—Review—

MF59® Adjuvanted Seasonal and Pandemic Influenza Vaccines

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MF59-adjuvanted seasonal trivalent inactivated (ATIV) vaccine licensed since 1997 and MF59-adjuvanted pandemic H1N1 vaccines have been distributed to approximately 80M persons. Addition of the emulsion adjuvant to inactivated vaccine formulations provides for higher levels of antibody to the viral hemagglutinin (HA) in less responsive older adults, infants and children which, in the case of the pandemic vaccine, allowed only 3.75 μg of the HA to be immunogenic. The adjuvant also stimulates production of more broadly-reactive antibodies against strains that are mismatched to those in the vaccine, a potential advantage in the face of perennial influenza virus antigenic drift. In a field trial, ATIV was 89% efficacious in preventing laboratory-confirmed influenza in 6–<72 month old children, 81% more efficacious than the unadjuvanted control split vaccine while, in older adults, ATIV reduced community-acquired pneumonia and influenza hospitalizations in adults >65 years old by 23% compared to unadjuvanted vaccine, in an observational study. The effectiveness of MF59 adjuvanted split pandemic H1N1 vaccine was 74% overall. Unadjuvanted pandemic vaccine was poorly immunogenic in HIV-infected persons, whereas their responses to MF59-adjuvanted vaccine were similar to those of healthy controls. Analyses of the clinical trials and pharmacovigilance databases and observational studies have shown that while MF59-adjuvanted influenza vaccines are more locally reactogenic, they have not been associated with an increased risk for various adverse effects (AE) of special interest, including unsolicited neurological or autoimmune events.

Key words——adjuvant; MF59; influenza vaccine

INTRODUCTION

The emulsion adjuvant, MF59, is a component of a seasonal influenza vaccine (Fluad®), licensed since 1997, and pandemic and pre-pandemic vaccines for H1N1 (Focetria® and Celtura®) and H5N1 (Aflunov®) viruses. This review summarizes clinical data for the MF59-adjuvanted seasonal trivalent inactivated vaccine (ATIV) and for MF59-adjuvanted pandemic vaccines.

MF59

MF59 is an oil-in-water emulsion adjuvant in which squalene oil is microfluidized into a citrate buffer, resulting in particles averaging 160 nm in diameter, held in suspension by the surfactants Tween 80 and Span 85.[1] As the droplets are only ~1.5 larger than an influenza virion, the emulsion can be filter-sterilized, is completely miscible with aqueous solutions and is stable for at least five years, allowing for the adjuvant to be stockpiled for subsequent formulation. Squalene is synthesized in the human liver (~1000 mg daily) as a precursor to cholesterol and also is consumed in foods (~100 mg daily), whereas a single dose of Fluad contains <10 mg of squalene.

The adjuvant effect is provided by the emulsion but not by its single components. Importantly, the emulsion only provides an adjuvant effect when co-administered with the antigen *i.e.*, administration of the adjuvant and antigen at separate sites results in no adjuvant activity. A local inflammatory response is established at the administration site, consisting of neutrophils, monocytes and macrophages which release chemokines that attract a further influx of immunoreactive cells.[2,3] The immunostimulatory milieu activates dendritic cells, increases their uptake of antigen, and their migration to local lymph nodes, where engagement with helper T cells induces a robust and rapid CD4 cell response.[4] While MF59 does not directly activate dendritic cells and its action is independent of toll-like receptor activation, or the NLP3 inflammasome, it requires the Myd88 adaptor and apoptotic speck protein containing a caspase recruitment domain (ASC).[5,6] The local effects are transient, as MF59 is cleared from the injection site within 6 h.

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In humans, the immune response induced by the adjuvant results in quantitatively higher antibody titers and, in the case of the influenza A (H5N1) and (H1N1 pandemic) viral hemagglutinins (HA), also a differential shaping of the antibody repertoire, resulting in an increased proportion of antibodies, as mapped by phage display, directed against epitopes on the HA1 subunit than to the stem HA2 (compared to unadjuvanted vaccine).[7,8] As the HA1 contains the viral receptor binding domain and principal neutralizing epitopes, this qualitative shift in the antibody repertoire may explain the more cross-reactive adjuvanted response to antigenically divergent influenza viral strains. In addition, the antibodies also exhibit a higher affinity for properly folded HA, seen in higher equilibrium levels and a tenfold increased resistance to dissociation under treatment with 7M urea, as measured by surface plasmon resonance. The slower dissociation was highly correlated with increasing neutralizing antibody titer.[7,8]

SEASONAL ATIV-OLDER ADULTS

ATIV was developed to improve the lower antibody response and suboptimal efficacy of TIV in older adults-estimated to be 50% in the only placebo-controlled trial in this age group.[9,10] Numerous small comparative trials in older adults (over 65 years of age) have shown that hemagglutination inhibition (HI) and microneutraliziation (MNT) antibody responses to ATIV are approximately 1.5 fold higher than to identical but unadjuvanted TIV, with strain to strain variability of the geometric mean ratios (GMR) ranging from 1.2–1.8 fold higher.[11]

Importantly, because influenza viruses undergo continual antigenic drift which can result in reduced efficacy of TIV when the vaccine is mismatched to circulating strains, ATIV provides higher levels of antibody to heterovariant strains compared with TIV.[12,13] Because H3N2, of all circulating subtypes, undergoes the most rapid and frequent antigenic change, and causes the most seasonal morbidity and mortality, observations that ATIV can provide a higher level of seroprotective HI titers against these heterovariant viruses than TIV, are particularly relevant. When serum samples from older adults vaccinees were stored and tested against H3N2 viruses that emerged and circulated two and three years later, while HI and MNT titers of TIV recipients declined as might be expected with antigenic drift, the responses of the ATIV recipients were produced to seroprotective levels (Fig. 1).[12,13] The more broadly reactive ATIV responses could mitigate the reduced efficacy of vaccination in years when strains included in the vaccine are mismatched to late-emerging heterovariant viruses.

The clinical effectiveness of ATIV was shown in observational studies that showed MF59-adjuvanted vaccine recipients, after adjustments for functional impairment, had 87%, 93% and 68% lower relative risks of hospitalizations for acute coronary syndrome, cerebrovascular disease, and pneumonia, during the peak period of influenza transmission, com-

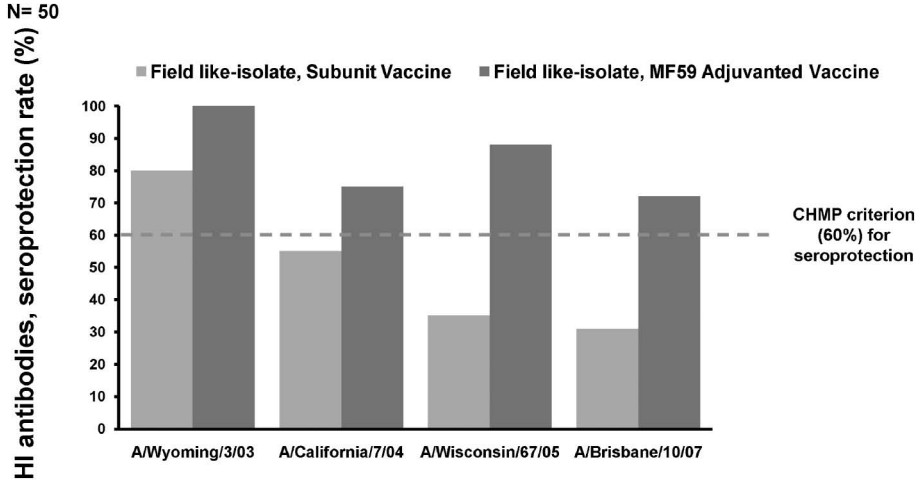


Fig. 1. Seroprotective Hemagglutination Inhibition Antibody Responses in ≥65 Year Old Recipients of Unadjuvanted or MF59-adjuvanted Seasonal Influenza Vaccine Containing A/Wyoming/3/2003 (H3N2) and to Future H3N2 Strains[12]
$n=50$.

pared with unvaccinated controls.[14,15] The comparative effectiveness of ATIV versus TIV in preventing influenza morbidity in older adults, however, is of greater public policy interest. A large-scale observational study in the Lombardy region of Italy compared hospitalizations for pneumonia and influenza (PI) among older adult recipients of ATIV and TIV across three consecutive influenza seasons. After adjustments for confounding factors, including the introduction of a propensity score in the analysis, during the peak weeks of influenza activity, ATIV recipients had a 23% lower risk for PI hospitalizations, compared to TIV vaccinees.[16] This reduction, however, underestimates the potential benefit of the adjuvanted vaccine because cardiovascular hospitalizations were not analysed, nor were outpatient encounters. A cost-benefit analysis for Italy, concluded that 66% ATIV coverage of the population of ≥65 year olds would result in annual health system cost savings of 74 M Euros compared to no vaccination.[17]

Cost-savings in Japan could be greater as a larger proportion of the Japanese elderly population remains employed than in many other countries. In an analysis of the cost effectiveness of influenza vaccination in Japan, under the assumption that 20.2% of adults over 65 years remained in the work force, working 153.1 h/month, with a further life expectancy of 13.3 years, the cost per year of life saved was only ¥516,332, a highly cost-effective intervention by most standards.[18]

The acute and long term safety of ATIV has been shown in analyses of the company pharmacovigilance database and of the MF59-adjuvanted vaccine clinical trials database (Table 1).[19–21] In a meta-analysis, ATIV was associated with a higher frequency of local pain, induration and erythema and, among systemic adverse events, myalgias, compared to unadjuvanted TIVs. The majority of these events were of mild to moderate severity and were transient *e.g.*, for pain, 84% were mild, 15% moderate, and 2% severe. To examine differences in risk for unsolicited events, another meta-analysis was conducted of the clinical databases of 38 trials encompassing ~33000 subjects (ranging in age from 6 m to 100 years) who received adjuvanted influenza vaccines (principally ATIV but also adjuvanted H5N1 vaccine) or their unadjuvanted counterparts.[19] There was no significant difference in reporting rates for all serious adverse events, hospitalizations, or deaths in the two groups but, interestingly, reporting rates for all unsolicited events, those related to a cardiac diagnosis and the new onsets of chronic diseases were lower among adjuvanted vaccine subjects compared with unadjuvanted vaccine subjects (Fig. 2). Although designed as a safety study, the latter observations were consistent with ATIV's increased effectiveness for these outcomes.

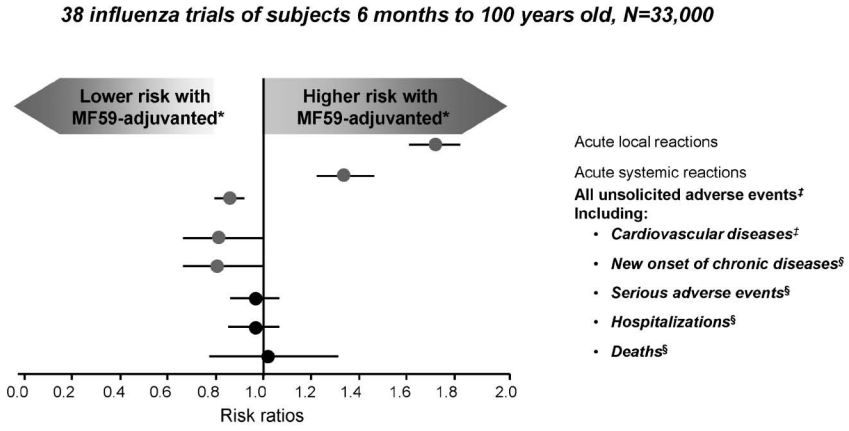
While ~55 M doses of ATIV have been distributed, an analysis of the pharmacovigilance database when ~32 M doses of ATIV had been administered to the indicated population of persons ≥65 years old, found spontaneous report rates of various adverse events of special interest (AESI), including Guillain-Barre syndrome, acute disseminated encephalomyelitis and related neurological disorders, vascular disorders (which would encompass vasculitis), SAEs and deaths, were low with respect to expected background rates.[22]

SEASONAL ATIV-YOUNG CHILDREN

As the importance of influenza in young children increasingly has been recognized, the inadequacy of available vaccines for young children has been brought into focus. The efficacy of TIV in children under 9 years is estimated to be ~55% and in children under 24 months, vaccine efficacy (VE) has not been shown conclusively.[23] While intranasally ad-

Table 1. Subjects in Clinical Trials of MF59-adjuvanted Vaccines, by Age and Underlying Disease

Age group	Influenza studies		Non-influenza studies		Combined	
	(+) MF59	(−) MF59	(+) MF59	(−) MF59	(+) MF59	(−) MF59
Children/Adolescents	5,918	4,602	19	4	5,937	4,606
Adults 18–64 yrs	12,762	1,990	5,442	268	18,204	2,258
Adults ≥65 yrs	15,301	6,993	18	0	15,319	6,993
Subjects with underlying medical conditions	291	235	N/A	N/A	291	235
Overall	34,272	13,820	5,479	272	39,751	14,092



Left arrow indicates statistical evidence of a decreased risk with MF59-adjuvanted vaccine; right arrow indicates statistical evidence of an increased risk with MF59-adjuvanted vaccines. Significance claimed if 95% CI excluded 1.
* Compared to non-adjuvanted vaccine; ‡ includes or§ excludes study V7P35 (ClinicalTrials.gov Identifier: NCT00481065);
Risk ratio: risk of developing a disease after exposure to a vaccine

Fig. 2. Risk Ratios of Specified Adverse Events in Recipients of MF59-and Unadjuvanted Vaccines in 38 Clinical Trials[19)]

ministered live attenuated influenza vaccine (LAIV) is more efficacious than TIV, it can only be administered to children older than 24 months of age because of an increased risk for wheezing and hospitalization and, even in children up to 5 years old, the vaccine should not be given to children with a wheezing history.[24)] Thus, no clearly effective vaccine is available for children <24 months old.

Phase I and II immunogenicity studies in children 6–<72 months of age showed that ATIV was well tolerated even in infants 6–36 months old and that it was more immunogenic than licensed split TIV.[25,26)] Accordingly, an efficacy field trial was undertaken comparing ATIV (n=1937) with split TIV (n=1772) and comparator non-influenza vaccines (meningococcal C conjugate and tickborne encephalitis vaccines (n=993)) over the 2007–2009 influenza seasons in Germany and Finland in vaccine-naive 6–<72 month olds.[27)] Two age appropriate doses of the vaccines were administered and occurrences of influenza-like illness were monitored three weeks after vaccination through the influenza season and confirmed by PCR.

ATIV was non-inferior to TIV in acute reactogenicity in infants 6<36 months old, meeting the primary safety objective. In older 36–<72 month olds, ATIV was slightly more reactogenic than TIV but fever was not elevated to a significant degree and the frequency of febrile convulsions was similar in all three groups. Unsolicited adverse events (AE) and serious AE (SAE) occurred in similar frequencies.

The VE of ATIV versus non-influenza vaccine comparator against vaccine matched strains was 89% while TIV had an efficacy of 45%, for a relative VE of ATIV over TIV of 80%. Age specific relative efficacies of ATIV over TIV for 6–24, 6–36 and 36–<732 month olds were 75%, 68% and 91%. The absolute VE of ATIV in 6–24 month olds of 75% versus 2% for TIV was noteworthy, as this was the first demonstration of the efficacy of an inactivated influenza vaccine in children of this age, as noted above. VE of ATIV was maintained through the season as well as elevated HI and MNT antibodies. ATIV produced significantly higher HI and MNT antibodies to all three vaccine subtypes compared to TIV and, importantly, responses to the A subtypes after one dose were higher than to two doses of TIV. Sera were tested against viral strains that were mismatched to the vaccine and ATIV responses, as shown in older adults, were more reactive against the heterovariants than TIV, even against a lineage-mismatched B virus.

The VE results were based principally on protection against the H3N2 virus (VE=89%) that predominated in the two study seasons. However, an estimate of efficacy against influenza virus B could be inferred from the small number of cases (all lineage-mismatched) that occurred in the two years. The point estimate of 79% (−5 to 96%), although insignificant, trended toward showing efficacy against B strains of the opposing lineage, while the estimate for TIV was 36% (−162–84%).

The field trial was highly important in demonstrating efficacy of ATIV in children <24 months old and also among children up to 6 years of age. Moreover, its relative efficacy over TIV was higher than the relative VE of LAIV over TIV.

Additional trials in infants as young as 2 months olds, and studies of a quadrivalent formulation containing strains from both B lineages are planned.

The overall safety of MF59 in children was assessed by age in a metanalysis similar to the previously mentioned study, comparing recipients of adjuvanted versus comparator unadjuvanted study vaccines. There was no difference in occurrence of all AEs, or possibly or probably related AEs in any age group; severe AEs (*e.g.*, grade 3 AEs interfering with daily activity) were reported more frequently only among the youngest cohort of ATIV compared to TIV recipients.[20]

H1N1 PANDEMIC VACCINES

Novartis distributed two adjuvanted pandemic vaccines (produced in eggs and in cell culture, respectively) and at the request of the US government, conducted clinical trials of an adjvuanted formulation of another egg-derived vaccine (Fluvirin®) licensed in the US. The latter ultimately was distributed only in an unadjuvanted, 15 μg HA/dose, formulation.

Reactogenicity of the adjuvanted H1N1 vaccines were similar or slightly higher compared to their unadjuvanted counterpart containing 15 μg of HA.[28–31] The immuogenicities of the respective vaccines shown in Figs. 2 and 3 illustrate three important advantages conferred by the adjuvant: higher levels of antibody in young children <9 years old and in older adults, at levels sufficient in those groups to meet regulatory criteria while two doses were needed with the unadjuvanted vaccine, and as a result of the higher antibody titers achieved, antigen sparing, allowing for licensed formulations containing 7.5 μg of HA (egg-derived Focetria, and 3.75 μg HA (MDCK cell-derived Celtura). In addition, although in young adults 15 μg of unadjuvanted antigen was sufficient to provide acute antibody responses meeting licensure criteria, after one year, those levels dropped below those maintained by the adjuvanted vaccine.

Approximately 25 million doses of the adjuvanted egg-derived pandemic vaccine, Focetria, were administered in various countries in Europe and Latin America. Vaccine effectiveness was estimated in two studies, a screening study of populations in two Italian health authorities that found a 92.9% effectiveness (49.3%–98.9%), and a small case-control study in the Netherlands that estimated effectiveness at 75% (−473%–99%).[32,33] In addition, MF59 was used in a licensed bedside-mix formulation with a Green Cross Corporation 7.5 μg of split H1N1 antigen for certain at-risk groups in Korea. In a case-control study, effectiveness was estimated to be 74% (49%–86%).[34] Of interest, was the age-specific vaccine effectiveness in older adults. Whereas in the U.S., where unadjuvanted vaccine was used, overall effectiveness was 63% and no effectiveness was demonstrated in this age group, the MF59-adjuvanted Korean vaccine was 88% effective in older adults ($p = 0.07$) (Table 2).[35] In contrast, both vaccines were similarly efficacious in young adults. Although these were independent studies, the juxtaposition of data suggest that the adjuvanted vaccine may have been more effective, especially in a usually poorly responsive group.

The adjuvanted vaccine was studied in several special groups, including infants who were born prematurely, patients with beta thalassemia, cystic fibrosis,

Table 2. Effectiveness of Pandemic H1N1 Influenza Vaccine: Unadjuvanted Inactivated 15 μg Hemagglutinin (HA) in US and MF59-adjuvanted 7.5 μg HA in Korea, by Age[34,35]

Effectiveness	US case-control study: cases=1011, controls=5746			Total
	<10 years	10–49 years	≥50 years	
14 d window	32% (−92–76)	89% (15–98)	67% (−231–66)	62% (25–80%)

Effectiveness	Korea case-control study: cases-207, controls-209				Total
	10–19 y	20–49 y	50–64 y	>65 y	
14d window	81% (49–93%)	75% (10–94%)	81% (−88–81%)	88% (−19–99%)	73% (49–86%)

and congenital Williams, or Cornelia deLange syndromes; no difference in antibody response was seen compared to healthy controls.[36–40] In children who had various immunocompromising conditions, two doses of the adjuvanted vaccine were recommended and provided an antibody response similar to a single dose of unadjuvanted vaccine; the response to one dose was not studied.[41] However, in some kidney transplant and rheumatic disease patients, antibody responses to the adjuvanted vaccine were blunted but satisfactory.[39,40] Interestingly, in independent studies of HIV-infected children and adults on combination antiretroviral therapy, responses to one dose of the adjuvanted vaccine were similar to those of healthy controls.[42–45] In contrast, when unadjuvanted vaccine (containing 15 μg of antigen) was administered to adult HIV-infected patients with similar CD4 cell counts, the response to one dose was suboptimal.[46] Although these juxtaposed studies were independent and their outcomes cannot be compared formally, the results suggest that the adjuvanted vaccine was able to overcome the poor response to unadjuvanted vaccine in HIV-infected patients who appeared to be similarly immunocompromised.

Because pregnant women were identified (correctly) as a group at risk for severe disease in the pandemic, many countries recommended and administered adjuvanted vaccine to them in large numbers. One uncontrolled study found that maternal antibodies were transferred to the infant and, in 81%, were maintained at seroprotective levels (HI titer $>$40) at 5 months.[47] The result is of interest as no influenza vaccine currently is licensed for infants $<$6 months old.

The safety of the adjuvanted pandemic vaccine was assessed in the general population and in special groups. The Novartis pharmacovigilance database of spontaneously reported AE found no difference in reporting rates for various AE, including neurological events and others AESI compared to those for seasonal influenza vaccines.[48] Italian pharmcovigilance and clinical studies also found no safety concerns.[49,50] Interestingly, a study in rheumatic disease patients found no change in disease activity 4–6 weeks after vaccination.[40] As three pandemic vaccines were distributed in Europe, Eudravigilance compared reported AEs for the three products and found that high fever and fever were reported disproportionately after AS03-adjuvanted vaccination.[51] Two large observational studies of Focetria safety in pregnant women were undertaken: among $\sim$19000 women in Argentina, where $\sim$650000 women were vaccinated during pregnancy and in the Netherlands and Italy (Novartis unpublished data).[52] Preliminary analyses of both studies, comparing vaccinated versus unvaccinated women have found no differences in outcomes of pregnancy or in specified adverse outcomes in their infants.

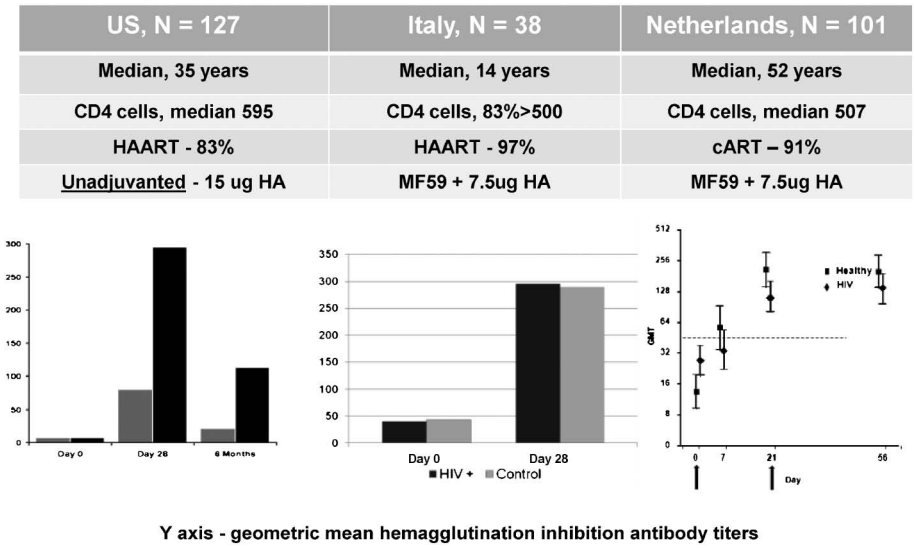


Fig. 3. Hemagglutination Inhibition Antibody Responses to Pandemic H1N1 Influenza Vaccine: Unadjuvanted 15 μg Hemagglutinin (HA) in US and MF59-adjuvanted 7.5 μg HA in Italy and Netherlands, HIV Infected Persons[42,45,46]
Y axis-geometric mean hemagglutination inhibition antibody titers.

H5N1 PRE-PANDEMIC VACCINE

Both egg-and MDCK cell culture-derived MF59-adjuvanted H5N1 vaccines have been studied with similar antigen sparing properties compared to unadjuvanted vaccine as described above.[53,54] In the only head-to-head study of alum versus MF59 in adjuvanting H5N1 antigen, the MF59-adjuvanted vaccine provided a clearly higher antibody response.[55] For both the egg- and cell-derived vaccines, two doses are needed to provide adequate antibody responses, albeit with 3.75–7.5 μg of HA[53,54] whereas, for an adjuvanted H9N2 vaccine, one dose was as immunogenic as two doses of unadjuvanted vaccine.[56] Importantly, the responses are broadly reactive to viruses in various H5N1 clades and rapid similarly broad anamnestic MNT responses in primed individuals can be elicited eight years later with a single dose of adjuvanted vaccine from a mismatched clade.[57,58]

The immune memory induced by the adjuvanted vaccine and the breadth of the anamestic response suggest the utility of such vaccines to prime individuals in the face of a future pandemic due to avian influenza virus.

CONCLUSION

MF59 adjuvanted influenza vaccines provide higher and more broadly reactive antibodies that persist longer at seroprotective levels than their unadjuvanted counterparts. A higher comparative clinical efficacy or effectiveness of the adjuvanted seasonal vaccine was shown in children and older adults and was suggested for the pandemic H1N1 vaccine. Assessments of the adjuvanted vaccincs' safety have disclosed no indications of an increased risk compared to their unadjuvanted counterparts.

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