**Prevention of vaccine-matched and mismatched influenza in children 6−35 months of age: a multinational randomized trial across five influenza seasons**

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# Research in context

## Evidence before this study

Influenza is associated with substantial disease burden in young children. Indeed, the World Health Organization recognizes that children <5 years of age, and particularly those <2 years, are a priority group to receive annual influenza vaccination.

Two antigenically distinct lineages of influenza B (Yamagata and Victoria) circulate worldwide. However, traditional vaccination strategies use a trivalent influenza vaccine containing two influenza A strains (H1N1 and H3N2) but only one B strain, meaning that they cannot provide adequate protection against influenza B viruses from both lineages. Quadrivalent influenza vaccines containing both B/Yamagata and B/Victoria lineages may offer broader protection and are increasingly used in vaccination programmes. The influenza virus strains included in the seasonal influenza vaccine are updated each year, but antigenic drift of the virus can result in a mismatch between the strains contained in the vaccine and the predominant circulating strains. This mismatch can cause substantially reduced vaccine effectiveness.

We searched PubMed from January 2000 to July 2017 for clinical trials reporting vaccine efficacy of inactivated influenza vaccine in children <5 years. We identified few studies, and those we found reported variable estimates of vaccine efficacy. A Cochrane systematic review has also concluded that little evidence is available to support influenza vaccine administration in children under 2 years of age. Given the importance of preventing influenza in young children, it is imperative to collect high quality data on vaccine prevention of illness in this age group.

## Added value of this study

Over five influenza seasons, we evaluated vaccine efficacy of an inactivated quadrivalent influenza vaccine in 12,000 children 6−35 months of age in 13 temperate and subtropical countries. Vaccine efficacy was 63% against laboratory-confirmed influenza disease of moderate-to-severe intensity, an endpoint that reflects the most important clinical outcomes of influenza such as lower respiratory infection, ear infection, and high fever. Vaccine efficacy against laboratory-confirmed influenza disease of any intensity was 50%. These levels of vaccine efficacy were seen despite substantial mismatch between the strains contained in the vaccine and those circulating in the community.

## Implications of all the available evidence

Routine vaccination of young children against influenza is recommended in many countries despite the lack of high quality evidence for the efficacy of inactivated influenza vaccine in this age group. This study provides data from different regions over multiple seasons and allows estimation of a typical vaccine efficacy value against influenza of any intensity and against moderate-to-severe disease, the most clinically important endpoint associated with greatest burden. The high vaccine efficacy estimate obtained despite considerable mismatch to circulating strains provides reassuring evidence to support universal vaccination of all children from 6 months of age.

# Abstract

**Background**: We evaluated efficacy of an inactivated quadrivalent influenza vaccine (IIV4) in children 6–35 months of age.

**Methods**: This phase III, observer-blind, multinational trial was conducted in five independent cohorts across five influenza seasons (2011–2014) in Europe, Central America and Asia. We randomized healthy children 1:1 to IIV4 or control vaccines. Primary endpoints were moderate-to-severe influenza or all influenza (regardless of disease severity) confirmed by reverse transcription polymerase chain reaction (RT-PCR) on nasal swabs. Cultured isolates were further characterized as antigenically matched/mismatched to vaccine strains. Efficacy was evaluated in the per-protocol cohort (PP; N=11,404) and total vaccinated cohort (TVC; N=12,018) (time-to-event analysis).

**Findings**: At least one RT-PCR-confirmed influenza case occurred in 356 (5∙9%) and 693 (11∙5%) children in the IIV4 and control groups, respectively. Influenza A/H3N2 and B/Yamagata were predominant (50∙3% and 30∙0%). Overall, 63∙6% of antigenically characterized isolates were vaccine-mismatched (A/H1N1: 15∙2%; A/H3N2: 97∙4%; B/Victoria: 85∙7%; B/Yamagata: 33∙4%). Vaccine efficacy (97∙5% confidence interval) against moderate-to-severe influenza and all influenza was, respectively, 63∙2% (51∙8–72∙3) and 49∙8% (41∙8–56∙8) (PP), and 63∙9% (52∙8–72∙8) and 49∙5% (41∙6–56∙5) (TVC). Compared with control, IIV4 reduced general practitioner/paediatrician visits (47%), antibiotic use (50%), emergency room visits (79%), and parental work or day-care absence (54% and 55%) associated with influenza. There were no clinically meaningful safety differences between IIV4 and control.

**Interpretation**: IIV4 prevented influenza A and B in children 6–35 months of age, despite high vaccine-mismatch levels. Vaccine efficacy was highest against moderate-to-severe disease, the most clinically important endpoint associated with greatest burden.

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# Introduction

Influenza is associated with substantial disease burden in children. The youngest children are at particular risk of severe disease, and influenza is an important cause of medical visits and hospitalization in this population.1-3 Children also play a substantial role in spreading influenza in the community, including the elderly.4-7 The World Health Organization (WHO) recognizes that children <5 years of age, especially those <2 years, are a priority for vaccination.8

Despite the importance of vaccinating children <5 years of age, few studies evaluating vaccine prevention of influenza have been reported in this age group. Evidence in children <2 years of age is particularly scarce.9 Estimates of the efficacy of inactivated influenza vaccine (IIV) among children vary. In a study in children 6–24 months of age, vaccine efficacy was 66% in the first study season, and -7% in the following season, due to an unexpectedly low influenza attack rate.10 A study in children 18–72 months of age showed vaccine efficacy of 51%, despite substantial mismatch of the vaccine strains to the circulating strains.11 In an age-stratified study, efficacy of 48% against vaccine-matched disease (mainly A/H3N2) was seen in children 36–71 months of age, but the 41% efficacy seen in children 6–35 months of age was not statistically significant.12 Vaccine efficacy of 45% was seen in children 24−60 months of age attending day care centres.13 A recent study of children 6−23 months of age in Bangladesh found overall vaccine efficacy of 31% across four vaccination seasons.14 In contrast, a study in children 6−24 months of age failed to show any benefit of IIV against influenza A infection compared with control.15

Both influenza A and influenza B viruses are subject to considerable antigenic drift. To ensure that the influenza strains used in the seasonal vaccine are closely related to the predominant circulating viruses, the WHO updates the strains used in the vaccine annually. Despite best efforts, however, antigenic drift can result in the vaccine strains not being closely related to the circulating strains. This is termed vaccine mismatch, and can cause substantially reduced vaccine effectiveness.8

Two antigenically distinct lineages of influenza B (Yamagata and Victoria) circulate worldwide. However, traditional vaccination strategies use a trivalent influenza vaccine containing two influenza A strains (H1N1 and H3N2) but only one B strain, meaning that they cannot provide adequate protection against influenza B viruses from both lineages when co-circulating. Quadrivalent influenza vaccines containing both B/Yamagata and B/Victoria lineages potentially offer broader protection and are increasingly used in vaccination programmes.

High quality data on influenza vaccine efficacy in young children are lacking and their acquisition is imperative. In the present study, we evaluated the efficacy and impact on healthcare utilization of an inactivated quadrivalent influenza vaccine (IIV4) (GSK) in children 6–35 months of age, in an endeavour to fill an evidence gap in this area. We made a particular effort to evaluate efficacy in relation to whether the vaccine was matched or mismatched to circulating influenza strains in the five influenza seasons covered by the study.

# Methods

This was a phase III, randomized, controlled, observer-blind, multinational trial in children 6–35 months of age, conducted across five influenza seasons in Europe, Central America and Asia. Its objective was to assess the efficacy of IIV4 in the prevention of laboratory-confirmed influenza. It was approved by independent ethics committees or institutional review boards, conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines, and regulatory requirements of participating countries. Parents or legally acceptable representatives provided written informed consent. The trial was sponsored by GlaxoSmithKline Biologicals SA (NCT01439360).

## Study design, randomization, and masking

The trial was conducted in 13 temperate and subtropical countries between October 2011 and December 2014. Children were recruited in five independent cohorts, each in a different influenza season (Figure 1). The study lasted for 6 to 8 months for each participant. This period included vaccination, surveillance for influenza, and safety monitoring (appendix; Figure S1).

Healthy children, as determined by clinical examination and medical history, were eligible regardless of influenza vaccination in previous seasons. The method of recruitment to the study varied between countries and was done according to local practice. Children were enrolled from a variety of clinics (e.g. private practice, academic centres, public hospitals). The protocol was applied uniformly in all countries and study centres. Exclusion criteria are shown in the appendix. Children were randomized 1:1 to IIV4 or control vaccine. The randomization sequence was generated at GSK using MATerial Excellence, a program developed by GSK for use in SAS (Cary, NC, US). The randomization algorithm used a minimization process accounting for country, centre, influenza vaccine priming status, age strata of 6–11, 12–23 and 24–35 months, attendance at day care or school, history of recurrent acute otitis media (AOM), and history of vaccination with the conjugated pneumococcal vaccine. Minimization factors had an equal weight in the algorithm. The algorithm ensured that approximately 11%, 33% and 56% of children were 6–11, 12–23 and 24–35 months of age, respectively. Treatment allocation was performed at the investigator site using a central randomization system on internet (SBIR). Personnel administering the vaccines did not participate in endpoint assessments.

## Vaccines

The IIV4 (*Fluarix Quadrivalent*, GSK, Dresden, Germany) contained 15 µg haemagglutinin antigen per strain per 0∙5 mL dose; strain composition followed WHO recommendations (Figure 1). It was administered intramuscularly. Vaccine-primed children received a single 0∙5 mL dose on Day 0; vaccine-unprimed children received two doses, 0∙5 mL each on Days 0 and 28 (appendix; Figure S1). Children were considered primed for influenza vaccination if they had previously received at least two doses of seasonal influenza vaccine separated by at least 28 days. Currently available influenza vaccines are not licensed in children <6 months of age, and children who had received an influenza vaccine within 6 months of the study start were excluded. Therefore, all children under 12 months of age were considered vaccine-unprimed.

Three possible control vaccines were used: pneumococcal polysaccharide conjugate vaccine (PCV), hepatitis A vaccine, or varicella vaccine. Control vaccine allocation was based on age and vaccine-priming status. The control vaccines provided benefit to the study participants and were approved in the study countries. Further details are provided in the appendix.

## Surveillance for illness and recording of symptoms

Surveillance for influenza-like episodes (ILE) began in each individual child from 14 days after they received their final vaccination until the end of the influenza season. Most children received two doses of vaccine, so surveillance was initiated approximately 6 weeks after they entered the study (Figure 1; appendix, Figure S1). The ILE surveillance period encompassed the peak of the influenza season in each country, based on available epidemiological data for the different participating countries or regions. These data were obtained from influenza surveillance activities conducted by public health bodies.16,17

ILE included influenza-like illness (ILI), physician-diagnosed acute otitis media (AOM) and lower respiratory infection (LRI). ILI was defined as a temperature ≥38∙0°C with at least one of cough, runny nose, nasal congestion or breathing difficulty. LRI was defined as physician-diagnosed pneumonia, lower respiratory tract infection, bronchiolitis, bronchitis, or croup (laryngotracheitis).

Parents were instructed to contact the study centre within 24 hours if the child developed an ILE. Study staff contacted parents weekly to check whether their child had experienced an ILE or medically attended visit since the last reminder. Parents recorded symptoms and any medication used to treat the ILE daily during the ILE episode using an internet-based system or a paper booklet (appendix). Study staff made a follow-up contact at the end of the episode to record the outcome of the episode, use of healthcare resource, use of supplementary oxygen therapy for >8 hours, and number of missed day-care days for the child and missed days of paid work for the parents if applicable.

## Virus detection in nasal swabs

A nasal swab was collected for each ILE reported within 7 days of onset, preferably within 24 hours of the episode being reported. No swabs were taken if the episode was not reported in time for a visit within 7 days of onset. All testing of nasal swabs was done at a central laboratory for each assay (appendix). Influenza A or B virus was confirmed by reverse transcription polymerase chain reaction (RT-PCR) (appendix; Figure S2). Samples confirmed as influenza A were further characterized by RT-PCR as A/H1N1 or A/H3N2 subtype. Samples confirmed as influenza B were also characterized by RT-PCR and additionally underwent a DNA sequencing step to classify the sample as B/Victoria or B/Yamagata lineage (appendix).

RT-PCR-positive samples underwent two subsequent tests: (1) viral culture with immunostaining; (2) antigenic characterization (appendix; Figure S2). To identify PCR-positive samples as either matched or mismatched compared with the vaccine strain, samples were amplified by serial passages and then antigenically characterized by a standard haemagglutination inhibition (HI) assay for A/H1N1 and B lineage. Following the emergence during the study of A/H3N2 strains with an impaired haemagglutination phenotype, antigenic characterization for A/H3N2 was performed by microneutralization (MN) assay.18

For each influenza A subtype or B lineage, the results were reported as either matched or mismatched to the vaccine strain. For samples that could not be evaluated for technical reasons (e.g. insufficient virus titre), the outcome was categorized as an indeterminate result. A vaccine-matched strain was defined as ≤4-fold difference in HI or MN titre relative to a reference serum and vaccine antigen; similarly, a mismatch was defined as a >4-fold difference in HI or MN titre relative to reference serum and vaccine antigen (appendix).

## Study endpoints

### Efficacy

The two primary endpoints were first occurrence of RT-PCR-confirmed moderate-to-severe influenza or all influenza (regardless of disease severity) associated with any seasonal influenza strain. Secondary endpoints were analyzed sequentially with pre-defined success criteria (appendix, Figure S3): LRI with RT-PCR-confirmed influenza; culture-confirmed influenza associated with antigenically-matching strains (1. moderate-to-severe; 2. all influenza); culture-confirmed influenza associated with any seasonal strain (1. moderate-to-severe; 2. all influenza); AOM with RT-PCR-confirmed influenza; RT-PCR-confirmed severe influenza. The order of the sequential analysis was set according to clinical relevance and expected frequency of the outcome. Exploratory efficacy endpoints were influenza associated with individual A subtypes or B lineages and according to age. Exploratory analyses of vaccine impact on daily activities/healthcare utilization (including antibiotic use) were also conducted.

Moderate-to-severe influenza was defined as any of: fever >39°C; physician-diagnosed AOM; physician-diagnosed LRI; physician-diagnosed serious extra-pulmonary complication (e.g. myositis, encephalitis, seizure, myocarditis/pericarditis or other serious medical condition); hospitalization in the intensive care unit (ICU); or supplemental oxygen for >8 hours. Any of the latter three criteria defined severe influenza.

### Immunogenicity and safety

HI antibody titre against each vaccine strain was measured using standard methods19 before vaccination and 28 days after last vaccination in a sub-cohort of children (appendix, Figure S1). Safety endpoints were evaluated in the TVC and included: solicited injection site and systemic symptoms (7-day post-vaccination period); spontaneously reported (unsolicited) adverse events (28-day post-vaccination period); medically-attended adverse events, serious adverse events and potential immune mediated disease (entire study period).

## Statistical analysis

Vaccine efficacy (VE) was calculated with a time-to-event model based on a Cox proportional hazard regression model (appendix). Vaccine efficacy with two-sided 97∙5% confidence intervals (CI) was calculated for both primary endpoints; efficacy was demonstrated if the lower limit of the CI was above 25% for moderate-to-severe influenza or above 15% for all influenza. If both primary endpoints were met, secondary efficacy endpoints were evaluated sequentially according to pre-specified success criteria, with 95% CIs (appendix, Figure S3).

Efficacy analyses of the primary and secondary endpoints were conducted in the per-protocol (PP) efficacy time-to-event cohort (primary analysis). Secondary analyses of the primary endpoints and analyses of the exploratory endpoints were conducted in the total vaccinated cohort (TVC). In the time-to-event model, data were censored or eliminated for certain protocol violations. In the PP time-to-event analysis, children were eliminated if they did not meet inclusion and exclusion criteria for the study, were not randomized into the correct group, did not receive the study vaccine in accordance with the protocol (or details were unknown), or withdrew from the study before the start of the influenza activity period.

In addition, children were censored from the PP time-to-event analysis for certain protocol violations only after the violation occurred (i.e. a child who experienced his or her first efficacy outcome event before the protocol violation took place was included in the efficacy analysis and disease-free duration was calculated accordingly). Children were censored for the following protocol violations: randomization code was broken by the investigator or sponsor, they received a concomitant vaccine or medication forbidden by the protocol, they had an underlying medical condition forbidden by the protocol, or they experienced a concomitant infection that might have influenced the immune response. The same censoring criteria were applied in the TVC time-to-event analysis.

Vaccine impact on daily activities/healthcare utilization and safety were evaluated in the TVC, which included all vaccinated children with available data. Immunogenicity was evaluated in the PP immunogenicity sub-cohort, which included children in the immunogenicity sub-cohort who complied with the protocol and had samples available. The immunogenicity sub-cohort was a convenience sample consisting of approximately 400 children from the IIV4 group and 200 children from the control group in the first two seasonal cohorts, approximately 150 children in the third seasonal cohort (approximately equal numbers from both vaccine groups), and up to 50 children from each participating country in the fourth and fifth seasonal cohorts (approximately equal numbers from both vaccine groups).

The power calculation is described in the appendix. SAS 9∙2 was used for statistical analysis.

## Role of the funding source

The trial was funded by GlaxoSmithKine Biologicals SA, who designed the study in collaboration with investigators and coordinated collection, analysis, and interpretation of data. All authors participated in the design or implementation or analysis, and interpretation of the study, and the development of this manuscript. All authors had full access to the data and gave final approval before submission.

# Results

The TVC included 12,018 children (IIV4: 6006; control: 6012) (appendix, Figure S4). Median age was 22 months, with approximately equal numbers of boys and girls (appendix, Table S1). Most children were of South East Asian, White European, Central/South Asian or Hispanic ancestry, and almost all (99∙2%) were vaccine-unprimed (appendix, Table S1). Mean duration of ILE surveillance was approximately 4 months in both groups, commensurate with expected influenza activity in the study regions.

**Occurrence of ILE and RT-PCR-confirmed influenza**

In the TVC, 2747 children in the IIV4 group experienced 4153 ILEs during the surveillance period; corresponding values in the control group were 2955 children with 4411 ILEs. Swabs were obtained within 7 days of ILE onset from 96∙4% and 96∙8% of ILEs in the IIV4 and control groups, respectively. A total of 356 children (5∙9%) in the IIV4 group and 693 (11∙5%) children in the control group had at least one RT-PCR-confirmed influenza infection in the TVC. Nine and 19 children in the IIV4 and control groups, respectively, had two infections. Strain distribution was A/H1N1: 13∙1%, A/H3N2: 50∙3%, B/Victoria: 6∙6%, and B/Yamagata: 30∙0%.

## Efficacy against influenza disease (time-to-event analysis)

In the time-to-event analysis, 353 (5∙9%) and 676 (11∙2%) children in the IIV4 and control groups, respectively, had RT-PCR-confirmed influenza in the TVC; corresponding values in the PP efficacy cohort were 344 (6∙0%) and 662 (11∙6%). Most antigenically characterized isolates were vaccine-mismatched (63∙6% in the TVC over the entire study; Figure 2).

The study met its two confirmatory primary objectives by demonstrating vaccine efficacy against RT-PCR-confirmed moderate-to-severe RT-PCR-confirmed influenza (63∙2% [97∙5% CI: 51∙8–72∙3]) and all RT-PCR-confirmed influenza (49∙8% [97∙5% CI: 41∙8–56∙8]) in the PP efficacy cohort (Table 1). Influenza of moderate-to-severe intensity accounted for 36∙6% of all influenza illness in the control group, but only 26∙2% in the IIV4 group (PP efficacy cohort). Efficacy was sustained throughout the surveillance period (appendix, Figure S5). We observed statistically significant vaccine efficacy against all sequentially evaluated secondary endpoints except for the last endpoint of severe influenza, of which only five cases were reported (Table 1).

Vaccine efficacy in the TVC was similar to the PP efficacy cohort (Table 1). The vaccine efficacy estimates in each seasonal cohort were all substantially positive, although observed values varied with the proportion of disease caused by vaccine-matching isolates (appendix, Figure S6). In a pre-specified exploratory analysis, we also observed vaccine efficacy against moderate-to-severe influenza associated with different individual influenza A subtypes and B lineages (Table 1). Efficacy was also seen against all influenza associated with both A subtypes and B/Yamagata but was lower for B/Victoria (Table 1). An exploratory analysis by age demonstrated vaccine efficacy in children 6–17 and 18–35 months of age (Table 1). There was a trend for higher efficacy in the 18–35 month age group, particularly against moderate-to-severe influenza.

## Impact on healthcare utilization and daily activities

The IIV4 reduced the impact of influenza on healthcare utilization and daily activities, approximately halving the risk of a general practitioner or paediatrician visit, antibiotic use, parental work absence, and missed day-care related to the current influenza illness (Table 2). Emergency room visits related to influenza were reduced by 79%.

## Immune response

All countries contributed to the immunogenicity sub-cohort; data for the vaccine strain subtype or lineage are pooled across seasonal cohorts. Compared with the TVC, children in the PP immunogenicity sub-cohort were of similar age, but the distribution of geographic ancestry was different (appendix, Table S2). Geometric mean titre of HI antibodies rose 9–17 fold against all vaccine strains after administration of IIV4 in the overall population (Figure 3). Post-vaccination, the seroconversion rate with IIV4 was 80%, 69%, 69% and 81% against A/H1N1, A/H3N2, B/Victoria and B/Yamagata, respectively (appendix, Table S3). Immunogenicity was higher in the 18–35 month age group (Figure 3).

## Safety

There were no clinically meaningful differences between IIV4 and control vaccine in safety outcomes (appendix, Table S4). Injection site symptoms (pain, redness and swelling) occurred in similar numbers in the IIV4 and control groups. Serious adverse events occurred in 3∙6% and 3∙3% of children in the IIV4 and control groups, respectively. Six (0∙1%) children experienced serious adverse events considered possibly related to vaccination in the IIV4 group (immune thrombocytopenic purpura, hypersensitivity, facial paralysis, febrile convulsion, febrile convulsion and apnoea [same child], and nephrotic syndrome), and two (0∙0%) in the control group (febrile convulsion, anoxic seizure). Five children in the IIV4 group experienced a potential immune mediated disease (appendix, Table S4); three cases (immune thrombocytopenic purpura, facial paralysis and nephrotic syndrome) were deemed possibly related to vaccination by the investigator. Review of the entirety of the cases by the sponsor identified in some cases confounding factors and in all cases insufficient evidence of a causal association. One child in the IIV4 group died as result of drowning. Two children in the control group also drowned, and a third died as a result of bronchitis and pneumonia complicated by pleural effusion. No deaths were considered related to vaccination.

# Discussion

To date, limited efficacy data for influenza vaccine in children <5 years of age are available.9-15 We conducted this study to comprehensively evaluate the value of IIV4 in children 6–35 months of age by collecting data from different regions over multiple seasons to provide a typical vaccine efficacy value.

We demonstrated vaccine efficacy of 49∙8% against all influenza and vaccine efficacy of 63∙2% against moderate-to-severe influenza. Higher vaccine efficacy estimates against moderate-to-severe illness were also observed for each influenza A subtype and B lineage, although CIs overlapped. These data are in line with those reported for a similar IIV4 in children 3–8 years of age, which also showed higher efficacy against moderate-to-severe illness.20 The moderate-to-severe endpoint is important because it reflects clinical outcomes of LRI, ear infection and fever that are most likely to result in medical consultations.21 Studies that fail to differentiate moderate-to-severe manifestations of influenza from mild illness cannot assess the vaccine’s ability to attenuate illness, and therefore undervalue its benefit to individuals.

The vaccine efficacy estimate of 49∙8% against all influenza included mild and moderate-to-severe illnesses. Because approximately 67% of RT-PCR-confirmed influenza in our study was classified as mild disease, this estimate primarily reflected efficacy against mild illness. The proportion of children with mild illness was higher in the IIV4 group than the control group, suggesting that breakthrough influenza illness is attenuated by the IIV4. Higher efficacy against moderate-to-severe illness is likely observed because it reflects both prevention of illness and attenuation of illness to a mild level of severity, whereas efficacy against all influenza largely reflects the additional prevention of mild illness. Although moderate-to-severe influenza has the greatest impact on individuals, it is less common than mild disease, and thus society is proportionally more burdened by mild disease. Prevention of all influenza, including mild illness, is therefore important at a population level by decreasing transmission. Children play a major role in spreading influenza, and immunizing children can benefit the whole population. In a cluster randomized trial among isolated Hutterite communities in Canada, communities in which children received influenza vaccination experienced lower levels of confirmed influenza illness even among non-vaccinated individuals compared with communities in which children were not vaccinated.4 Non-randomized community comparisons and household contact studies have shown similar results.5,6

We also demonstrated that IIV4 prevents influenza in infants 6–17 months of age, with efficacy of 48∙8% against moderate-to-severe influenza, although caution must be applied to this conclusion about a sub-group of the study population. Previously, a systematic review concluded that little evidence is available to support influenza vaccine administration in children under 2 years of age.9 Demonstration of vaccine efficacy in this group is important, as young children are at high risk of severe influenza outcomes.2,3 Interestingly, our study found the incidence of influenza to be approximately 50% higher in children 18–35 months of age than in children 6–17 months of age. The most plausible explanation for our finding is that older children mix more with other children and therefore are more exposed to the influenza virus. Other studies have demonstrated a higher incidence of influenza-related outpatient presentation in younger children.1,2

Notably, substantial vaccine efficacy was observed despite two-thirds of isolates associated with influenza being vaccine-mismatched. In fact, almost all A/H3N2 isolates were vaccine-mismatched, accounting for half of all isolates. A US analysis reported a mismatch, defined as at least 40% of characterized strains not matched to the vaccine strain, in four and six of the past 15 seasons for influenza A/H3N2 and B, respectively.22 For the A/H3N2- and B-mismatched seasons, previous estimates of population-level vaccine effectiveness range between 10–37% and 21–47%, respectively, compared with 41–60% for matched seasons.23 The efficacy observed in our study, despite mismatch of essentially all A/H3N2 isolates, probably reflects the broad immune response normally achieved in vaccine-naïve children following vaccination, including against drifted subtypes. Most children in the study had not been previously vaccinated against influenza.

A substantial burden of healthcare utilization and impact on daily activities is associated with childhood influenza.1-3,21,24 Compared with control vaccines, the IIV4 reduced general medical visits, emergency room visits, and hospitalization associated with influenza illness by 47%, 79%, and 57%, respectively. The proportion of children with a medical visit reflects consultations requested by parents rather than study procedures because study staff were instructed to differentially record the two. However, a high proportion (>80%) of children with an influenza infection underwent a medical consultation, and we cannot rule out some confounding in this regard. Antibiotic use associated with influenza illness was reduced by 50%. This is an important finding because antibiotic use in childhood respiratory infection is associated with antibiotic resistance.25,26 In addition, IIV4 halved absence from parental work or day-care related to influenza illness.

IIV4 was immunogenic against each vaccine strain, as seen in other studies in children of the same age.27-31 The IIV4 was given in a 0∙5 mL dose containing 15 µg haemagglutinin antigen per strain instead of the 0∙25 mL dose containing 7∙5 µg per strain that is usually administered to young children in influenza vaccines from other manufacturers. A previous study demonstrated improved immunogenicity of a 0∙5 mL dose of IIV4 versus a 0∙25 mL dose in some young children, with no impact on safety or reactogenicity.30 In our study, the safety profile of IIV4 was similar to that of licensed, commonly used control vaccines (hepatitis A, pneumococcal and varicella). Injection site pain occurred at a similar frequency in both groups. Few children experienced post-vaccination fever (≥38°C). This confirms the tolerability of the IIV4 in young children, as seen in previous studies.27-31

Study strengths included its design as a large randomized trial conducted in five independent cohorts during five different influenza seasons in temperate and subtropical countries, use of active surveillance and RT-PCR assay, and application of moderate-to-severe influenza endpoints that capture the most clinically relevant illness. Limitations included heterogeneous access to hospital care and antibiotics in different countries in the study. The low incidence of the B/Victoria strain during the study resulted in insufficient power to evaluate vaccine efficacy for this strain. Almost all children were considered to be unprimed for influenza vaccination and the study provides no information about the effect of repeat immunization. However, the anamnestic response to the IIV4 has been evaluated in a follow-up immunogenicity study in which a sub-cohort of the first seasonal cohort was revaccinated the following season (data to be published separately). We also observed a lower incidence of hospitalization than expected based on general population rates, probably because we recruited healthy children without underlying risk factors who were less likely to be hospitalized for influenza than the general population.1-3 Our definition of severe influenza was particularly stringent (ICU admission, supplemental oxygen for >8 hours or serious extra-pulmonary complication of influenza), and differed from the WHO definition (fever, cough and requiring hospitalization).32 This resulted in few cases meeting our definition of severe, impacting on the vaccine efficacy estimate for this endpoint. Finally, our selection of a PCV as control in children <12 months of age may have negatively impacted the vaccine efficacy estimate. A study in South African infants showed that PCV immunization reduces pneumonia associated with influenza A in hospitalized children by 45%.33 We cannot rule out that the PCV used in our study may have reduced susceptibility to influenza in children who received it.

In conclusion, the IIV4 prevented influenza A and B in children 6–35 months of age, despite high levels of vaccine-mismatch. Vaccine efficacy was observed against RT-PCR-confirmed moderate-to-severe influenza and all influenza. Efficacy estimates were highest against moderate-to-severe disease, the most clinically important endpoint associated with the greatest medical and socioeconomic burden, and the IIV4 attenuated breakthrough illness. Our study adds to the body of existing evidence to support universal vaccination of all children from 6 months of age, regardless of risk status, to prevent influenza in this age group and to reduce the spread of influenza in the general population.

# Disclosures

## Trademark statement

*Fluarix Quadrivalent* is a trademark of the GSK group of companies.

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**Contributorship**

All authors participated in the design or implementation or analysis, and interpretation of the study; and the development of this manuscript. All authors had full access to the data and gave final approval before submission.

**Conflict of interest statement**

Claeys C, Izu A, Schuind A, Chandrashekaran V, Corsaro B, Friel D, Malvaux L and Soni J are employed by the GSK group of companies. Claeys C, Izu A, Schuind A, Chandrashekaran V, Corsaro B, Friel D, Li P, Jain VK and Innis BL hold shares in the GSK group of companies. Zaman K reports payments to his institution from the GSK group of companies for the conduct of the study. Dbaibo G reports his institution received payments from the GSK group of companies for the conduct of this study.Li P reports she was employed by the GSK group of companies at the time of the study and is currently employed by Pfizer. Kosalaraksa P reports he received payments from the GSK group of companies for the conduct of the study and payments from the GSK group of companies for attending academic meeting and training workshop.Acosta B reports his institution received payments from the GSK group of companies for the conduct of this study. Arroba Basanta ML reports the GSK group of companies supplied drugs/equipment. Cabanero MA reports payments from the GSK group of companies during the conduct of the study and payments from the GSK group of companies and from Sanofi Pasteur MSD outside the submitted work. Diez-Domingo J reports payments from the GSK group of companies and Sanofi Pasteur MSD for advisory board, and payments from Sanofi Pasteur MSD outside the submitted work. Dinleyici EC reports payments from the GSK group of companies and MSD for consulting and speakers bureaux, outside this submitted work. Faust SN reports his institution received payments from the GSK group of companies for the conduct of this study, and from Pfizer, Astra Zeneca, Cubist, Sanofi Pasteur, the GSK group of companies, Novartis, Alios and Ablynx for speakers or advisory board or clinical trials on behalf of his institution. Garcia-Sicilia J reports payments to his institution from the GSK group of companies for the conduct of this study, and from the GSK group of companies, Novartis, Sanofi Pasteur and Pfizer outside this submitted work. Gomez-Go GD reports payments from the GSK group of companies for the conduct of the study. Gonzales MLA reports payments and non-financial support from the GSK group of companies for the conduction of this study, lectures and participation in speakers bureaux, and for travels to attend conferences. Lucero M reports payments from the GSK group of companies. Mares Bermudez J reports payments from the GSK group of companies for the conduct of this study and payments from the GSK group of companies, Sanofi Pasteur MSD, Novartis and Pfizer for lectures including service on speakers bureaux, and payments from Sanofi Pasteur for conducting clinical trials outside the submitted work. Martinon-Torres F reports payments from the GSK group of companies for the conduct of this study and payments from the GSK group of companies, Novartis and Pfizer outside the submitted work. Montellano M received payment for lectures from MSD, Sanofi Pasteur and UMED, and received payment from the GSK group of companies and Sanofi Pasteur for the conduct of clinical trials, outside of submitted work. Prymula R reports payments from the GSK group of companies for the conduct of this study and payments from the GSK group of companies, Novartis and Sanofi Pasteur outside the submitted work. Puthanakit T reports payments to her institution from the GSK group of companies for the conduct of this study, and payments from the GSK group of companies, MSD and Pfizer outside of this submitted work. Ruzkova R reports payments to her institution from the GSK group of companies for the conduct of this study. Sokal E reports payments from Promethera Biosciens, Abbvie and Alexion, outside the submitted work. Ulied A reports payments to her institution from the GSK group of companies for the conduct of this study, and from MSD, Novartis and Pfizer outside this submitted work. Jain VK reports she was employed by the GSK group of companies at the time of the study and is currently employed by Bill and Melinda Gates Foundation. Innis BL reports he was employed by the GSK group of companies at the time of the study and is currently employed by PATH. Rivera L, Aziz A, Cousin L, Diaz A, Hughes SM, Jackowska T, Kant S, Miranda M, Peix Sambola MA, Sadowska-Krawczenko I, Salamanca de la Cueva I and Szymanski H have nothing to disclose.

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Table 1. Vaccine efficacy against RT-PCR-confirmed and culture-confirmed influenza

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **IIV4** | | **Control** | | **Vaccine efficacy, % (CI†‡)** |
|  | **n** | **Attack rate, %** | **n** | **Attack rate, %** |  |
| **Per-protocol cohort (time-to-event)** | **N=5707** | | **N=5697** | |  |
| Moderate-to-severe RT-PCR-confirmed influenza | 90 | 1∙58 | 242 | 4∙25 | 63∙2 (51∙8 to 72∙3)† |
| All RT-PCR-confirmed influenza | 344 | 6∙03 | 662 | 11∙62 | 49∙8 (41∙8 to 56∙8)† |
| LRI associated with RT-PCR-confirmed influenza | 28 | 0∙49 | 61 | 1∙07 | 54∙0 (28∙9 to 71∙0)‡ |
| Moderate-to-severe culture-confirmed influenza associated with antigenically-matching strains | 20 | 0∙35 | 88 | 1∙54 | 77∙6 (64∙3 to 86∙6)‡ |
| All culture-confirmed influenza associated with antigenically-matching strains | 88 | 1∙54 | 216 | 3∙79 | 60∙1 (49∙1 to 69∙0)‡ |
| Moderate-to-severe culture-confirmed influenza associated with any seasonal strain | 79 | 1∙38 | 216 | 3∙79 | 63∙8 (53∙4 to 72∙2)‡ |
| All culture-confirmed influenza associated with any seasonal strain | 303 | 5∙31 | 602 | 10∙57 | 51∙2 (44∙1 to 57∙6)‡ |
| AOM associated with RT-PCR-confirmed influenza | 12 | 0∙21 | 28 | 0∙49 | 56∙6 (16∙7 to 78∙8)‡ |
| Severe RT-PCR-confirmed influenza | 2 | 0∙04 | 3 | 0∙05 | 34∙2 (-297∙3 to 91∙3)‡ |
| **TVC (time-to-event)** | **N=6006** | | **N=6012** | |  |
| Moderate-to-severe RT-PCR-confirmed influenza | 91 | 1∙52 | 249 | 4∙14 | 63∙9 (52∙8 to 72∙8)† |
| Influenza A | 67 | 1∙12 | 159 | 2∙64 | 58∙1 (44∙5 to 68∙7) ‡ |
| A/H1N1 | 13 | 0∙22 | 46 | 0∙77 | 72∙1 (49∙9 to 85∙5)‡ |
| A/H3N2 | 53 | 0∙88 | 112 | 1∙86 | 52∙7 (34∙8 to 66∙1)‡ |
| Influenza B | 26 | 0∙43 | 93 | 1∙55 | 72∙3 (57∙9 to 82∙4)‡ |
| B/Victoria | 3 | 0∙05 | 15 | 0∙25 | 80∙1 (39∙7 to 95∙4)‡ |
| B/Yamagata | 22 | 0∙37 | 73 | 1∙21 | 70∙1 (52∙7 to 81∙9)‡ |
| By age |  |  |  |  |  |
| 6–17 months | 30 | 1∙53 | 59 | 2∙98 | 48∙8 (21∙2 to 67∙4)‡ |
| 18–35 months | 61 | 1∙51 | 190 | 4∙71 | 68∙5 (58∙2 to 76∙5)‡ |
| All RT-PCR-confirmed influenza | 353 | 5∙88 | 676 | 11∙24 | 49∙5 (41∙6 to 56∙5)† |
| Influenza A | 221 | 3∙68 | 434 | 7∙22 | 50∙3 (41∙6 to 57∙8)‡ |
| A/H1N1 | 35 | 0∙58 | 103 | 1∙71 | 66∙7 (51∙7 to 77∙6)‡ |
| A/H3N2 | 183 | 3∙05 | 331 | 5∙51 | 45∙6 (35∙0 to 54∙7)‡ |
| Influenza B | 139 | 2∙31 | 258 | 4∙29 | 47∙1 (35∙1 to 57∙1)‡ |
| B/Victoria | 29 | 0∙48 | 38 | 0∙63 | 24∙4 (-22∙3 to 53∙7)‡ |
| B/Yamagata | 106 | 1∙76 | 210 | 3∙49 | 50∙4 (37∙5 to 60∙9)‡ |
| By age |  |  |  |  |  |
| 6–17 months | 100 | 5∙11 | 177 | 8∙94 | 43∙3 (27∙8 to 55∙8)‡ |
| 18–35 months | 253 | 6∙25 | 499 | 12∙37 | 51∙6 (43∙7 to 58∙4)‡ |

†97∙5% CI; ‡95% CI

N: number of children in the analysis cohort; n: number of children with a case

If multiple events were observed in the same child, only the first event was considered in the vaccine efficacy calculation. However, in the exploratory analysis of VE by influenza A subtype and B lineage, the first subsequent occurrence of an event associated with a different subtype or lineage than the first event was included.

AOM: acute otitis media; CI: confidence interval; LRI: lower respiratory infection; IIV4: inactivated quadrivalent influenza vaccine; RT-PCR: reverse transcription polymerase chain reaction; TVC: total vaccinated cohort

Table 2. Impact of all RT-PCR-confirmed influenza on healthcare utilization and daily activities related to the current influenza illness (total vaccinated cohort)

|  | **IIV4 N=6006** | **Control N=6012** | **RR (95% CI)** |
| --- | --- | --- | --- |
| **Number of children with at least one RT-PCR-confirmed influenza case** | 356 | 693 |  |
| **Healthcare utilization** |  |  |  |
| General practitioner or paediatrician visit |  |  |  |
| Number (%) of children affected | 310 (5∙2) | 583 (9∙7) | 0∙53 (0∙46 to 0∙61) |
| Mean (SD) number of visits | 1∙1 (0∙36) | 1∙2 (0∙50) |  |
| Antibiotic use |  |  |  |
| Number (%) of children affected | 172 (2∙9) | 341 (5∙7) | 0∙50 (0∙42 to 0∙60) |
| Emergency room visit |  |  |  |
| Number (%) of children affected | 7 (0∙1) | 33 (0∙5) | 0∙21 (0∙09 to 0∙47) |
| Mean (SD) number of visits | 1∙0 (0) | 1∙2 (0∙48) |  |
| Hospitalization |  |  |  |
| Number (%) of children affected | 3 (0∙0) | 7 (0∙1) | 0∙43 (0∙11 to 1∙66) |
| Mean (SD) duration of visit, days | 8∙0 (9∙64) | 4∙6 (1∙72) |  |
| **Impact on daily activities** |  |  |  |
| Parental absence from paid work |  |  |  |
| Number of relevant RT-PCR-confirmed cases1 | 173 | 390 |  |
| Number (%) of families affected | 24 (0∙4) | 52 (0∙9) | 0∙46 (0∙28 to 0∙75) |
| Mean (SD) duration of absence, days | 2∙2 (1∙96) | 2∙5 (2∙43) |  |
| Child absence from day-care |  |  |  |
| Number of relevant RT-PCR-confirmed cases2 | 113 | 290 |  |
| Number (%) of children affected | 49 (0∙8) | 108 (1∙8) | 0∙45 (0∙32 to 0∙63) |
| Mean (SD) duration of absence, days | 3∙9 (3∙22) | 4∙3 (3∙53) |  |

1Only families in which both parents were in paid work were included in the analysis

2Only children who attended day-care were included in the analysis

N: number of children in the analysis cohort

CI: confidence interval; IIV4: inactivated quadrivalent influenza vaccine; RR: risk ratio; RT-PCR: reverse transcription polymerase chain reaction; SD: standard deviation

Figure 1. Study cohorts

Figure 2. Proportion of antigenically characterized isolates from children with RT-PCR-confirmed influenza that was vaccine-matched or -mismatched (total vaccinated cohort, time-to-event analysis)



Percentage shown in bars is the proportion of antigenically characterized samples that were mismatched to the vaccine strain

N value shown above bars is the number of samples that were antigenically characterized i.e. had (1) either an A subtype identified by RT-PCR or a B lineage identified by sequence analysis and (2) an antigenic characterization result reported

A/H1N1: 138 samples available; 105 samples (76∙1%) were characterized; 33 samples (23∙9%) had an indeterminate result

A/H3N2: 514 samples available; 378 samples (73∙5%) were characterized; 136 samples (26∙5%) had an indeterminate result

B/Victoria: 67 samples available; 63 samples (94∙0%) were characterized; 4 samples (6∙0%) had an indeterminate result

B/Yamagata: 316 samples available; 302 samples (95∙6%) were characterized; 14 samples (4∙4%) had an indeterminate result

Figure 3. Immunogenicity according to strain (per-protocol immunogenicity sub-cohort)

**Overall population**



**6–17 months**



**18–35 months**



Immunogenicity data were pooled across the five seasonal cohorts for each influenza A subtype and B lineage used in the seasonal vaccine

Baseline: before vaccination; Day 28: 28 days after last vaccination (Day 28 for primed children who received one vaccine dose and Day 56 for unprimed children who received two vaccine doses)

Antibody levels below the assay cut-off value (10 1/DIL) were assigned an arbitrary value of half the cut-off for the purpose of the calculation

GMT: antilog of the arithmetic mean of the log10-transformed titre

CI: confidence interval; GMT: geometric mean titre; HI: haemagglutination inhibition; IIV4: inactivated quadrivalent influenza vaccine