ORIGINAL ARTICLE

# Oil-in-Water Emulsion Adjuvant with Influenza Vaccine in Young Children

Timo Vesikari, M.D., Markus Knuf, M.D., Peter Wutzler, M.D., Aino Karvonen, M.D., Dorothee Kieninger-Baum, M.D., Heinz-Josef Schmitt, M.D., Frank Baehner, M.D., Astrid Borkowski, M.D., Theodore F. Tsai, M.D., and Ralf Clemens, M.D.

## ABSTRACT

### BACKGROUND

The efficacy of inactivated influenza vaccines is known to be poor in infants and young children.

### METHODS

We studied the effect of the adjuvant MF59, an oil-in-water emulsion, on the efficacy of trivalent inactivated influenza vaccine (TIV) in 4707 healthy children 6 to less than 72 months of age who had not previously been vaccinated against influenza. The children were randomly assigned to three study groups, each of which received the assigned vaccines in two doses, 28 days apart, during two consecutive influenza seasons. Two of the groups were given age-appropriate doses of TIV either with or without the MF59 adjuvant, and the third group was given control (noninfluenza) vaccines to assess their absolute and relative efficacy against influenza-like illness, as confirmed by means of polymerase-chain-reaction (PCR) assay.

### RESULTS

Attack rates of influenza-like illness across both influenza seasons were 0.7%, 2.8%, and 4.7% in the adjuvant, nonadjuvant, and control vaccine groups, respectively. The absolute vaccine efficacy rates against all influenza strains (94 of 110 cases were due to vaccine-matched H3N2 viruses) were 86% (95% confidence interval [CI], 74 to 93) for the MF59-adjuvant vaccine (ATIV) and 43% (95% CI, 15 to 61) for the vaccine without the adjuvant (TIV); the relative vaccine efficacy rate for ATIV versus TIV was 75% (95% CI, 55 to 87). The efficacy rates for ATIV were 79% (95% CI, 55 to 90) in children 6 to less than 36 months of age and 92% (95% CI, 77 to 97) in those 36 to less than 72 months of age, as compared with 40% (95% CI, −6 to 66) and 45% (95% CI, 6 to 68), respectively, for TIV. Antibody responses were higher with ATIV and remained so through day 181. The rates of systemic and local reactions to the influenza vaccines with and without the adjuvant were similar in the younger age group (relative risk, 1.04; 95% CI, 0.98 to 1.09), but systemic events in the older age group were more frequent after administration of ATIV (63%) than after administration of TIV (44%) or the control vaccine (50%). Serious adverse events were distributed evenly across the three vaccine groups.

### CONCLUSIONS

Influenza vaccine with the MF59 adjuvant is efficacious against PCR-confirmed influenza in infants and young children. (Funded by Novartis Vaccines and Diagnostics; ClinicalTrials.gov number, NCT00644059.)

From the University of Tampere Medical School, Tampere, Finland (T.V., A.K.); and Zentrum für Kinder- und Jugendmedizin, Universitätsmedizin, Mainz (M.K., D.K.-B., H.-J.S.); Institute of Virology and Antiviral Therapy, Jena University Hospital–Friedrich Schiller University, Jena (P.W.); and Novartis Vaccines and Diagnostics, Marburg (F.B., A.B., T.F.T., R.C.) — all in Germany. Address reprint requests to Dr. Vesikari at the University of Tampere Medical School, Biokatu 10, 33520 Tampere, Finland, or at timo.vesikari@uta.fi.

N Engl J Med 2011;365:1406-16.

Children have the highest rates of seasonal influenza infection and illness, with amplification of community viral transmission. Thus, numerous countries recommend routine seasonal vaccination to protect children directly and the entire population indirectly.[1-9] Parenteral trivalent inactivated influenza vaccine (TIV) is poorly immunogenic in young children, with an efficacy of only 59.0% in children older than 2 years of age.[10-12] Although intranasal live attenuated influenza vaccine has an efficacy of 69.2 to 95.6% in children 2 to 7 years of age, it cannot be used in children under 2 years of age because of the increased risk of hospitalization or in children under 5 years of age who have a history of wheezing, because of the increased risk of wheezing.[13,14]

MF59 is an oil-in-water emulsion that augments the immune response when combined with vaccine antigens. It has been used since 1997 as a TIV adjuvant (ATIV) for seasonal vaccination in older adults and is licensed for such use in 27 countries.[15-17] We previously found that ATIV induced greater immune responses than did TIV in children 6 to 36 months of age,[18,19] and we report here the efficacy of these two types of vaccines in a field trial in children 6 to less than 72 months of age who had not previously received influenza vaccine.

## Methods

### Study Design and Objectives

We conducted this phase 3 study during two influenza seasons: 2007–2008 in Germany (654 children) and 2008–2009 in Germany (2104 children) and Finland (1949 children). Participants were stratified according to age (6 to <36 months and 36 to <72 months) and were randomly assigned to one of three study groups. In year 1, they were assigned to ATIV, subunit TIV, or control (non-influenza) vaccine in a ratio of 2:1:1; in study year 2, they were assigned to ATIV, split TIV, or control vaccine in a ratio of 2:2:1. The subunit influenza vaccine consisted of purified hemagglutinin and neuraminidase viral-surface proteins; the split vaccine consisted of purified disrupted virions. Vaccines were administered in two doses, 28 days apart, with or without concomitant routine vaccines.

The study was approved by the ethics committee at each participating study center and was conducted according to the International Conference on Harmonisation Good Clinical Practice guidelines. Although planned to last 3 years, the study was terminated in 2009 after requests by the ethics committees to substitute seasonal vaccine with monovalent influenza A (H1N1) vaccine; any children who were recruited and vaccinated with pandemic (A/H1N1) or nonpandemic vaccines in year 3 were not included in this analysis.

The principal study objectives were to show that in children 6 to less than 36 months of age, ATIV has an acceptable level of combined local and systemic reactogenicity, as compared with TIV, and superior protective efficacy, as compared with control vaccine, against vaccine-matched influenza illness confirmed by means of real-time polymerase-chain-reaction (PCR) assay. The study participants were healthy children who had not previously received influenza vaccine, who had no contraindications to vaccination, and whose parents gave written informed consent. Solicited reactions were recorded on diary cards for 7 days, with active follow-up for 28 days after each vaccination for unsolicited adverse events and for 6 months in study year 1 and for 12 months in study year 2 to collect data on adverse events that were serious or led to withdrawal from the study. During the two consecutive influenza seasons, beginning 3 weeks after administration of the second vaccine dose, if a child became ill, the parents were asked to bring the child to the clinic for examination within 7 days after the onset of the illness, at which time nasopharyngeal aspirates or swabs were obtained for confirmation of the influenza diagnosis and for strain identification by means of real-time PCR and gene-sequencing techniques developed and conducted at the Universitätsklinikum Jena.[1,20] Events occurring within the preceding 7 days were recorded as unsolicited adverse events.

In study year 2, in a convenience sample of 793 children who were recruited at 14 sites in Finland, we obtained at least one prevaccination blood sample (on day 1) and three postvaccination samples (on days 29, 51, and 181). Hemagglutination-inhibition antibody titers were measured against three homologous 2008–2009 vaccine strains and against three heterologous vaccine strains — A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/15/2009 (H3N2), and B/Brisbane/60/2008 — from influenza seasons 2007–2008, 2010–2011, and 2009–2010, respectively (see the Methods section in the Supplementary Appendix, available with the full text of this article at NEJM.org).[21] Seropro-

tection was defined as a hemagglutination-inhibition antibody titer of 40 or greater.

The study was designed and conducted by the sponsor, Novartis Vaccines, in collaboration with the study investigators, who gathered the clinical data. Novartis Vaccines conducted the data analysis, and the manuscript was written by the first author, with the assistance of one of the industry authors and a medical writer with support from the sponsor. The decision to submit the manuscript for publication was made by the first author. All authors vouch for the accuracy and completeness of the analyses presented and the adherence of the study and this report to the protocol. The complete protocol is available at NEJM.org.

### ACTIVE VACCINES AND CONTROL VACCINES

In the 2007–2008 season, ATIV (Fluad, Novartis Vaccines), which included MF59 emulsion adjuvant, and subunit TIV (Agrippal S1, Novartis Vaccines) contained World Health Organization reference strains A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004). In the 2008–2009 season, ATIV and split TIV (Influsplit SSW, GlaxoSmithKline Biologicals) contained A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), and B/Florida/4/2006 strains. Vaccines contained 15 $\mu$g of hemagglutinin from each of three viral subtypes in each 0.5-ml dose. Children 6 to less than 36 months of age received 0.25-ml doses, and children 36 to less than 72 months of age received 0.5-ml doses. Control vaccines were meningococcal C conjugate vaccine (Menjugate, Novartis Vaccines), given in 0.25-ml doses in children 6 to less than 12 months of age, and tickborne encephalitis vaccine (Encepur Children, Novartis Vaccines), given in 0.5-ml doses in children 12 to less than 72 months of age.

### STATISTICAL ANALYSIS

We planned to enroll approximately 8000 children over three seasons in order to have 90% power for the safety objective and up to 80% power for the efficacy objective. Demographic characteristics were tabulated with the use of descriptive statistics. The relative risk of real-time PCR–confirmed influenza-like illness was estimated with the use of a Poisson regression model that included season, region, and age cohort (when applicable) as independent variables, with $\log_{10}$-transformed values for time at risk as an offset variable (ignoring family and household relationships of the study participants). The ratio of likelihood-based estimates and two-sided 97.7% confidence intervals were adjusted for the primary efficacy and safety analyses and one interim analysis; for other analyses, 95% confidence intervals were calculated. The Cochran–Mantel–Haenszel approach was used for the safety analysis and selected vaccine efficacy results, for robustness. Vaccine efficacy was calculated as (1−the relative risk) × 100%. The two primary analyses were adjusted for multiplicity, so that in 240 comparative analyses (e.g., of vaccine efficacy in four age cohorts, as well as solid-phase and geometric mean titers in two age cohorts each), 12 significant findings could be expected by chance. To evaluate immunogenicity end points, least-squares geometric mean titers and ratios and associated two-sided 95% confidence intervals were determined by means of analysis of variance (with $\log_{10}$ transformation).

## RESULTS

### STUDY POPULATION AND FOLLOW-UP

Baseline characteristics of the 4707 enrolled children were similar among the three vaccine groups (Fig. 1). Safety data were available for 4692 children, 4513 of whom completed the study; 13 children withdrew because of adverse events.

### ASSESSMENTS OF EFFICACY

Of 47 cases of influenza-like illness in the control group (Table 1), 4 occurred among 164 children in study year 1 (attack rate, 2.4%) and 43 among 829 children in study year 2 (attack rate, 5.2%). Vaccine efficacy was not calculated for year 1, since there were only 5 cases, 3 of which were due to B/Florida/4/2006–like viruses (Yamagata lineage, lineage-mismatched to the vaccine strain) and 2 of which were due to viruses with unknown B lineage.

In study year 2, a total of 94 of the 105 isolates from children with influenza-like illness were A/Brisbane/10/2007(H3N2)–like viruses (matched to the vaccine strain), 4 were A-type viruses (subtype unknown), 5 were B/Brisbane/60/2008–like viruses (Victoria lineage, lineage mismatched to the vaccine strain), and 2 were B-type viruses (lineage unknown). No H1N1 viruses were detected. In study year 2, the vaccine efficacy was 86% (95% confidence interval [CI], 73 to 92) for ATIV, as compared with 40% (95% CI, 11 to 60) for split TIV, for a relative vaccine efficacy of 76% (95% CI, 55 to 87).

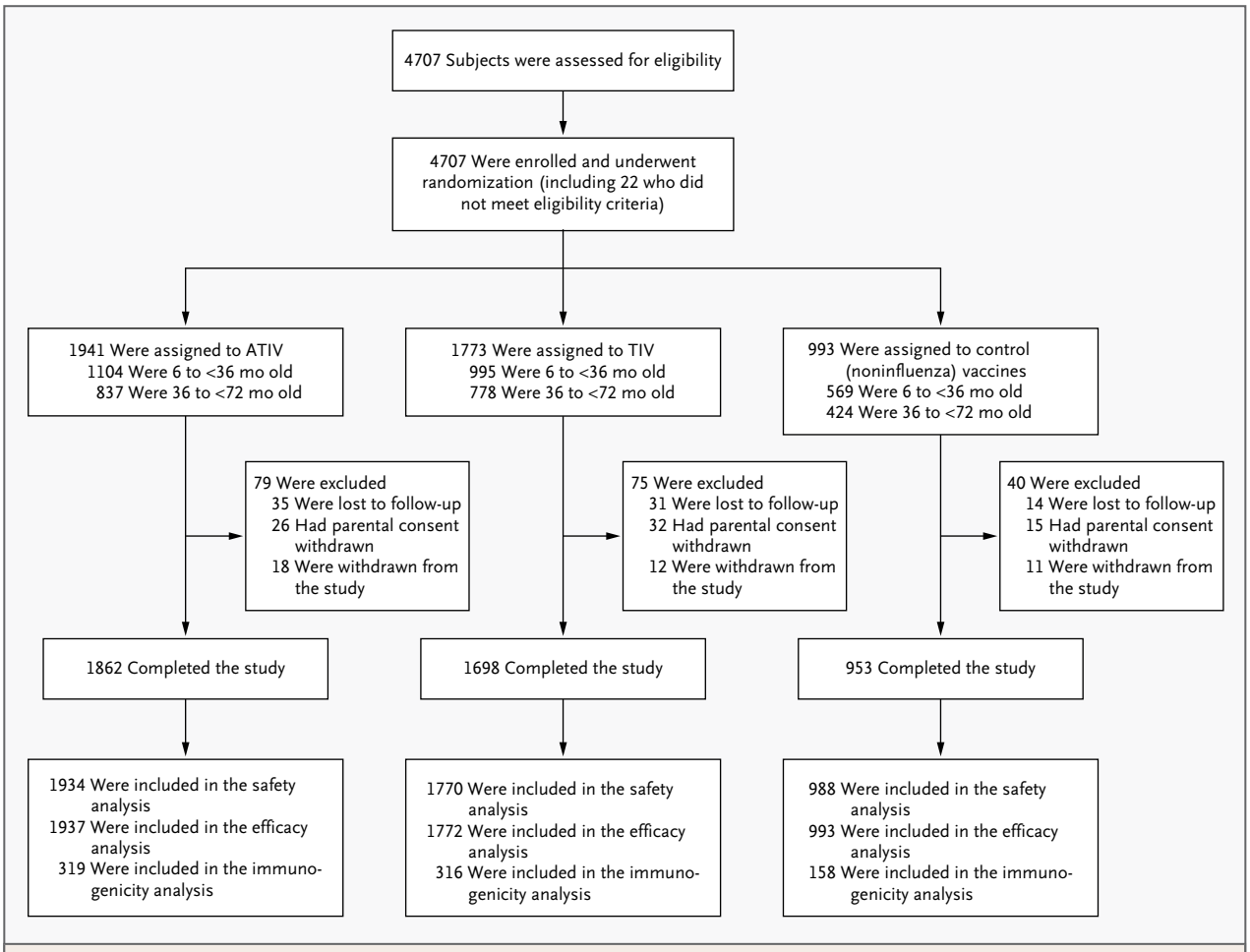


**Figure 1. Numbers of Children Enrolled, Assigned to a Study Group, and Included in the Analyses.**
A total of 22 children who did not meet the entry criteria were enrolled and participated in the study. ATIV denotes trivalent inactivated influenza vaccine with the MF59 adjuvant, and TIV trivalent inactivated influenza vaccine without the adjuvant.

The absolute efficacy of ATIV across both influenza seasons was 86% (95% CI, 74 to 93) against all strains, 89% (95% CI, 78 to 95) against vaccine-matched strains and H3N2 virus, and 79% (95% CI, –5 to 96) against B strains (Table 1). In the subanalysis, ATIV was efficacious in both age groups. In children who were 6 to less than 36 months of age, vaccine efficacy was 79% against all strains and 81% against vaccine-matched strains (with lower 95% CI limits of 55% and 49%, respectively), and in children 36 to less than 72 months of age, vaccine efficacy was 92% against all strains and 96% against vaccine-matched strains (with lower 95% CI limits of 77% and 81%, respectively), fulfilling the primary study objective.

In contrast, TIV had an efficacy of 43% (95% CI, 15 to 61) against all strains, 45% (95% CI, 16 to 64) against vaccine-matched strains and the H3N2 virus, and 36% (95% CI, –162 to 84) against B strains, with similar point estimates in the age subgroups but with the 95% CI overlapping zero for children who were 6 to less than 36 months of age. On the basis of these results, ATIV had higher relative efficacy than TIV, with relative efficacy rates of 75% (95% CI, 55 to 87) against all strains, 80% (95% CI, 59 to 90) against vaccine-matched strains and the H3N2 virus, and 66% (95% CI, –103 to 94) against B strains, in the entire cohort. The relative efficacy rates for ATIV according to age group were 64% against all strains and 68% against matched strains among children who were 6 to less than 36 months of age and 86% and 91%, respectively, among those who were 36 to less than 72 months of age. In a post hoc analysis of vaccine efficacy in children 6 to less than 24 months of age, the overall efficacy of ATIV against matched

**Table 1. Efficacy of MF59-Adjuvant Trivalent Influenza Vaccine (ATIV), Trivalent Influenza Vaccine without Adjuvant (TIV), and Control (Noninfluenza) Vaccine against Confirmed Influenza over Two Seasons (2007–2008 and 2008–2009) in Finland and Germany.***

| Age Group and Vaccine | Confirmed Cases of Influenza | Relative Efficacy (95% CI)† |
|---|---|---|
| | *no. of children/total no.* | *percent* |
| **Efficacy against all strains** | | |
| 6 to <72 mo | | |
| ATIV vs. control | 13/1937 vs. 47/993 | 86 (74 to 93) |
| TIV vs. control | 50/1772 vs. 47/993 | 43 (15 to 61) |
| ATIV vs. TIV | 13/1937 vs. 50/1772 | 75 (55 to 87) |
| 36 to <72 mo | | |
| ATIV vs. control | 4/834 vs. 25/427 | 92 (77 to 97) |
| TIV vs. control | 25/777 vs. 25/427 | 45 (6 to 68) |
| ATIV vs. TIV | 4/834 vs. 25/777 | 86 (59 to 95) |
| 6 to <36 mo | | |
| ATIV vs. control | 9/1103 vs. 22/566 | 79 (55 to 90) |
| TIV vs. control | 25/995 vs. 22/566 | 40 (–6 to 66) |
| ATIV vs. TIV | 9/1103 vs. 25/995 | 64 (23 to 83) |
| 6 to <24 mo | | |
| ATIV vs. control‡ | 5/820 vs. 11/401 | 77 (37 to 92) |
| TIV vs. control‡ | 18/706 vs. 11/401 | 11 (–89 to 58) |
| ATIV vs. TIV‡ | 5/820 vs. 18/706 | 73 (29 to 90) |
| **Efficacy against vaccine-matched strains** | | |
| 6 to <72 mo | | |
| ATIV vs. control | 9/1937 vs. 41/993 | 89 (78 to 95) |
| TIV vs. control | 44/1772 vs. 41/993 | 45 (16 to 64) |
| ATIV vs. TIV | 9/1937 vs. 44/1772 | 80 (59 to 90) |
| 36 to <72 mo | | |
| ATIV vs. control | 2/834 vs. 22/427 | 96 (81 to 99) |
| TIV vs. control | 22/777 vs. 22/427 | 48 (8 to 71) |
| ATIV vs. TIV | 2/834 vs. 22/777 | 91 (63 to 98) |
| 6 to <36 mo | | |
| ATIV vs. control | 7/1103 vs. 19/566 | 81 (49 to 93) |
| TIV vs. control | 22/995 vs. 19/566 | 41 (–9 to 68) |
| ATIV vs. TIV‡ | 7/1103 vs. 22/995 | 68 (27 to 86) |
| 6 to <24 mo | | |
| ATIV vs. control‡ | 4/820 vs. 8/401 | 75 (20 to 92) |
| TIV vs. control‡ | 15/706 vs. 8/401 | 2 (–129 to 58) |
| ATIV vs. TIV‡ | 4/820 vs. 15/706 | 75 (25 to 91) |

* Efficacy was calculated for children who received two age-appropriate doses of the assigned vaccine. Cases of influenza were confirmed with the use of a polymerase-chain-reaction assay.

† Confidence intervals were estimated with the use of the Poisson regression method unless otherwise indicated; 95% confidence intervals were calculated for all efficacy comparisons except the primary comparison (ATIV vs. control vaccine in children 6 to less than 36 months of age), for which a 97.7% confidence interval was calculated.

‡ The confidence interval was estimated with the use of the Mantel–Haenszel method.

strains was 75% (95% CI, 20 to 92), whereas TIV had almost no efficacy (2%) (95% CI, −129 to 58). The time–cumulative efficacy calculations showed that the estimates of vaccine efficacy for ATIV and TIV were robust throughout the 5-month surveillance period (Fig. 2).

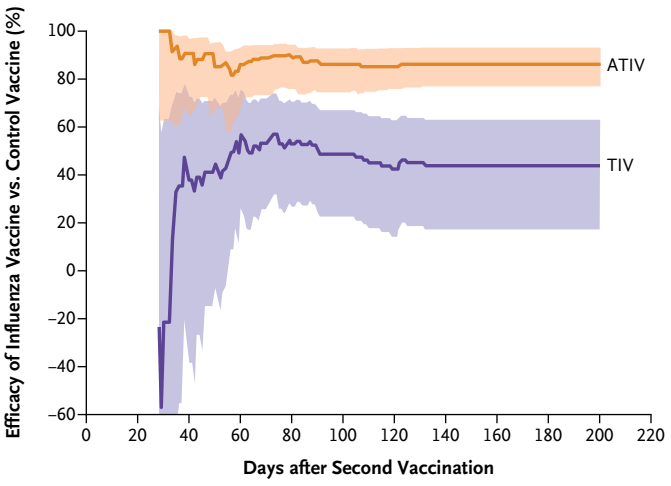


**Figure 2. Efficacy of Influenza Vaccines versus Control Vaccine over Time.**
The cumulative efficacy of ATIV and of TIV, as compared with control (non-influenza) vaccine, is shown. The data are for efficacy against all viral strains over time after the second dose of vaccine in children 6 to less than 72 months of age. Shaded areas represent 95% confidence intervals.

### IMMUNOGENICITY

After one dose of vaccine, the proportions of children 6 to less than 36 months of age who had seroprotective hemagglutination-inhibition antibody titers (≥40) against the homologous influenza A H1N1 and H3N2 strains were 92% and 95%, respectively, among the ATIV recipients and 20% and 12%, respectively, among the TIV recipients. Among children who were 36 to less than 72 months of age, the respective proportions were 100% and 97% for ATIV and 63% and 60% for TIV (Fig. 3). Moreover, 98 to 100% of all ATIV recipients still had seroprotective titers against these strains at day 181, whereas the respective proportions for TIV recipients were 49% and 65%.

Two doses of ATIV (day 50) elicited seroprotective antibody titers (≥40) against the homologous influenza B strain in 88% of the younger children and in 99% of the older children, with the proportions declining to 40% and 64%, respectively, by day 181. The proportions of TIV recipients who had robust hemagglutination-inhibition antibody titers against the B strain were lower even after two doses; only 19% of the younger children and 60% of the older children had seroprotective responses, and the proportions were only 13% and 33%, respectively, at day 181. The median interval between doses for the three vaccine groups was 30 days (standard deviation, 6.1 to 6.9).

Two doses of ATIV produced seroprotective titers (≥40) against heterologous H1N1 and H3N2 antigens in 95 to 100% of recipients in the two age groups, and these levels were maintained at day 181 in 61% or more of the younger children and in 85% or more of the older children. The B-lineage mismatch was not effectively overcome by ATIV; only 5 to 15% of recipients had a seroprotective titer against the Victoria lineage strain. In those given TIV, the responses to heterologous strains were low, with the exception of the heterovariant H3N2 virus. The geometric mean titers against A subtype strains in response to heterologous antigens with ATIV exceeded those in response to homologous antigens with TIV; in both age groups, these titers in response to all homologous and heterologous antigens after two doses of TIV were exceeded or matched after one dose of ATIV (Fig. 1 in the Supplementary Appendix).

### SAFETY AND REACTOGENICITY

Among the children who were 6 to less than 36 months of age, the relative risk of solicited adverse events after one or two doses of ATIV, as compared with TIV, was 1.04 (95% CI, 0.98 to 1.09), fulfilling the primary safety objective. Local adverse events were reported more frequently among ATIV recipients (54%) and recipients of control vaccine (52%) than among TIV recipients (46%), but rates of systemic adverse events were similar (68%, 61%, and 66%, respectively). Among the children who were 36 to less than 72 months of age, local adverse events were reported in 68%, 60%, and 55% of recipients of ATIV, TIV, and control vaccine, respectively, whereas systemic adverse events were more frequent among ATIV recipients (63%) and control-vaccine recipients (50%) than among TIV recipients (44%). The majority of local and systemic solicited events, including fever, were mild or moderate and were transient (Tables 1 and 2 in the Supplementary Appendix). Febrile convulsions were reported in five ATIV recipients, five TIV recipients, and four recipients of control vaccine.

Rates of all unsolicited or serious adverse events

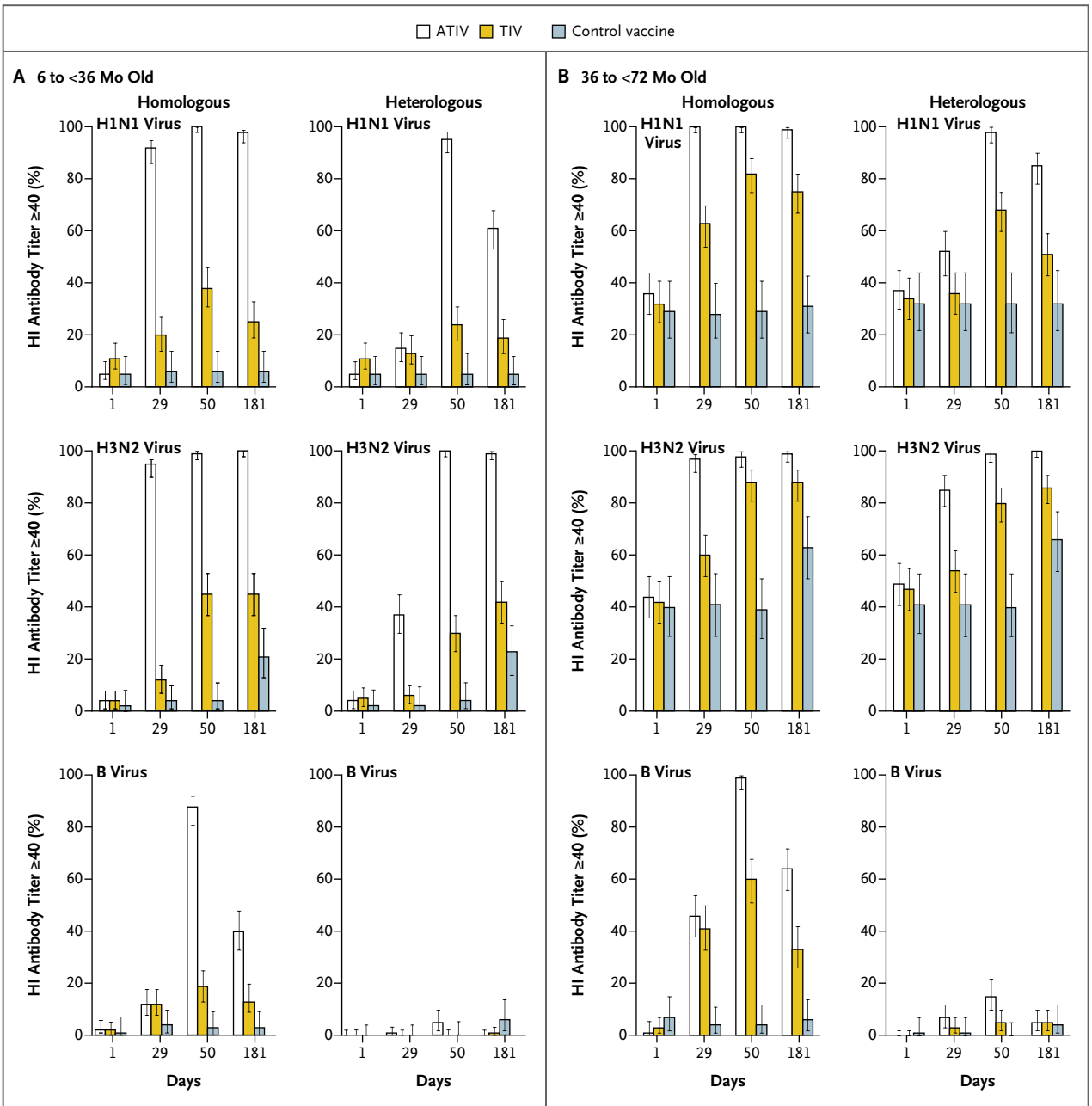


**Figure 3. Seroprotective Hemagglutination-Inhibition (HI) Antibody Titers, According to Age Group, Vaccine Group, and Virus Strain.**

Results are shown for children 6 to less than 36 months of age (Panel A) and for children 36 to less than 72 months of age (Panel B). Responses are shown before vaccination, at 29 days (4 weeks after the first dose), at 50 days (4 weeks after the second dose), and at 6 months after vaccination. HI antibody titers of 40 or higher were considered to be seroprotective.

over the course of the whole study were similar in the ATIV, TIV, and control-vaccine groups; no deaths and no cases of narcolepsy were reported. Serious adverse events were reported in 8% of ATIV recipients, 10% of TIV recipients, and 11% of control-vaccine recipients, among children who were 6 to less than 36 months of age, and in 4%, 8%, and 11%, respectively, of those who were 36 to less than 72 months of age (Table 2). A total of 13 children were withdrawn from the study because of serious adverse events. In addition, 2 children in each of the three vaccination groups had seri-

**Table 2. Serious Adverse Events during Treatment and Those Leading to Withdrawal from the Study, According to Vaccine and Age Group.***

| Event | ATIV | | TIV | | Control (Noninfluenza) Vaccine | |
|---|---|---|---|---|---|---|
| | 6 to <36 mo (N=1177) | 36 to <72 mo (N=835) | 6 to <36 mo (N=1069) | 36 to <72 mo (N=777) | 6 to <36 mo (N=607) | 36 to <72 mo (N=422) |
| | *number of children (percent)* | | | | | |
| Total (any adverse event) | 91 (8) | 31 (4) | 104 (10) | 65 (8) | 65 (11) | 45 (11) |
| Blood and lymph system disorders | 1 (<1) | 0 | 1 (<1) | 1 (<1) | 0 | 0 |
| Cardiac disorders | 1 (<1) | 0 | 1 (<1) | 0 | 0 | 0 |
| Congenital and genetic disorders | 0 | 1 (<1) | 0 | 0 | 0 | 0 |
| Endocrine system disorders | 0 | 0 | 0 | 1 (<1) | 0 | 0 |
| Gastrointestinal system disorders | 4 (<1) | 1 (<1) | 6 (1) | 2 (<1) | 0 | 2 (<1) |
| General and vaccination-site reactions | 3 (<1) | 2 (<1) | 2 (<1) | 2 (<1) | 0 | 1 (<1) |
| Immune system disorders | 0 | 0 | 0 | 1 (<1) | 1 (<1) | 0 |
| Infections and infestations | 57 (5) | 19 (2) | 83 (8) | 49 (6) | 56 (9) | 38 (9) |
| Influenza | 12 (1) | 8 (1) | 39 (4) | 40 (5) | 33 (5) | 31 (7) |
| Injury and poisoning | 13 (1) | 6 (1) | 8 (1) | 6 (1) | 7 (1) | 3 (1) |
| Investigations | 2 (<1) | 0 | 0 | 0 | 0 | 0 |
| Metabolic and nutritional disorders | 2 (<1) | 2 (<1) | 2 (<1) | 1 (<1) | 0 | 1 (<1) |
| Muscle, connective-tissue, and bone disorders | 2 (<1) | 0 | 0 | 1 (<1) | 1 (<1) | 0 |
| Neoplasia, benign or malignant | 1 (<1) | 0 | 0 | 0 | 0 | 0 |
| Nervous system disorders | 5 (<1) | 2 (<1) | 5 (<1) | 1 (<1) | 2 (<1) | 1 (<1) |
| Psychiatric disorders | 0 | 0 | 1 (<1) | 1 (<1) | 1 (<1) | 0 |
| Renal and urinary tract disorders | 0 | 0 | 0 | 1 (<1) | 0 | 1 (<1) |
| Reproductive system disorders | 0 | 0 | 1 (<1) | 0 | 0 | 0 |
| Respiratory, thoracic, and mediastinal disorders | 4 (<1) | 1 (<1) | 3 (<1) | 3 (<1) | 2 (<1) | 2 (<1) |
| Skin and subcutaneous disorders | 5 (<1) | 0 | 0 | 0 | 0 | 0 |
| Social circumstances | 0 | 0 | 1 (<1) | 0 | 0 | 0 |
| Surgical and medical procedures | 2 (<1) | 2 (<1) | 0 | 1 (<1) | 0 | 0 |

* Events are listed according to the terminology from the Medical Dictionary for Regulatory Activities, which is organized by system, organ, and class. Thirteen events led to withdrawal from the study: one case each of strabismus, growth retardation, brain tumor, and diarrhea in the group of younger children who received the trivalent influenza vaccine with the MF59 adjuvant (ATIV); one case each of food allergy, acquired epileptic aphasia, pyrexia, and otitis media in the group of older ATIV recipients; one case each of ataxia on walking, rash, asthma, and lymphadenopathy in the group of younger children who received the trivalent influenza vaccine without the MF59 adjuvant (TIV); and one case of asthma in the group of older children who received control vaccine.

ous adverse events that were judged to be possibly or probably related to the vaccination (for details, see the Supplementary Appendix).

## DISCUSSION

ATIV was efficacious against laboratory-confirmed influenza caused by all circulating influenza viral strains during the two study years (86% efficacy rate), with higher efficacy against vaccine-matched strains (89%). In contrast, the respective efficacy rates for TIV were 43% and 45%, resulting in relative efficacy rates of 75% and 80% for ATIV as compared with TIV. The higher efficacy rates were significant in the predefined age cohorts (i.e., 6 to less than 36 months and 36 to less than 72 months) and also in a post hoc analysis of children who were 6 to less than 24 months of age. The latter finding is noteworthy because evidence of the efficacy of TIV in children younger than 24 months of age is limited.[10,11,22]

Since the results of this study were principally from year 2, when vaccine-like H3N2 viruses predominated (in 94 of 110 culture-confirmed cases),

the reported overall vaccine efficacy is, in effect, an H3N2-specific observation.[23] Only 12 infections with influenza B virus occurred, all involving strains that were not characterized for vaccine matching or that were lineage-mismatched to the vaccine strains, so the B virus–specific efficacy of ATIV and TIV could not be meaningfully assessed (observed efficacy, 79% [95% CI, −5 to 96] and 36% [95% CI, −162 to 84], respectively). B-lineage mismatches present a greater obstacle to vaccine efficacy than do mismatches due to antigenic drift because cross-lineage hemagglutination-inhibition antibodies are not effectively induced by vaccination, especially in children in whom priming has not been carried out.[1,24-26]

Immune responses to ATIV were significantly greater than immune responses to TIV and were maintained for up to 181 days, both against homologous (vaccine) and heterologous strains. Because children under 9 years of age need to receive two priming doses of TIV, it was notable that responses to a single ATIV dose met the conventional seroprotection threshold (hemagglutination-inhibition antibody titer ≥40) for both A-subtype viruses, a finding that is consistent with previous observations of seasonal and pandemic influenza vaccines with the MF59 adjuvant.[1,18,27-31] Although responses to B strains were lower, 88 to 99% of all children had titers of 40 or higher after two doses of ATIV, as compared with 19 to 60% of TIV recipients. Low responses to the B strain in children are of particular concern, since this age group has a disproportionate share of influenza B virus infections.[1,32] Moreover, B strains are frequently responsible for late-season (spring) influenza outbreaks, so the high proportion of children with seroprotective hemagglutination-inhibition antibody titers at day 181 (64%) and the maintenance of vaccine efficacy through the end of the influenza season are potential advantages of ATIV over TIV, particularly with the start of vaccine deliveries and immunizations in August.[24,30,33]

In previous studies of seasonal and H5N1 vaccines, formulations that included the MF59 adjuvant elicited higher levels of cross-reactive antibodies to antigenically related viruses than did their counterparts without the adjuvant.[18,19,34,35] We confirmed this observation in the current study for heterovariant A-subtype strains, including an H3N2 strain that did not circulate extensively in the Northern Hemisphere until after the study's completion — a prospective benefit when vaccine and circulating viruses are mismatched. Since the H3N2 viruses circulating in the second study year were vaccinelike, there was no opportunity to show clinical efficacy against a drift variant.

Vaccine-related adverse events were generally mild to moderate in both age cohorts, with no important differences in solicited reactogenic events among the three vaccine groups after either dose in the younger cohort. In older children, systemic reactions, including mild fever, were slightly more frequent after receipt of ATIV, as compared with the other vaccines, but these reactions were mostly mild and of short duration. Similarly, rates of unsolicited adverse events and serious adverse events were not disproportionate in the ATIV group, confirming previous experience with various vaccines having the MF59 adjuvant in trials involving approximately 33,000 children ranging in age from neonates to teenagers under 17 years of age.[18,19,36,37] Although clinically controlled experience with MF59 in children is limited, information from the commercial distribution of more than 50 million Fluad doses since 1997 for administration in elderly persons, as well as interim observations from approximately 100 million doses of pandemic influenza A (H1N1) vaccines with the MF59 adjuvant that were distributed in 2009–2010 in all age groups (and also in pregnant women), has not revealed any safety concerns.[38-40]

In this controlled trial, the efficacy of an inactivated influenza vaccine in children 6 to less than 72 months of age was relatively high. Since protection was principally against the subtype H3N2 virus, no conclusions could be reached with regard to the efficacy of ATIV or TIV against H1N1 and B viruses. The immunogenicity analysis indicated that ATIV elicits higher, more persistent, and more cross-reactive antibody responses than TIV. There was no opportunity to confirm the clinical effect of the heterovariant antibodies.

The modest efficacy of TIV against matched strains (45%) in our study is consistent with the results of previous studies in children 1 to 5 years of age (44 to 49%) and in children 6 to 24 months of age (66% [95% CI, 34 to 82] in the first year and −7% [95% CI, −247 to 67] in the second year), as well as with the results of most effectiveness studies.[10-12,22,25,27,28,41] In infants 6 to 59 months of age, administration of a live attenuated influenza vaccine led to 54.9% fewer cases of culture-

confirmed influenza, as compared with TIV; however, live attenuated influenza vaccine is not indicated for children under 24 months of age or for older children with a history of wheezing.[13,14] The use of TIV with the MF59 adjuvant, which we studied, is a potentially effective option for children 6 to less than 72 months of age, with the additional potential advantages of increased heterovariant coverage, a longer duration of protection, and, for some strains, protection after a single dose.

Supported by Novartis Vaccines and Diagnostics.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Gerlind Anders, Giovanni della Cioppa, Delphine Goux, Nicola Groth, Ralf Jaeger, Uwe Nicolay, Michele Pellegrini, Keith Veitch, and Nyree Williamson of Novartis Vaccines and Linda Gibbs of 4Clinics for their contributions in conducting the study, analyzing the data, and assisting in the preparation of the manuscript.

## REFERENCES

1. Fiore AE, Uyeki TM, Broder K, et al. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010. MMWR Recomm Rep 2010; 59(RR-8):1-62. [Errata, MMWR Recomm Rep 2010;59:993, 1147.]
2. Barker WH. Excess pneumonia and influenza associated hospitalization during influenza epidemics in the United States, 1970-78. Am J Public Health 1986;76:761-5.
3. Neuzil KM, Mellen BG, Wright PF, Mitchel EF Jr, Griffin MR. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. N Engl J Med 2000;342:225-31.
4. Poehling KA, Edwards KM, Weinberg GA, et al. The underrecognized burden of influenza in young children. N Engl J Med 2006;355:31-40.
5. Paget WJ, Balderston C, Casas I, et al. Assessing the burden of paediatric influenza in Europe: the European Paediatric Influenza Analysis (EPIA) project. Eur J Pediatr 2010;169:997-1008.
6. Glezen WP, Couch RB. Interpandemic influenza in the Houston area, 1974–76. N Engl J Med 1978;298:587-92.
7. Monto AS, Davenport FM, Napier JA, Francis T Jr. Modification of an outbreak of influenza in Tecumseh, Michigan, by vaccination of schoolchildren. J Infect Dis 1970;122:16-25.
8. Loeb M, Russell ML, Moss L, et al. Effect of influenza vaccination of children on infection rates in Hutterite communities: a randomized trial. JAMA 2010;303: 943-50.
9. Heikkinen T, Booy R, Campins M, et al. Should healthy children be vaccinated against influenza? A consensus report of the Summits of Independent European Vaccination Experts. Eur J Pediatr 2006;165: 223-8.
10. Jefferson T, Rivetti A, Harnden A, Di Pietrantonj C, Demicheli V. Vaccines for preventing influenza in healthy children. Cochrane Database Syst Rev 2008;2: CD004879.
11. Jefferson T, Smith S, Demicheli V, Harnden A, Rivetti A, Di Pietrantonj C. Assessment of the efficacy and effectiveness of influenza vaccines in healthy children: systematic review. Lancet 2005;365: 773-80.
12. Neuzil KM, Dupont WD, Wright PF, Edwards KM. Efficacy of inactivated and cold-adapted vaccines against influenza A infection, 1985 to 1990: the pediatric experience. Pediatr Infect Dis J 2001;20:733-40.
13. Rhorer J, Ambrose CS, Dickinson S, et al. Efficacy of live attenuated influenza vaccine in children: a meta-analysis of nine randomized clinical trials. Vaccine 2009;27:1101-10.
14. Belshe RB, Edwards KM, Vesikari T, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. N Engl J Med 2007;356:685-96.
15. Podda A. The adjuvanted influenza vaccines with novel adjuvants: experience with the MF59-adjuvanted vaccine. Vaccine 2001;19:2673-80.
16. O'Hagan DT. MF59 is a safe and potent vaccine adjuvant that enhances protection against influenza virus infection. Expert Rev Vaccines 2007;6:699-710.
17. Mannino S, Villa M, Weiss N, Apolone G, Rothman KJ. Effectiveness of influenza vaccination with Fluad versus a subunit influenza vaccine. Am J Epidemiol 2010;171:Suppl:S157. abstract.
18. Vesikari T, Pellegrini M, Karvonen A, et al. Enhanced immunogenicity of seasonal influenza vaccines in young children using MF59 adjuvant. Pediatr Infect Dis J 2009;28:563-71.
19. Vesikari T, Groth N, Karvonen A, Borkowski A, Pellegrini M. MF59-adjuvanted influenza vaccine (FLUAD) in children: safety and immunogenicity following a second year seasonal vaccination. Vaccine 2009;27:6291-5.
20. Puppe W, Weigl JA, Aron G, et al. Evaluation of a multiplex reverse transcriptase PCR ELISA for the detection of nine respiratory tract pathogens. J Clin Virol 2004;30:165-74.
21. Haemagglutination inhibition. In: Kendal AP, Pereira MS, Skehel JJ. Concepts and procedures for laboratory-based influenza surveillance. Atlanta: Centers for Disease Control and Prevention, 1982:B17-B35.
22. Hoberman A, Greenberg DP, Paradise JL, et al. Effectiveness of inactivated influenza vaccine in preventing acute otitis media in young children: a randomized controlled trial. JAMA 2003;290:1608-16.
23. World Health Organization. Influenza season 2010-2011. (http://www.eurofluorg/cgi-files/bulletin_v2.cgi.)
24. Levandowski RA, Regnery HL, Staton E, Burgess BG, Williams MS, Groothuis JR. Antibody responses to influenza B viruses in immunologically unprimed children. Pediatrics 1991;88:1031-6.
25. Sugaya N, Herome K, Ishida M, Matsumoto M, Mitamura K, Nirasawa M. Efficacy of inactivated vaccine in preventing antigenically drifted influenza type A and well-matched type B. JAMA 1994;272: 1122-6.
26. Belshe RB, Coelingh K, Ambrose CS, Woo JC, Wu X. Efficacy of live attenuated influenza vaccine in children against influenza B viruses by lineage and antigenic similarity. Vaccine 2010;28:2149-56.
27. Ritzwoller DP, Bridges CB, Shetterly S, Yamasaki K, Kolczak M, France EK. Effectiveness of the 2003-2004 influenza vaccine among children 6 months to 8 years of age, with 1 vs 2 doses. Pediatrics 2005; 116:153-9.
28. Allison MA, Daley MF, Crane LA, et al. Influenza vaccine effectiveness in healthy 6- to 21-month-old children during the 2003-2004 season. J Pediatr 2006;149: 755-62.
29. Clark TW, Pareek M, Hoschler K, et al. Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. N Engl J Med 2009;361:2424-35.
30. Arguedas A, Soley C, Lindert K. Responses to 2009 H1N1 vaccine in children 3 to 17 years of age. N Engl J Med 2010; 362:370-2.
31. Atmar RL, Keitel WA, Patel SM, et al. Safety and immunogenicity of nonadjuvanted and MF59-adjuvanted influenza A/H9N2 vaccine preparations. Clin Infect Dis 2006;43:1135-42.
32. Reyes F, Macey JF, Aziz S, et al. Influenza in Canada: 2005-2006 season. Can Commun Dis Rep 2007;33:21-41.
33. Ambrose CS, Wu X, Belshe RB. The efficacy of live attenuated and inactivated influenza vaccines in children as a function of time postvaccination. Pediatr Infect Dis J 2010;29:806-11.
34. Ansaldi F, Zancolli M, Durando P, et al. Antibody response against heterogeneous circulating influenza virus strains elicited by MF59- and non-adjuvanted vaccines during seasons with good or partial

matching between vaccine strain and clinical isolates. Vaccine 2010;28:4123-9.
**35.** Banzhoff A, Gasparini R, Laghi-Pasini F, et al. MF59-adjuvanted H5N1 vaccine induces immunologic memory and heterotypic antibody responses in non-elderly and elderly adults. PLoS One 2009;4(2):e4384.
**36.** Vesikari T, Karvonen A, Tilman S, et al. Immunogenicity and safety of MF59-adjuvanted H5N1 influenza vaccine from infancy to adolescence. Pediatrics 2010;126(4):e762-e770.
**37.** Pellegrini M, Nicolay U, Lindert K, Groth N, Della Cioppa G. MF59-adjuvanted versus non-adjuvanted influenza vaccines: integrated analysis from a large safety database. Vaccine 2009;27:6959-65.
**38.** Schultze V, D'Agosto V, Wack A, Novicki D, Zorn J, Hennig R. Safety of MF59 adjuvant. Vaccine 2008;26:3209-22.
**39.** Banzhoff A, Haertel S, Praus M. Passive surveillance of adverse events of an MF59-adjuvanted H1N1v vaccine during the pandemic mass vaccinations. Hum Vaccin 2011;7:539-48.
**40.** Black S, Della Cioppa G, Malfroot A, et al. Safety of MF59-adjuvanted versus non-adjuvanted influenza vaccines in children and adolescents: an integrated analysis. Vaccine 2010;28:7331-6.
**41.** Heinonen S, Silvennoinen H, Lehtinen P, Vainionpää R, Ziegler T, Heikkinen T. Effectiveness of inactivated influenza vaccine in children aged 9 months to 3 years: an observational cohort study. Lancet Infect Dis 2011;11:23-9.