5138 *vs* 3242 [64%] of 5056; appendix). Most solicited adverse events were mild to moderate in severity, and most reported fevers were less than 39°C. The appendix shows the numbers and proportions of participants with solicited and unsolicited adverse events. Despite absolute differences in the proportions of participants reporting adverse events in younger and older children, the relative distribution between the vaccine groups was consistent across the age groups. A greater proportion of participants in the aIIV4 group than in the non-adjuvanted vaccine group reported tenderness, fever

(≥38°C), or use of analgesic or antipyretic agents (appendix).

The proportion of participants who reported any unsolicited adverse event was similar for the aIIV4 and the comparator vaccine groups (3576 [68%] of 5243 *vs* 3543 [69%] of 5161; appendix). A similar pattern was also observed for possibly related adverse events, any unsolicited serious adverse events, adverse events of special interest, unsolicited adverse events leading to death, unsolicited adverse events leading to withdrawal from the study or vaccine, admissions to hospital, and new-onset chronic disease for all age groups. The most commonly reported unsolicited adverse events in either vaccine group were influenza-like illness and upper respiratory tract infection (appendix). Nine adverse events of special interest occurred in the study: five in participants who received aIIV4 and four in those who received the comparator; however, none were considered to be related to the vaccine by the study investigators.

Discussion

Our study, in which aIIV4 and the comparator were administered at a 0·25 mL dose in children aged less than 3 years, showed that aIIV4 had significantly greater efficacy in preventing influenza versus a comparator without an adjuvant in children aged 6 through 23 months, and similar efficacy in older children, with age emerging as a significant effect modifier. The immune response to vaccination with aIIV4 was superior across all ages, with significantly greater GMT ratios and seroconversion against both homologous and heterologous strains relative to the comparator. The difference in immune response was greatest in children younger than 2 years compared with older children. After vaccination with aIIV4, more children achieved a haemagglutination inhibition titre threshold of 1:629 or more, which was previously associated with 90% protection against influenza in children,[19] and this enhancement was most apparent in children aged 6 through 23 months. These age-related differences in the immune response to vaccination provide a biologically plausible rationale for the increase in efficacy seen in younger children.

aIIV4 had greater relative vaccine efficacy after a single dose but before the second vaccine dose in children considered vaccine-naive to previous vaccination, a finding supported by higher GMTs seen after the first dose of aIIV4 versus comparator. This result has implications for children who have not been previously vaccinated or exposed to influenza, especially when vaccines are administered late in the influenza season or the season starts early. In routine paediatric practice, many vaccine-naive children receive only a single vaccination.[21]

Overall, the safety profiles of the aIIV4 and comparator vaccine were similar except for a greater incidence of solicited adverse events reported by participants who received aIIV4, most of which were mild to moderate in severity and of short duration. A greater proportion of children in the aIIV4 group reported tenderness (most cases were assessed as mild to moderate in severity) and fever (most <39°C). The increased reports of solicited adverse events in aIIV4 are consistent with the study of aIIV3 in this age group.[20] The proportion of participants who reported unsolicited adverse events, including serious adverse events, new-onset chronic disease, and adverse events of special interest, were similar between the two vaccine groups.

It is important to consider why the results of this study are different from an earlier study that showed greater vaccine efficacy after vaccination with aIIV3 in children aged 6 months through 5 years.[20] The ages of children enrolled in the two studies were similar; however, all children in the earlier trial were considered naive with regard to previous influenza vaccination, whereas children who had been previously vaccinated were included in the current study. Consequently, all participants in the earlier trial received two doses of aIIV3 or IIV3, but those in the current study received either one or two doses of aIIV4 or either IIV3 or IIV4. In the current study, nearly half of the children had baseline haemagglutination inhibition titres of 1:40 or more against either A/H1N1 or A/H3N2, which was more than twice the percentage in the previous aIIV3 study. In the earlier study, the proportion of children with baseline haemagglutination inhibition titres of 1:40 or more in those aged 6 months through 5 years against all three vaccine strains was similar to that of children aged 6 through 23 months in the current study. The differences in study population might explain the greater GMT ratios favouring aIIV3 seen in the earlier study compared with the GMT ratios favouring aIIV4 in the current study. In this trial, age was more closely associated with immunological naivety than the historical assessment of vaccine naivety in the overall study population and might explain why additional clinical benefit was shown in the youngest age group but not in naive children of all ages. MF59 increases the magnitude of immune response to vaccination with influenza, and this effect was most evident in children at any age with low baseline haemagglutination inhibition titres to the strain of virus in the vaccine. The different findings in the two studies might therefore be explained by differences in previous exposure to influenza and are consistent with the proposed mechanism of action of MF59 in infants, which skews the immune system towards a more mature immune response.[22,23]

Another explanation for the differences in the results of the two studies is the epidemiology of influenza during the seasons when the trials were done. Most influenza cases in the earlier trial were caused by a virus strain that was antigenically similar to the strain

used in the vaccine. The relative efficacy of aIIV3 compared with IIV3 (Influsplit) was 75% (95% CI 55–87) in children aged 6 months through 5 years and 73% (29–90) in those aged 6 through 23 months.[20] By contrast, the majority of influenza cases in the current trial were reported during the 2014–15 season, when the predominant circulating virus was a strain of A/H3N2 that was antigenically distinct from the vaccine strain. In the setting of substantial antigenic drift, which occurred during the current study, we hypothesise that MF59 was particularly effective at enhancing the vaccine response in the youngest children because they were immunologically naive to influenza. This apparent cross-protection in our current trial is supported by evidence of epitope spreading shown in earlier studies of an MF59-adjuvanted H5N1 vaccine.[24]

A limitation to the current study is that most children were enrolled in one season, and the overwhelming majority of influenza cases included in the final analysis was derived from this single season, in which the most prominent circulating strain (A/H3N2) was antigenically distinct from the vaccine strain. Despite this disadvantage, aIIV4 was still more effective than the comparator in preventing influenza in the youngest age group. Although this subgroup was a prespecified objective in the trial, multiplicity adjustment for type I error was not accounted for in the statistical design. However, tests for interaction (Breslow-Day test and a proportional hazards regression that used age as an interaction term) returned significant p values; therefore, age was a modifier of the vaccine effect, and analyses by age subgroups are a necessary consideration.

In conclusion, this study showed that aIIV4 provided significantly superior efficacy and immunogenicity than a non-adjuvanted influenza vaccine in a prespecified age group of children aged 6 through 23 months, and superior immunogenicity and similar safety profile in all children aged 6 months through 5 years. The broader and more persistent immune response to aIIV4 versus comparator in the youngest children and those without previous influenza vaccination are consistent with past studies of MF59-adjuvanted seasonal and pandemic influenza vaccines in young children.[12,20] Furthermore, the differences between the current study and the earlier study of aIIV3 offer important insight into the mechanism of action of MF59 in the context of the developing immune system. Despite the poor effectiveness of licensed vaccines in 2014–15 attributed to substantial antigenic drift, aIIV4 had greater efficacy in the youngest, most vulnerable population during this period.

Contributors

TV and EH conceived the study. TV, MdB, and EH participated in the study design. BL, MdB, and EH participated in the statistical design. TV, JK, GDG, BL, MER, LI, MdB, and EH conducted the study, including acquisition, analysis, or interpretation of data. TV, BL, LI, MdB, JO, and EH did the statistical analysis or interpretation of the data, or both. TV drafted the manuscript. All authors critically reviewed, edited, and approved the manuscript and made the decision to submit for publication. All authors assume responsibility for the accuracy and completeness of the data and for the fidelity of the study to the protocol.

Declaration of Interests

TV has received lecture fees and travel support from Seqirus, and his institution has received research grants from Seqirus. JK's institution received financial support from Seqirus during the conduct of the trial. GDG has received research grants and honoraria from Seqirus. BL, MER, and LI are employees of Seqirus. MdB, JO, and EH are employees of Seqirus Netherlands BV.

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