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Trivalent and quadrivalent MF59®-adjuvanted influenza vaccine in young children: A dose- and schedule-finding study☆

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ABSTRACT

Young children are at increased risk for influenza infections and related complications. The protection offered to children by conventional trivalent inactivated influenza vaccines (TIV) is suboptimal, due to poor immunogenicity and a higher exposure to infection and complications in this age group, particularly from influenza B strains. In this dose-ranging, factorial design trial, we report the safety and immunogenicity of different combinations of adjuvanted (ATIV) and non-adjuvanted trivalent (TIV) and quadrivalent (QIV) influenza vaccines in 480 healthy children 6 to <36 months of age.

The results show that the second B strain added to TIV was immunogenic and did not affect immunogenicity of the other strains. The addition of the MF59® adjuvant promoted robust immune responses with notable elevations in antibodies observed even after one dose. A dose–response relationship was observed between the antibody response and MF59 adjuvant. No patterns in safety and tolerability emerged with different adjuvant and antigen doses nor with the addition of a second B strain. MF59-adjuvanted QIV offers potential advantages to young children.

1. Introduction

Influenza is associated with substantial morbidity in children [1,2], who also play a major role in the transmission and spread of influenza in households and the community during epidemics [3,4]. Vaccination of children against influenza brings important health benefits, directly to the children themselves and indirectly to persons in other age groups, by helping to control the spread of influenza [5,6]. However, meta-analyses reveal that immune responses conferred by conventional, nonadjuvanted, trivalent inactivated influenza vaccines (TIVs) do not appear to effectively induce protective antibodies in young unprimed children, resulting in only modest efficacy [7]. This trend appears particularly evident for the influenza B strain for which conventional nonadjuvanted vaccines generally exhibit lower immunogenicity to the B strain than to the influenza A antigens [8], although this may also be a consequence of lower sensitivity for antibodies to the B strain in the standard test [9]. Immune responses in young children can, however, be effectively enhanced with the use of the oil-in-water emulsion adjuvant MF59® (Novartis Vaccines and Diagnostics) [8].

Since the early 1980s, two antigenically dissimilar strains of influenza B virus have been present in the human population, one lineage represented by B/Victoria/2/87-like and the other by B/Yamagata/16/88-like viruses [10]. Notably, since 1988, both have simultaneously been associated with significant numbers of infections in various locations worldwide [10,11]. A recent study demonstrated that influenza B infections accounted for approximately one fifth of all influenza-related hospitalizations in children under 16 years of age in Finland [12]. Moreover, proportionately more influenza B strain infections occur in children compared with older populations [13]. In 5 of the past 10 influenza seasons in the United States (2001–2010), the predominant circulating influenza B virus lineage did not match the strain contained in the vaccine [14]. Hence, as two influenza B virus lineages cocirculate, and there is little cross-protection between the two lineages [15,16], the protection offered by only one B virus lineage in each year's TIV exposes children to insufficient protection in case of a mismatch [17–19]. Recent observations of influenza trends demonstrate that

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in years in which the recommended influenza B strain in TIV is poorly matched to the circulating B strain, up to 29% of influenza cases in a population might be attributed to the circulation of the other influenza B strain which had not been included in the vaccine [20]. A potential strategy to cover this still unmet medical need is a quadrivalent formulation that includes a second B strain from the alternative lineage.

The present study in healthy children 6 to <36 months of age is a dose-ranging factorial design trial to evaluate the safety, tolerability and immunogenicity of different vaccine formulations with different doses of MF59 adjuvant and/or a second B strain (quadrivalent influenza vaccine [QIV]) when added to either high or low doses of a purified subunit influenza vaccine.

2. Methods

This multicenter, randomized, observer-blind, dose-ranging, factorial design, clinical trial in healthy children aged 6 to <36 months was performed in 10 study centers in Finland and 5 centers in Belgium. Data were collected between October 2008 and March 2009. The study was undertaken in compliance with Good Clinical Practice guidelines and the Declaration of Helsinki, and the study protocol was approved by the National Ethics Committee of Finland and the individual ethics review committees of participating centers in Belgium. Before enrollment, written informed consent was obtained from parents or legal guardians.

2.1. Objectives

Immunogenicity objectives of this study were (i) to evaluate whether a one-dose schedule of any formulation provides a similar immunogenicity to a two-dose schedule (two 0.25-mL vaccinations of adjuvanted [7.5 μg TIV/QIV + 50% MF59] (ATIV) and nonadjuvanted [7.5 μg TIV/QIV + 0% MF59] TIV formulations); (ii) to evaluate the immunogenicity of the study vaccine formulations according to the European Medicines Agency (EMEA) recommendations (CPMP/BWP/214/96); (iii) to determine whether the addition of a second influenza B strain to a TIV is immunogenic against the second B strain and whether it affects the immune response against the first influenza B strain or the A/H3N2 and A/H1N1 strains; (iv) to assess the dose–response relationship between the MF59 dose and strain-specific antibodies; (v) to compare the study vaccine formulations to an approved nonadjuvanted pediatric TIV (Vaxigrip®, Sanofi Pasteur) and to the vaccine formulated with 7.5 μg per antigen and 50% of the approved MF59 dose in elderly adults (ATIV). Safety and tolerability objectives were to evaluate solicited local and systemic reactogenicity and spontaneously reported adverse events (AEs) and serious AEs (SAEs).

2.2. Subjects

A total of 595 healthy subjects of 6 to <36 months of age were planned to be enrolled in this study. Ultimately, 410 subjects were randomized owing to the slower than expected recruitment, which was stopped close to the middle of the influenza season. The main exclusion criteria were any history or ongoing chronic illness likely to interfere with the results; any history or likelihood of anaphylaxis or adverse reactions to vaccine components; any known or suspected impairment/alteration of the immune system (including use of immunosuppressive therapy, receipt of immunostimulants, blood, blood products, and/or plasma derivatives or any parenteral immunoglobulin preparation in the past 12 weeks before enrollment); and any history of progressive or severe neurologic disorder, nonfebrile seizures, clinically suspected development delay, or bleeding diathesis. Additional exclusion criteria were receipt of any other vaccine within 2 weeks (inactivated vaccines) or 4 weeks (live vaccines) before enrollment, of influenza vaccine or laboratory confirmed influenza within 6 months before the enrollment visit, of any other investigational product within 90 days of enrollment, rectal temperature ≥38.5 °C, and/or any acute illness within 3 days before enrollment visit, unwillingness to refuse participation in another clinical trial before study completion, or a surgery planned during the study period that would interfere with the study visit schedule.

2.3. Study procedures

Subjects were randomly assigned on a 1:1 ratio to one of the 17 study groups (A–Q) as listed in Table 1. The vaccine study groups included combinations of 7.5-μg or 15-μg doses of each TIV strain and 0%, 12.5%, 25%, 50%, or 100% of the MF59 adjuvant dose approved in Europe for elderly adults (Fluad®, Novartis Vaccines and Diagnostics) and, for QIV, the addition of 7.5 μg or 15 μg of a second influenza B strain. Group Q refers to the marketed pediatric TIV comparator, Vaxigrip. All participating children were to be followed up through Day 29 (study groups O and P) or Day 50 (study groups A–N and Q). Subjects in groups A–N and Q were to receive two doses of the assigned study vaccine 4 weeks apart, on Days 1 and 29, whereas participants in study groups O and P with full-dose MF59 adjuvant were to receive one vaccination on Day 1 only. Vaccines were to be administered intramuscularly, preferably in the deltoid muscle of the nondominant arm (children 24–35 mo of age) or the anterolateral aspect of the thigh (children <24 mo of age). Time points for immunogenicity assessments were on Day 1 (prevaccination), Day 29 (4 wk after dose 1), and Day 50 (3 wk after dose 2). Blood samples of approximately 5 mL were obtained by venipuncture for immunogenicity analyses. Time points for safety assessments were Day 1 (prevaccination baseline to 30 min after vaccination), Day 8 (1 wk after dose 1), Day 29 (4 wk after dose 1), Day 36 (1 wk after dose 2), and Day 50 (3 wk after dose 2). Solicited local and systemic adverse reactions were to be recorded on a diary card for 7 consecutive days following the first and second vaccinations. All reports of spontaneous AEs and concomitant medications were to be collected throughout the entire study period and reported at the subsequent study visit. Any SAE was to be reported immediately to the study sponsor.

2.4. Vaccines

Trivalent influenza vaccines each contained 7.5mcg or 15mcg of hemagglutinin antigen (HA) from each of the three World Health Organization recommended influenza strains for the 2008–2009 influenza season in the northern hemisphere: influenza A/Brisbane/59/2007 (A/H1N1)-like virus, influenza A/Brisbane/10/2007 (A/H3N2)-like virus, and influenza B/Florida/4/2006-like virus (of the influenza B/Yamagata lineage). Influenza B/Malaysia/2506/2004-like antigen virus (Victoria lineage) was selected as the second B strain at 7.5mcg or 15mcg dose. The MF59 adjuvant is an oil-in-water emulsion, first licensed for use in seasonal influenza vaccines in 1997 and of which more than 50 million doses have been delivered in Europe. At the time of this publication, influenza vaccines including MF59 adjuvant have been administered to over 4100 children in clinical studies (Novartis Vaccines and Diagnostics, data on file). In these studies, the safety profile of the adjuvanted vaccine was similar to one of the nonadjuvanted comparator influenza vaccines [21–23], except for increases in mostly mild and local reactogenicity. A standard dose of the adjuvant MF59, as used in one 0.5-mL dose of the commercially available seasonal influenza vaccine Fluad, contains 9.75 mg squalene, 1.175 mg polysorbate 80, 1.175 mg sorbitan trioleate, 0.66 mg sodium citrate, 0.04 mg citric acid, and water for injection. Each TIV or QIV formulation contained one of the following four MF59 doses (as % of commercial ATIV for eldrely adults,

Table 1
Vaccine formulations and study groups.

	Dose of second B strain (μg)	Dose of each TIV strain (μg)	Adjuvant (MF59)				
			0% of adult dose	12.5% (1/8) of adult dose	25% (1/4) of adult dose	50% (1/2) of adult dose	100% (1/1) of adult dose
TIV	0	7.5	Group A (2 × 0.25 mL)	Group E (2 × 0.25 mL)	Group G (2 × 0.25 mL)	Group K (2 × 0.25 mL)	–
	0	15.0	Group B Agrippal[a] (2 × 0.50 mL)	–	Group H (2 × 0.50 mL)	Group L (2 × 0.50 mL)	Group O Fluad[a] (1 × 0.50 mL)
Quadrivalent influenza vaccine	7.5	7.5	Group C (2 × 0.25 mL)	Group F (2 × 0.25 mL)	Group I (2 × 0.25 mL)	Group M (2 × 0.25 mL)	–
	15.0	15.0	Group D (2 × 0.50 mL)	–	Group J (2 × 0.50 mL)	Group N (2 × 0.50 mL)	Group P (1 × 0.50 mL)
Marketed pediatric TIV comparator	0	7.5	Group Q Vaxigrip[a] (2 × 0.25 mL)	–	–	–	–

[a] Commercially available vaccines: group B: Aggripal (adult), group O: Fluad (adult), group Q: Vaxigrip (pediatric).
TIV: trivalent influenza vaccine.

Fluad@ Novartis Vaccines and Diagnostics): 0% (non adjuvanted), 25%, 50%, 100%. Vaccines were supplied in monodose, prefilled syringes filled with either approximately 0.5 mL or 0.25 mL of vaccine, depending on the vaccine formulation (Table 1). The vaccine formulations were prepared and administered by blinded study personnel, who otherwise did not participate in evaluation of the subjects during the study.

2.5. Immunogenicity assessment

Blood samples taken for immunologic assays were centrifuged, and sera were stored at −18 °C or below until shipped to the Novartis Vaccines Clinical Serology Laboratory in Marburg, Germany, for analysis. Antibody responses were measured by hemagglutination inhibition (HI), according to standard methods [24]. HI antibody responses on Days 1, 29, and 50 were expressed as geometric mean titer (GMT), geometric mean ratio (GMR) of the postvaccination to prevaccination titer (Day 29/Day 1 titer and Day 50/Day 1 titer); seroprotection rates were defined as the percentage of subjects with HI titers ≥40, and seroconversion rates were defined as the percentage of subjects per group achieving at least a 4-fold increase in HI titer from a seropositive prevaccination titer (≥10) or a rise from <10 to ≥40 in those who were prevaccination seronegative.

2.6. Safety and tolerability assessment

Solicited local reactions reported via diary card included ecchymosis, erythema, induration, swelling, and tenderness at injection site; solicited systemic reactions included sleepiness, diarrhea, vomiting, irritability, change in eating habits, shivering, and unusual crying. Other indicators of reactogenicity were body temperature (preferably rectal temperature measurement) and use of analgesics/antipyretic medication for vaccination-induced fever. Local and systemic reactions were graded for severity according to standardized scales, following the Center for Biologics Evaluation and Research (CBER) Guidance [25,26]. The severity of adverse reactions was categorized as none, mild (grade 1), moderate (grade 2), severe (grade 3), or potentially life threatening (grade 4).

2.7. Statistical analyses

2.7.1. Sample size

Assuming log-normal distributed antibody titer, a minimum relevant difference of factor 2.5, a common standard deviation of 0.45 for the $\log_{10}$ titer, a 1-sided experiment-wise error of 5%, an 80% any-pair power, and a 9% dropout rate resulted in a total of 595 subjects ($n = 35$ in each study group) [27]. As groups could be combined, detectable minimum relevant differences (80% marginal power; 2-sided alpha 5%) ranged between 1.64 ($n = 70$/group) and 1.80 ($n = 70$/group) when using t tests and the same assumptions as above. Population characteristics were summarized per vaccine group.

2.7.2. Immunogenicity analyses

Immunogenicity analyses were run on the per-protocol set (PPS), whereas safety was analyzed for all subjects exposed. Log_{10}-transformed antibody titers were modeled using analysis of covariance (ANCOVA) for each strain and time point separately. Vaccine groups were included as qualitative factor, and strain-specific prevaccination titers served as covariate. Point estimates (geometric means, ratio of geometric means), their 2-sided 95% confidence intervals, and p values for testing noninferiority and superiority hypotheses were derived from the models. Noninferiority and superiority margins of 0.67 and 1.00, respectively, were prespecified, and significance was declared if the 1-sided p value was below 0.025. For the analysis of differences, a 2-sided

p value less than 0.05 was required. Immunogenicity data were also analyzed based on HI licensure criteria according to EMEA recommendations (CPMP/BWP/214/96) [28]. There are no criteria for immunogenicity in children established by EMEA; the closest standards are those used for an adult population. For the analyses of the TIV strains, vaccine groups were combined (pooled by MF59 doses and across identical TIV antigen levels) after having demonstrated that the second B strain had no impact on the antibody response of the TIV strains. A similar pooling was done for the analysis of the second B strain as the TIV antigen levels had no impact on the antibody response to the second B strain. The dose–response relationship was modeled using two approaches: (1) linear regression analysis with $\log_{10}$-transformed titer as dependent and MF59 dose as independent quantitative variable and by means of a (2) prevaccination titer adjusted ANCOVA with 3 pooled vaccine groups, each consisting of both TIV and both QIV formulations, for 0%, 25%, and 50% MF59 doses. MF59 doses of 12.5% and 100% could not be used because they were either employed in only one TIV and one QIV formulation or only one vaccination was given.

2.7.3. Safety and tolerability analyses

Safety and tolerability data were summarized by vaccine group providing the percentage of subjects reporting an event.

3. Results

3.1. study subjects

A total of 410 healthy children 6 to <36 months old were enrolled in the study. All subjects were vaccinated: 395 (96%) subjects were included for immunogenicity analyses (PPS) at baseline, 322 (79%) subjects were included at Day 29, and 282 (77.9% of enrolled subjects that were to receive second vaccination) subjects were included at Day 50. All subjects were evaluated for safety. Subjects were predominantly white, mean age ranged from 14.2 to 20 months across study groups, and boys and girls were evenly distributed in most groups (Table 2). There were no noteworthy differences in weight and height. A total of 11 children (2.7% of overall population) had previously received an influenza vaccination, and all participants met the entry criteria.

3.2. Immunogenicity

Prevaccination, there were low levels of antibodies against the four vaccine strains in the 395 subjects tested, with 14.7%, 10.6%, 10.4% and 1.0% having titers $\geq$10 against the H1N1, H3N2, B Florida and B Malaysia strains, respectively.

(i) In the assessment of whether a one-dose schedule of any formulation provides similar immunogenicity to two 0.25-mL vaccinations of adjuvanted (7.5 μg TIV/QIV + 50% MF59) or nonadjuvanted (7.5 μg TIV/QIV) formulations, it was found that no formulation was noninferior after one vaccination compared with two vaccinations of the adjuvanted comparator (7.5 μg TIV/QIV + 50% MF59). Vice versa, all adjuvanted formulations were noninferior after one vaccination compared with two vaccinations of the nonadjuvanted comparator (7.5 μg TIV/QIV) for the H1N1 and H3N2 strains. For the first B strain, only the adjuvanted formulations with either 50% or 100% MF59 were noninferior after one vaccination, and for the second B strain, only 15 μg + 100% MF59 were noninferior after one vaccination compared with two vaccinations of the nonadjuvanted comparator (7.5 μg TIV/QIV).

(ii) Evaluation of GMRs, seroprotection, and seroconversion rates according to Committee for Medicinal Products for Human Use (CHMP) criteria across vaccine groups and virus strains are presented in Table 3 (A/H1N1, A/H3N2, first B strain, second B strain). On Day 29, 4 weeks after the first vaccination, strong immune responses for the A strains were evident for the adjuvanted formulations, and all three CHMP criteria were met for all the adjuvanted vaccine groups. For the first B strain (Florida), only 15 μg TIV/QIV formulated with a half or full MF59 dose met any of the criteria (GMR) after the first vaccination. After two vaccinations, all three CHMP criteria were met for the three TIV strains by all adjuvanted formulations. None of the nonadjuvanted formulations – 7.5 and 15 μg TIV/QIV or the licensed pediatric comparator Vaxigrip (7.5 μg TIV) – met all CHMP criteria after either first or second vaccination. For the second B strain (Malaysia), after one vaccination none of the QIV formulations met any CHMP criterion; after the second vaccination, all adjuvanted QIV formulations met all CHMP criteria, whereas none of the nonadjuvanted QIV formulation met any CHMP criterion.

(iii) As expected, antibody responses to the second influenza B strains were greater in children who were vaccinated with QIV rather than TIV strains. The addition of the B/Malaysia strain to TIV did not significantly impact ($p > 0.05$ for all strains and visits) the antibody responses against A/H1N1, A/H3N2, or B/Florida strains. GMT ratios of pooled QIV to pooled TIV antigen groups for Day 29 and Day 50 were in the following ranges: 0.99–1.23 for A/H1N1, 0.71–0.83 for A/H3N2, and 0.85–1.07 for B/Florida.

(iv) Linear regression analyses showed significant increases in antibody response with increasing MF59 dose for all four influenza strains. The slopes and corresponding CI for lower (7.5 μg) and higher (15 μg) antigen doses are specified in terms of factors. Increasing the MF59 content from 0% to 100% translates for lower (7.5 μg) and higher (15 μg) antigen dose, respectively, into an increase in antibody titer (95% CI) of 33.1 (9.3–117.5) and 117.5 (38–363.1) for A/H1N1, 30.2 (9.3–97.7), and 93.3 (32.4–269.2) for H3N2, 20.4 (8.1–52.5), and 91.2 (37–229.1) for first B strain, and 26.9 (6.8–114.8) and 52.5 (14.8–186.2) for second B strain, respectively. The slopes measured at the higher antigen dose were consistently greater than those measured at the lower antigen dose, suggesting that increasing antigen dose in combination with increasing MF59 dose is associated with higher antibody responses to these influenza strains.

All low-and high-antigen dose levels for 0%, 25%, and 50% MF59 doses were pooled to increase sample size. Pooling analyses could not be performed for the remaining two MF59 doses, 12.5% and 100%, because these had either only low or only high antigen dose. Substantially increased GMTs are evident for pooled 25% MF59 dose groups compared with nonadjuvanted pooled groups, as shown by a 5- to 7-fold increase in GMTs at Day 50 for all virus strains (Fig. 1A–D). When comparing the pooled group with 25% MF59 doses with the pooled group with 50% MF59 dose, GMTs at Day 29 show additional increases of approximately 28% for A/H1N1, 29% for A/H3N2, 24% for B/Florida, and 8% for B/Malaysia, and at Day 50, GMTs show additional increases of approximately 23% for H1N1, 13% for H3N2, 36% for B\Florida, and 9% for B\Malaysia (Fig. 1A–D).

(v) All MF59-adjuvanted formulations but none of the nonadjuvanted formulations induced superior antibody responses after the second vaccination against all virus strains compared with the pediatric nonadjuvanted licensed comparator Vaxigrip (7.5 μg TIV). None of the other study vaccine groups met the noninferiority criterion for GMT against any of the four strains (H1N1, H3N1, B/Florida, B/Malaysia) when compared with the vaccine formulated with 7.5-μg TIV/QIV adjuvanted with 50% MF59 dose.

Table 2
Demographic characteristics.

Vaccine group	Age (months)	Sex boys/girls (%)	Weight (kg)	Height (cm)	Race Asian/black/white (%)
Group A (n = 25) 7.5 μg TIV + 0% MF59	20 (7)	44/56	11.4 (1.7)	84.1 (8.3)	0/8/88
Group B (n = 22) 15 μg TIV + 0% MF59	15 (8.8)	73/27	11 (2.9)	79.5 (8.7)	0/9/82
Group C (n = 25) 7.5 μg QIV + 0% MF59	18 (8.9)	64/36	11.3 (2.4)	81.8 (8.7)	4/4/88
Group D (n = 28) 15 μg QIV + 0% MF59	15.2 (7.8)	54/46	10.8 (2.2)	80 (9.1)	4/4/89
Group E (n = 24) 7.5 μg TIV + 12.5% MF59	18.5 (9.3)	54/46	11.3 (2.3)	82.1 (8.3)	4/4/92
Group F (n = 23) 7.5 μg QIV + 12.5% MF59	17 (9)	39/61	10.8 (2.1)	80.6 (8.7)	0/17/78
Group G (n = 23) 7.5 μg TIV + 25% MF59	16.4 (7.4)	43/57	11 (2.1)	80.3 (7.3)	0/0/91
Group H (n = 21) 15 μg TIV + 25% MF59	15.4 (7.6)	38/62	10.8 (2.3)	79.1 (8.2)	0/5/95
Group I (n = 24) 7.5 μg QIV + 25% MF59	16.6 (8.8)	46/54	11 (2.3)	81.2 (8.9)	4/4/92
Group J (n = 24) 15 μg QIV + 25% MF59	16 (9.4)	50/50	10.8 (2.7)	80 (10.4)	0/4/96
Group K (n = 27) 7.5 μg TIV + 50% MF59	19 (9.4)	59/41	11 (2.5)	82.1 (9.5)	4/7/89
Group L (n = 23) 15 μg TIV + 50% MF59	18.3 (8.7)	35/65	11.1 (2.4)	81 (9)	9/13/78
Group M (n = 22) 7.5 μg QIV + 50% MF59	16.3 (9)	41/59	10.5 (2.2)	80.5 (8.1)	0/5/95
Group N (n = 25) 15 μg TIV + 50% MF59	15.4 (9.2)	52/48	10.4 (2.4)	78.5 (9.9)	0/0/100
Group O (n = 26) 15 μg TIV + 100% MF59	14.2 (7.1)	42/58	10.6 (1.8)	78.7 (8.2)	8/12/77
Group P (n = 22) 15 μg QIV + 100% MF59	17.8 (8.7)	73/27	11.1 (2.4)	80.8 (7.8)	5/5/91
Group Q (n = 26) 7.5 μg TIV + 0% MF59	16.1 (8.5)	50/50	10.8 (1.8)	80 (8.2)	0/12/81

TIV: trivalent inactivated vaccine, QIV: quadrivalent inactivated vaccine.
For age, weight and height the standard deviation is given in parentheses.

3.3. Safety and tolerability

All 410 enrolled subjects were included in the data set for the assessment of safety and tolerability. The proportions of subjects per antigen dose and per MF59 adjuvant dose with reported local or systemic reactions after the first or second vaccine doses are illustrated in Fig. 2A and B, respectively. As already mentioned, the 100% MF59 groups received only one vaccination; all other groups received two. The percentages of children with any local reaction ranged from 29% to 59% across vaccine groups (50% for the comparator licensed vaccine, Vaxigrip); percentages of children with any systemic reactions ranged from 35% to 68% (46% for Vaxigrip). There was no tendency for an increase in local and systemic solicited reactions with increasing MF59 content, either in

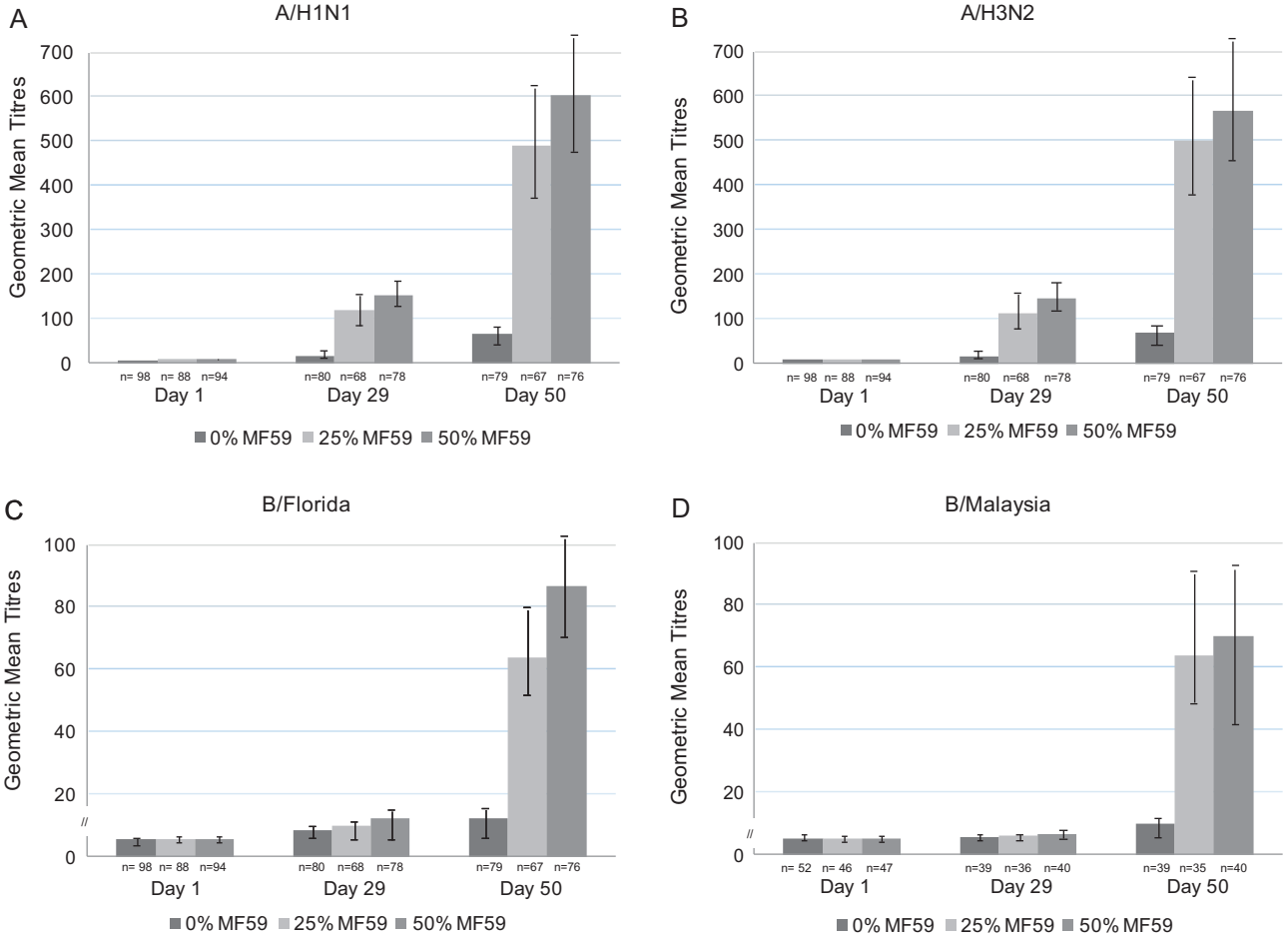

Fig. 1. Prevaccination titer adjusted geometric mean titers (GMTs) at Days 29 and 50, and corresponding 95% CIs for MF59 at 0%, 25%, and 50% of adult Fluad dose across pooled TIV and QIV formulations for A/H1N1 (A), A/H3N2 (B), B/Florida (C) and B/Malaysia (D) influenza strains.

Table 3
Immunogenicity evaluation according to CHMP criteria against A/H1N1, A/H3N2, first B strain (Florida), and second B strain (Malaysia).

Vacc.	7.5 μg TIV		7.5 μg TIV/QIV[1]		15 μg TIV/QIV[2]		7.5 μg TIV/QIV 12.5% MF59[3]		7.5 μg TIV/QIV 25% MF59[4]		15 μg TIV/QIV 25% MF59[5]		7.5 μg TIV/QIV 50% MF59[6]		15 μg TIV/QIV 50% MF59[7]		15 μg TIV+ 100% MF59[8]	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
A/H1N1																		
N	**23**	**24**	**37**	**37**	**43**	**42**	**36–37**	**35–36**	**34–35**	**33–34**	**33**	**33**	**41**	**40**	**36–37**	**35–36**	**36**	NA
SC[c] or SI[d]	39%	**96%**	14%	**62%**	21%	**79%**	**81%**	**97%**	**79%**	**100%**	**85%**	**100%**	**88%**	**100%**	**94%**	**100%**	**94%**	NA
SP[a]	43%	**96%**	16%	65%	21%	**79%**	**81%**	**97%**	**77%**	**100%**	**85%**	**100%**	**88%**	**100%**	**95%**	**100%**	**94%**	NA
GMR[b]	**4.44**	**25**	1.98	**8.15**	1.83	**10**	**14**	**72**	**14**	**61**	**19**	**65**	**16**	**78**	**25**	**84**	**24**	NA
A/H3N2																		
SC[c] or SI[d]	35%	**92%**	22%	**62%**	9%	**71%**	**81%**	**97%**	**85%**	**94%**	**88%**	**100%**	**98%**	**100%**	**92%**	**100%**	**94%**	NA
SP[a]	35%	**92%**	30%	70%	12%	**71%**	**84%**	**100%**	**89%**	**100%**	**88%**	**100%**	**98%**	**100%**	**92%**	**100%**	**97%**	NA
GMR[b]	**4.31**	**27**	2.22	**8.7**	1.91	**9.91**	**10**	**61**	**13**	**57**	**17**	**77**	**18**	**72**	**21**	**76**	**23**	NA
1st B strain																		
SC[c] or SI[d]	22%	**42%**	14%	19%	7%	12%	11%	**83%**	15%	**88%**	9%	**85%**	12%	**90%**	22%	**97%**	17%	NA
SP[a]	22%	42%	14%	19%	7%	12%	11%	**83%**	14%	**88%**	9%	**85%**	12%	**90%**	22%	**97%**	17%	NA
GMR[b]	1.8	**4.06**	1.54	2.39	1.28	2.07	1.73	**11**	1.18	**10**	1.62	**13**	1.67	**14**	**3.3**	**18**	**2.54**	NA

Vacc.	7.5 μg QIV[10]		15 μg QIV[11]		7.5 μg QIV 12.5% MF59[12]		7.5 μg QIV 25% MF59[13]		15 μg QIV 25% MF59[14]		7.5 μg QIV 50% MF59[15]		15 μg QIV 50% MF59[16]		15 μg QIV 100% MF59[17]	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
2nd B strain																
N	**17–18**	**18**	**22**	**21**	**17–18**	**17–18**	**17–18**	**17–18**	**18**	**17**	**19**	**19**	**21**	**21**	**18–21**	NA
SC[c] or SI[d]	6%	17%	0%	14%	0%	**76%**	0%	**82%**	6%	**76%**	0%	**79%**	5%	**95%**	17%	NA
SP[a]	6%	17%	0%	14%	0%	**72%**	0%	**83%**	6%	**76%**	0%	**79%**	5%	**95%**	17%	NA
GMR[b]	1.28	2.16	1	1.94	1.18	**8.85**	1.18	**13**	1.26	**12**	1.24	**14**	1.44	**14**	2.12	NA

[1]Groups A and C; [2]groups B and D; [3]groups E and F; [4]groups G and I; [5]groups H and J; [6]groups K and M; [7]groups L and N; [8]group O and P; [9]group Q; [10]group C; [11]group D; [12]group F; [13]group I; [14]group M; [15]group J; [16]group N; [17]group P. NA: not applicable because only one vaccination given.

Bold: CHMP criteria met (i.e., seroprotection rate > 70%, seroconversion or significant increase rate > 40%, geometric mean ratio > 2.5).

[a] SP refers to seroprotection and is defined as an HI titer ≥40.

[b] GMR: ratios of Day 50/Day 1 or Day 29/Day 1 geometric mean HI titers.

[c] SC refers to seroconversion and is defined as negative prevaccination serum (i.e., HI titer < 10) and post-vaccination HI titer ≥40.

[d] SI refers to significant increase and is defined at least a 4-fold increase from non-negative (≥10) prevaccination HI titer.

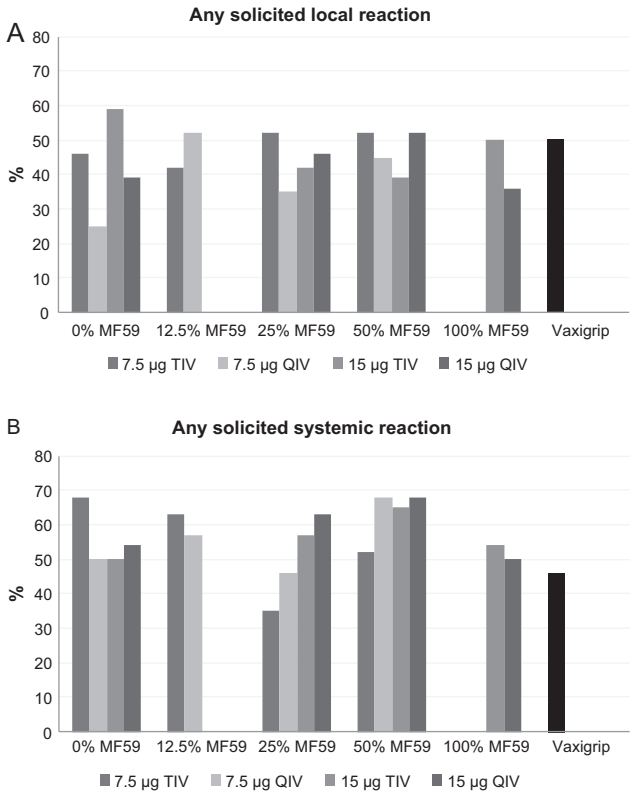

Fig. 2. Proportion of subjects with any solicited local (A) and systemic (B) reactions after first or second vaccination (combined) per 7.5-μg and 15-μg trivalent and quadrivalent vaccine strains, and per 0%, 12.5%, 25%, 50%, or 100% MF59 dose. 100% MF59 dose only received one vaccination. Black bar refers to the licensed 7.5-μg trivalent pediatric comparator Vaxigrip.

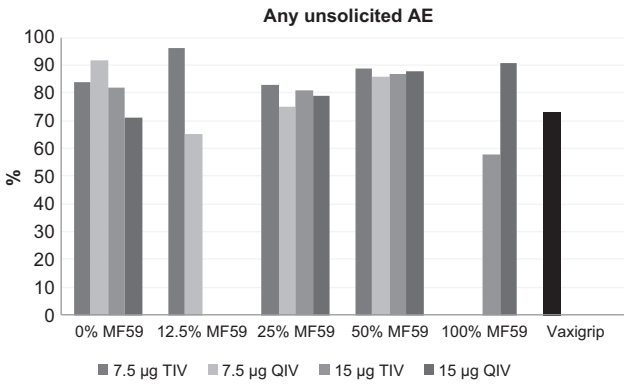

Fig. 3. Proportion of subjects with any unsolicited adverse events after first or second vaccination (combined) per 7.5-μg and 15-μg trivalent and quadrivalent vaccine strains, and per 0%, 12.5%, 25%, 50%, or 100% MF59 dose. 100% MF59 dose only received one vaccination. Black bar refers to the licensed 7.5-μg trivalent pediatric comparator Vaxigrip.

terms of frequency or severity. Reactogenicity of the 7.5-μg TIV/QIV formulations was slightly lower than for the corresponding 15-μg formulations. Most commonly reported local reactions were erythema (12–44% across groups), followed by tenderness (5–41% across groups). Local reactions were all mild or moderate, transient, and mostly observed within 1–4 days following vaccination, with first onset peaking at 6 h postvaccination, and resolving within 7 days. Irritability was the most frequently reported systemic reaction (12–43% across groups). For each solicited systemic reaction, the frequency and duration was as expected in a study of influenza vaccination in a pediatric population.

Spontaneously reported AEs occurred in 58–96% of children across vaccine groups (Fig. 3) of which 4–36% were considered at least possibly related. There was no dose-response pattern for MF59 adjuvant and antigen dose for unsolicited AEs. Overall, the most frequently observed unsolicited AEs were upper respiratory tract infection, cough, pyrexia, crying, diarrhea, and irritability. Of those considered at least possibly related to vaccination, the most common were pyrexia, cough, and irritability. All possibly and probably related AEs reported were known common side effects of influenza vaccination in children. Furthermore, the majority of unsolicited AEs were mild or moderate in severity. Two subjects in the MF59-adjuvanted vaccine groups experienced hypersensitivity events, that is, allergic reaction and worsening of allergic reaction, which lasted 1 and 4 days, respectively; both events were rated as mild and assessed as not related to the study vaccine. A total of nine SAEs were reported and were distributed across vaccine groups with no clustering related to adjuvant or antigen content. One SAE (lymphadenitis) was reported in the nonadjuvanted 7.5-μg QIV group, 1 SAE (gastroenteritis) in the nonadjuvanted 15-μg QIV group, 1 SAE (pyelonephritis) in the 7.5-μg TIV + 12.5% MF59 group, 1 SAE (pneumonia) in the 15-μg QIV + 25% MF59 group, 2 SAEs (laryngitis, gastroenteritis) in the 15-μg TIV + 50% MF59 group, 1 SAE (gastroenteritis) in the 7.5-μg QIV + 50% MF59 group, 1 SAE (gastroenteritis rotavirus) in the 15-μg QIV + 50% MF59 group, and 1 SAE (gastroenteritis rotavirus) in the nonadjuvanted 7.5-μg TIV pediatric comparator group. None of the SAEs was considered to be related to the study vaccine. There were no deaths. One subject discontinued from the study owing to an AE (an ear infection), which was considered unrelated to vaccination.

4. Discussion

Previous reports have shown that unprimed young children respond suboptimally to influenza vaccines. Indeed, a recent Cochrane analysis implies that conventional TIVs have limited efficacy in young children and toddlers [7], who are at higher risk for influenza-related complications [1]. Vaccine efficacy may be even further compromised when there is a mismatch between the virus strains included in the vaccine and the actual strains in circulation [29]. Since the early 1980s, two distinct lineages of B influenza strains have often cocirculated in humans, and there is no cross-protection between these lineages. Adding an additional B strain to the traditional TIV is expected to significantly broaden the coverage of the vaccine, while adding an adjuvant such as MF59 has been shown to enhance the immune responses in general and broaden it to cover heterologous strains [30–32].

In this study we found that the addition of the second B strain improved immunologic response to the matched antigen and did not negatively affect immunogenicity of the other virus strains. Furthermore, the MF59-adjuvanted vaccine formulations induced superior antibody responses compared with nonadjuvanted comparators for all virus strains. Both 7.5-μg and 15-μg MF59-adjuvanted formulations met all three EU CHMP licensure criteria specified for adults after the first vaccination for A/H1N1 and A/H3N2 strains and after the second vaccination for the two B strains. In contrast, none of the nonadjuvanted vaccine formulations – 7.5 and 15 μg TIV/QIV or the licensed pediatric comparator Vaxigrip (7.5 μg TIV) – met CHMP criteria for licensure after the first or second vaccinations in this population of children aged 6 to <36 months in this study. Further, results show that a single vaccination of MF59-adjuvanted vaccine might induce generally high antibody responses to influenza A strains, which may have important clinical implications, for example, in case of vaccination late in the influenza season or in case of poor compliance with the two-dose schedule.

Adjuvants have been developed to improve the performance of vaccines, and MF59, an oil-in-water emulsion containing naturally occurring squalene, has been approved for human use since 1997 as an influenza subunit vaccine adjuvant for elderly adults [23]. Previous clinical studies have demonstrated that MF59-adjuvanted influenza vaccines induced higher and broader antibody responses than nonadjuvanted vaccines, especially in subjects with low pre-vaccination antibody titers, in vulnerable populations including the elderly and those with underlying chronic conditions, and, most recently, in young children [8,22,33–35]. We observed a dose–response trend for immunogenicity with increasing MF59 dose for all influenza stains. GMTs at Day 29 and Day 50 for pooled MF59 dose groups (7.5 and 15 μg antigens combined) showed additional increases of 28% and 23% for A/H1N1, 29% and 13% for A/H3N2, 24% and 36% for B/Florida, and 8% and 9% for B/Malaysia, respectively, for the pooled group with 50% over the one with 25% of the adult MF59 dose group. Also, results from regression analyses suggest that increasing antigen dose (15 μg vs 0 and 7.5 μg) in combination with increasing MF59 dose was associated with higher antibody responses for A/H1N1, A/H3N2, and first B strains.

The overall safety and tolerability profiles were as expected in this age group and were in line with previous studies using MF59-adjuvanted vaccines in young children [8,34]. There was no tendency for an increase in local or systemic solicited reactions nor in unsolicited AEs with increasing MF59 content in terms of either frequency or severity. However, the reactogenicity and safety profile of the full MF59 dose level could not be adequately assessed because only one vaccination was given in the corresponding groups and because of limited experience with high MF59 doses in young children. Reactogenicity of the 7.5-μg TIV/QIV formulations was slightly lower than for the corresponding 15-μg formulations, but inclusion of the second B strain did not appear to affect reactogenicity. Altogether, the safety results did not reveal an increased risk associated with MF59 dose, antigen dose, or the addition of a second B strain.

In conclusion, this study confirms that the immunogenicity of nonadjuvanted influenza vaccines is suboptimal in young children and indicates that

- Addition of MF59 adjuvant promotes HI antibody responses to levels associated with protection in adults to an extent not achieved by nonadjuvanted vaccines and with no impact on reactogenicity and safety in these young children
- While the combination of the 7.5-μg antigen and 50% MF59 appears to offer the best balance between significantly improved immunogenicity and good tolerability, the incremental increase over 25% MF59 was relatively small, and this dose may be considered if future experience raises any concerns over reactogenicity
- A second influenza B strain combined with the traditional TIV vaccine is immunogenic and does not affect immunogenicity of the other three influenza strains
- The MF59-adjuvanted TIV and QIV vaccines already show a meaningful immune response to influenza A strains after one dose, which may be beneficial in real-life clinical practice where a second dose is often missed, but the two-dose vaccination schedule must continue to be recommended in young children to provide protection against influenza B strains.

Further studies of quadrivalent and MF59-adjuvanted influenza vaccines in young children, including efficacy trials, are warranted.

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References

[1] Thompson WW, Shay DK, Weintraub E, Brammer L, Bridges CB, Cox NJ, et al. Influenza-associated hospitalizations in the United States. JAMA 2004;15(292):1333–40.
[2] Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 2003;289:179–86.
[3] Fiore AE, Shay DK, Broder K, Iskander JK, Uyeki TM, Mootrey G, et al. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2008;57(RR-7): 1–60.
[4] Poehling KA, Edwards KM, Weinberg GA, Szilagyi P, Staat MA, Iwane MK, et al. The underrecognized burden of influenza in young children. N Engl J Med 2006;355:31–40.
[5] Heikkinen T. Influenza in children. Acta Paediatr 2006;95:778–84.
[6] Reichert TA, Sugaya N, Fedson DS, Glezen WP, Simonsen L, Tashiro M. The Japanese experience with vaccinating schoolchildren against influenza. N Engl J Med 2001;344:889–96.
[7] 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.
[8] Vesikari T, Pellegrini M, Karvonen A, Groth N, Borkowski A, O'Hagan DT, et al. Enhanced immunogenicity of seasonal influenza vaccines in young children using MF59 adjuvant. Pediatr Infect Dis J 2009;28:563–71.
[9] Kendal AP, Cate TR. Increased sensitivity and reduced specificity of hemagglutination inhibition tests with ether-treated influenza B/Singapore/222/79. J Clin Microbiol 1983;18:930–4.
[10] Rota PA, Wallis TR, Harmon MW, Rota JS, Kendal AP, Nerome K. Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. Virology 1990;175:59–68.
[11] Leads from the MMWR. Update: influenza activity – worldwide. JAMA 1989;261:687.
[12] Silvennoinen H, Peltola V, Vinionpaa R, Ruuskanen O, Heikkinen T. Incidence of influenza-related hospitalizations in different age groups of children in Finland. 16-year study. Pediatr Infect Dis J 2011;30:e24–8.
[13] United States Centers for Disease control and Prevention. Seasonal influenza activity surveillance reports: 2000–2001 to 2009–2010 seasons. Available from: www.cdc.gov/flu/weekly/fluactivity.htm.
[14] Reyes F, Macey JF, Aziz S, Li Y, Watkins K, Winchester B, et al. Influenza in Canada: 2005–2006 season. Can Commun Dis Rep 2007;33:21–41.
[15] 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.
[16] Rota PA, Hemphill ML, Whistler T, Regnery HL, Kendal AP. Antigenic and genetic characterization of the haemagglutinins of recent cocirculating strains of influenza B virus. J Gen Virol 1992;73:2737–42.
[17] Langley JM, Faughnan ME. Prevention of influenza in the general population. CMAJ 2004;171:1213–22.
[18] Munoz ET, Deem MW. Epitope analysis for influenza vaccine design. Vaccine 2005;23:1144–8.
[19] Skowronski DM, Masaro C, Kwindt TL, Mak A, Petric M, Li Y, et al. Estimating vaccine effectiveness against laboratory-confirmed influenza using a sentinel physician network: results from the 2005–2006 season of dual A and B vaccine mismatch in Canada. Vaccine 2007;25:2842–51.
[20] Tsai T. Novartis Vaccines and Diagnostics Inc-based on calculations from CHC website performed in 2007 via the following link: http://www.cdc.gov/flu/weekly/fluactivity.htm.
[21] Black S, Della Cioppa G, Malfroot A, Nacci P, Nicolay U, Pellegrini M, et al. Safety of MF59-adjuvanted versus non-adjuvanted influenza vaccines in children and adolescents: an integrated analysis. Vaccine 2010;28:7331–6.
[22] 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.
[23] Schultze V, D'Agosto V, Wack A, Novicki D, Zorn J, Hennig R. Safety of MF59 adjuvant. Vaccine 2008;26:3209–22.
[24] Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol 1999;37: 937–43.
[25] Bonhoeffer J, Bentsi-Enchill A, Chen RT, Fisher MC, Gold MS, Hartman K, et al. Guidelines for collection, analysis and presentation of vaccine safety data in pre- and post-licensure clinical studies. Vaccine 2009;27:2282–8.
[26] Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research. September 2007. Available at: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074775.htm.
[27] Horn M, Vollandt R. Multiple Tests und Auswahlverfahren. Stuttgart, Jena, New York: Gustav Fischer Verlag; 1995.
[28] Committee for proprietary medicinal products. Note for guidance on harmonisation of requirements for influenza vaccines. 12 March 1997, CPMP/BWP/214/96. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf.
[29] Carrat F, Flahault A. Influenza vaccine: the challenge of antigenic drift. Vaccine 2007;25:6852–62.

[30] Banzhoff A, Gasparini R, Laghi-Pasini F, Staniscia T, Durando P, Montomoli E, et al. MF59-adjuvanted H5N1 vaccine induces immunologic memory and heterotypic antibody responses in non-elderly and elderly adults. PLoS One 2009;4:e4384.
[31] Leroux-Roels I, Borkowski A, Vanwolleghem T, Drame M, Clement F, Hons E, et al. Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine: a randomised controlled trial. Lancet 2007;370:580–9.
[32] Stephenson I, Nicholson KG, Hoschler K, Zambon MC, Hancock K, DeVos J, et al. Antigenically distinct MF59-adjuvanted vaccine to boost immunity to H5N1. N Engl J Med 2008;359:1631–3.
[33] Banzhoff A, Nacci P, Podda A. A new MF59-adjuvanted influenza vaccine enhances the immune response in the elderly with chronic diseases: results from an immunogenicity meta-analysis. Gerontology 2003;49: 177–84.
[34] Esposito S, D'Angelo E, Daleno C, Peia F, Scala A, Serra D, et al. Immunogenicity, safety and tolerability of monovalent 2009 pandemic influenza A/H1N1 MF59-adjuvanted vaccine in patients with beta-thalassemia major. Vaccine 2010;28:7825–8.
[35] 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.