MF59-adjuvanted vaccines: increased immunogenicity with an optimal safety profile

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The need to enhance the immunogenicity of purified subunit antigens has prompted the development of several new adjuvants. However, many of these new molecules have demonstrated a reactogenicity profile that is not suitable for their inclusion in vaccines for human use. In this context, the adjuvant emulsion MF59™ has been developed, tested in combination with different antigens in several animal models and subsequently evaluated in humans. Clinical trials with several MF59-adjuvanted vaccines have been performed in different age groups (from newborns to the elderly) and have shown an increased immunogenicity of coadministered antigens, associated with a high level of safety and tolerability. MF59 has been the first adjuvant to be licensed for human use after alum and, as part of an enhanced influenza vaccine for the elderly, is now available in the marketplace of several countries worldwide.

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Vaccines containing highly purified recombinant or subunit antigens are generally poorly immunogenic and therefore they need to be formulated with an adjuvant to increase their immunogenicity. In the past decades, major efforts have been made to develop new vaccine adjuvants and several of them have even been evaluated in animal models and in humans in association with different vaccine candidates [1]. Nevertheless, alum, introduced for the first time as a vaccine adjuvant in the 1920s, is still the most frequently used adjuvant for human vaccines. A major limitation to the acceptance of new adjuvants candidates has been the unsatisfactory safety profile of many of them. In the 1990s, following an extensive preclinical and clinical investigation, the MF59™ (Chiron Vaccines, CA, USA) adjuvant emulsion, in combination with a subunit influenza vaccine, has been the first new adjuvant to be licensed for human use by European regulatory agencies.

This review shall briefly summarize the characteristics of the MF59 adjuvant and describe the most significant preclinical and clinical findings with several MF59-adjuvanted investigational vaccines, focusing particularly on the clinical profile of vaccines containing the new adjuvant.

The MF59 adjuvant

MF59 is an oil-in-water emulsion consisting of small (<250 nm in diameter), uniform and stable droplets. Their main ingredient is the fully metabolizable oil squalene, which is obtained from shark liver and is also found in humans as a natural metabolite of cholesterol and as a normal component of cell membranes. Squalene droplets are stabilized by the addition of two emulsifiers, a water-soluble surfactant, the polyoxyethylene sorbitan monooleate (Tween 80) and an oil-soluble surfactant, sorbitan trioleate (Span 85) [2] (FIGURE 1).

There are still several uncertainties concerning the adjuvant effect of adjuvants. This is also basically true for MF59. As shown in studies with fluorescent-labelled MF59, the MF59 emulsion does not have a depot effect. In fact, antigens coadministered with MF59 (e.g., herpes simplex virus [HSV]-2 gD) are cleared from the site of injection independently of MF59, suggesting that the presence of the adjuvant does not influence the distribution of the antigen [3,4]. Using similar techniques, it has been also shown that the administration of MF59 induces a significant influx of macrophages to the site of injection [3,4].

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Despite the uncertainties regarding the mechanism of action of MF59, preclinical data clearly indicate that this adjuvant typically induces a T-helper (Th)2-type immune response, as shown by the immunoglobulin (Ig) isotypes and the cytokines induced following immunization in mice. In fact, mice immunized with MF59-adjuvanted influenza vaccine exhibit a significant increase in the levels of interleukin (IL)-5 and IL-6, (but not of interferon [IFN]-γ), and produce higher titers of antigen-specific IgG1 than IgG2a [5,6]

Adjuvanticity in animals

The adjuvant emulsion alone and/or in combination with different antigens (e.g., influenza, HSV, HIV and others) has been tested in several toxicological studies aimed at evaluating the safety profile of this compound before commencing clinical trials. Toxicological studies, performed in rats, rabbits, dogs and other animal species, included acute toxicity studies, repeated-dose toxicity studies, mutagenicity studies and teratology studies. The overall conclusion from this extensive toxicological evaluation was that MF59, either alone or in combination with different antigens, has an acceptable safety profile and can be safely administered to humans. Based on these data, several MF59-adjuvanted vaccines have been included in extensive clinical programs.

From a pharmacological perspective, MF59 has been used as a vaccine adjuvant to enhance the immunogenicity and the efficacy of several antigens (mostly viral, such as influenza, HSV-2, HIV, hepatitis B virus [HBV], hepatitis C virus [HCV], cytomegalovirus [CMV] and others, but also bacterial antigens such as the polysaccharides of *Haemophilus influenzae* and *Neisseria meningitides*) in various animal models (TABLE 1). In several cases, the positive results obtained from these preclinical studies in animals have prompted the evaluation of the respective vaccines in humans.

Although the focus of this paper is on the clinical data, we will provide hereinafter a short description of the most significant preclinical data, using the examples of flu, HBV, HSV and HIV vaccines.

Conventional, nonadjuvanted influenza vaccines are commonly available and their use is cost effective. However, particularly in the elderly, there is a need to enhance their ability to stimulate the immune system and to obtain a stronger and more efficacious response. Alum does not increase the immunogenicity of influenza antigens and therefore the possible use of MF59 to this purpose was tested in several animal species. Particularly interesting is the experience in old mice, which respond very poorly to immunization with a conventional subunit vaccine, as compared with young, fully immunocompetent mice. However, when immunized with an MF59-adjuvanted vaccine, their antibody titers increased to levels normally reached only in young animals [6]. Additional studies demonstrated the ability of MF59 to reduce the amount of antigen needed per dose, to decrease the viral load in the lungs of mice and to significantly protect mice against a challenge with a wild virus [7].

The addition of MF59 to a Chinese hamster ovary (CHO)-derived HBV vaccine containing the pre-S2 component induced a very strong increase in anti-HBs antibody titers in baboons (up to 127-fold than those obtained with conventional vaccines) [8]. Similarly, immunization of monkeys with the MF59-adjuvanted HCV E1–E2 envelope glycoproteins, derived from a eukaryotic expression system, showed a strong antigen-specific antibody response and protection against an infectious challenge with the homologous virus [9].

Immunization with the HSV gD2 recombinant proteins along with MF59 efficiently protected guinea-pigs against recurrent genital disease both therapeutically [10] and prophylactically [11]. Most of the chimpanzees immunized with recombinant HIV gp120 administered with MF59, either as such [12], or as a boost to a primary immunization provided with adenoviruses expressing the gp120 [13], were protected against a challenge with homologous HIV. Similar effective levels of protection were achieved in immunized rhesus monkeys subsequently challenged with simian immunodeficiency virus–HIV chimeric virus (SHIV) [14].
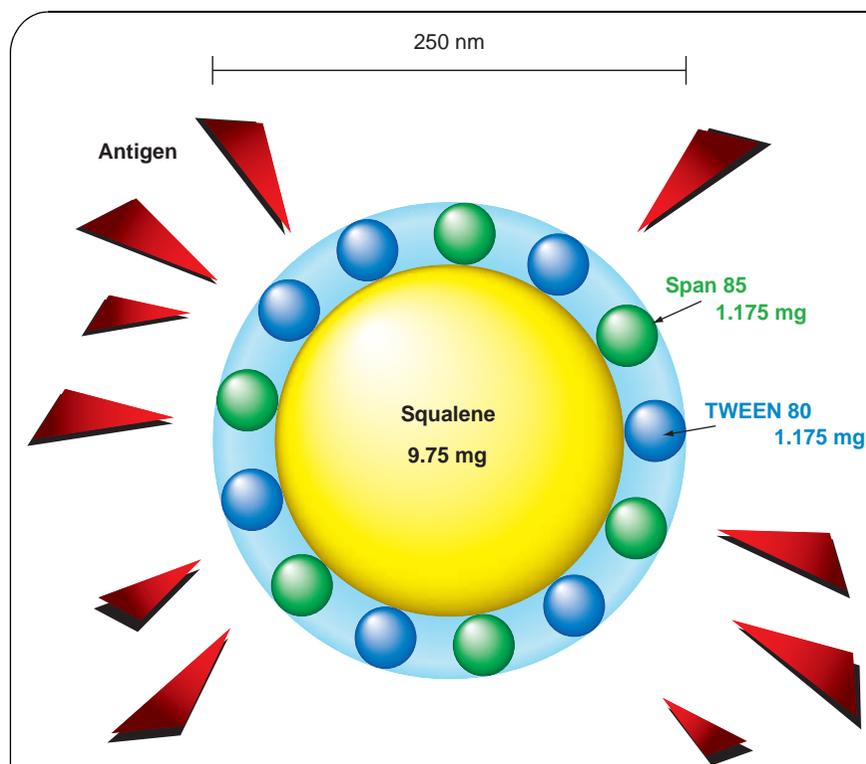

Figure 1. Schematic picture of the MF59 oil-in-water adjuvant emulsion.

250 nm

Antigen

Span 85
1.175 mg

Squalene
9.75 mg

TWEEN 80
1.175 mg

Table 1. MF59 adjuvanted antigens tested in different animal models

Species	Models
Mice	Flu
Guinea-pigs	Herpes simplex virus
Rabbits	Cytomegalovirus
Pigs	Papillomavirus
Goats	Hepatitis B virus
Monkeys	Hepatitis C virus
	HIV
	Plasmodium species
	Uropathogenic *Escherichia coli*
	Haemophilus influenzae b
	Neisseria meningitidis

MF59 adjuvanted vaccines in humans

The clinical experience to date with several MF59-adjuvanted vaccines ranges across all age groups. Depending on the type of vaccine, clinical trials with MF59-adjuvanted vaccines have been performed in elderly subjects, younger adults, adolescents, children and even newborns in some cases.

Immunization of adults

Vaccines against interpandemic & pandemic influenza

As previously mentioned, although the efficacy of conventional influenza vaccines is high (70–90%) in young adults, it decreases significantly in elderly subjects [15–18]. The reduced immunogenicity of these vaccines in elderly subjects [19,20] might be one of the reasons for their decreased efficacy in this age group [18,21,22]. The preclinical data with MF59 showing a significant increase in the immune response to influenza vaccination in old mice with depressed antibody and cellular immune response, as well as altered cytokine response [2,6] prompted the evaluation of this vaccine in elderly human subjects [23].

The clinical trials with MF59-adjuvanted influenza vaccine were aimed to compare the safety and immunogenicity of the new vaccine with that of nonadjuvanted conventional vaccines.

Safety

Overall, the clinical database includes more than 12,000 elderly subjects immunized with the adjuvanted influenza vaccine. In this population, the vaccine was safe and very well-tolerated. Although the presence of the adjuvant is associated with an increased rate of local adverse reactions, particularly local pain, most of these reactions were mild in nature, of short duration and qualitatively similar to those induced by control vaccines [23–28]. Since influenza vaccines are normally administered every year because of the frequent change in their antigenic composition, the safety profile of the new vaccine was carefully evaluated also after repeated administration. This approach demonstrated that re-immunization of elderly subjects with the adjuvanted vaccine across subsequent influenza seasons does not induce any significant increase of reactogenicity [24,25]. More clinically relevant adverse events, including those requiring a physician visit or intervention in the first week after immunization, were rare and quantitatively similar to those induced or temporally associated with control vaccines (TABLE 2) [23].

Immunogenicity

The adjuvant effect of MF59, resulting in an increased immunogenicity, was consistently demonstrated in elderly subjects compared with conventional vaccines; this finding was particularly relevant for the A/H3N2 and B strains [23–28]. The enhanced immunogenicity of MF59-adjuvanted flu vaccines was also demonstrated after repeated immunization [24,25]. Subset immunogenicity analyses showed that the adjuvant effect of MF59 was greater in elderly subjects with a low titer of specific antibodies before immunization (FIGURE 2) and in subjects affected by chronic underlying conditions, such as respiratory and cardiovascular diseases and/or diabetes mellitus [23]. This finding is clinically relevant since these subjects are normally more likely to develop influenza and its most serious complications and therefore are the obvious target for influenza immunization.

Influenza pandemic vaccines

Following the prepandemic alert associated with the A/H5N1 outbreak in Hong Kong in 1997, the role of MF59 has also been explored as a potential adjuvant for more effective pandemic influenza vaccines, to be used not only in elderly but also in younger populations. Clinical studies performed in young adults, immunized with increasing concentrations of A/H5N3 antigen with or without MF59, clearly demonstrated that the addition of MF59 was instrumental to the significant increase of protective antibodies compared with conventional nonadjuvanted subunit vaccines, which were very poorly immunogenic against the homologous vaccine strain A/H5N3 and even less against the heterologous wild A/H5N1 Hong Kong strain [29,30].

Table 2. Low rate of clinically significant adverse events. The rate of adverse events requiring a physician visit within 1 week of immunization with the MF59-adjuvanted influenza vaccine is low and similar to that associated with a licensed control subunit vaccine.

Vaccine	Number of subjects	Number of AEs§	AE rate (%)	RR (%)
FLUAD	9171	94	1.0	0.92
Control	4550	51	1.1	(0.66–1.30)

§When restricting to possibly related events the respective numbers were 30 (0.3%) for FLUAD and 18 (0.4%) for the control vaccine.
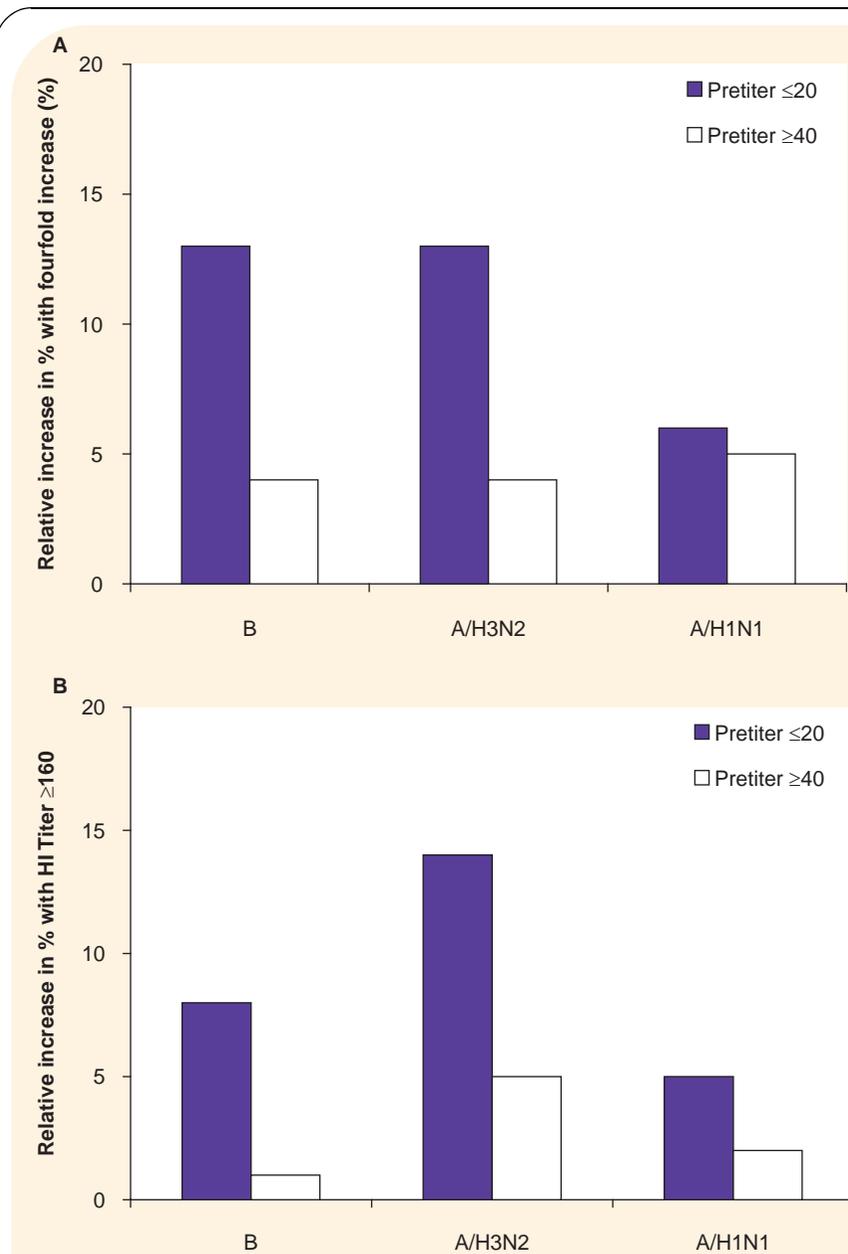
Figure 2. Increased adjuvant effect in elderly subjects susceptible to influenza. **A**. Proportion of subjects with 4-fold increase or seroconversion. **B**. Proportion of subjects with HI Titre ≥160. Both the proportion of subjects with fourfold increase or seroconversion and that of subjects with highly protective postimmunization titers (i.e., ≥160) are significantly higher in subjects with low preimmunization titers (i.e., ≤20) following immunization with subunit influenza antigens combined with MF59.

From a safety point of view, the addition of MF59 was very well-tolerated and did not provide any evidence of clinically important reactogenicity. These data underline the major role that an innovative adjuvant such as MF59 can play in a public health emergency like an influenza pandemic.

HBV vaccine

Hepatitis B vaccines are licensed worldwide and largely available alone or in combination with other vaccines. However, the current schedule of immunization, requiring three doses at 0, 1 and 6 months and the immunogenicity profile of the vaccine, particularly in some high-risk populations, might still be improved. For this reason, due to the very good data obtained in baboons [8] with a vaccine containing HBV PreS2 + S antigens, produced in CHO cells and adjuvanted with MF59, this vaccine was evaluated in seronegative human adult volunteers and compared with a licensed vaccine adjuvanted with aluminum hydroxide using different immunization schedules [31].

As expected, due to the presence of MF59, the adjuvanted vaccine induced a higher rate of mild local reactions (pain at the injection site was the most frequent reaction). However, the incidence of systemic reactions did not significantly differ between MF59-adjuvanted and alum-adjuvanted vaccines. From an immunogenicity perspective, after the first immunization, 89% of recipients of the adjuvanted vaccine had protective (≥10 mIU/ml) anti-HBs antibodies compared with 12% of subjects immunized with the licensed vaccine [31]. The geometric mean titer after the first dose was more than 100-fold greater than that of the licensed vaccine. Additionally, antibody responses following the second and third immunizations were still significantly higher in the MF59 group compared with the licensed vaccine. In particular, it was clinically important that all subjects in the MF59 group, after the second dose, regardless of its timing (1, 2 or 6 months after the first dose), had titers tenfold greater than the protective threshold of 10 mIU/ml. In summary, the clinical experience with the PreS2 + S hepatitis B virus vaccine adjuvanted with MF59 highlights the adjuvant effect of MF59 which, with a very reasonable safety profile, increases the immunogenicity of the vaccine, allows the use of a two-dose schedule over a short period of time and might be considered for a better prophylaxis of hypo- or nonresponders to the current vaccine.

HSV vaccine

The recombinant HSV vaccine formulated with MF59 included the two recombinant glycoproteins gD2 and gB2, which represent the target of specific antibodies with neutralizing activity. Three immunizations with these proteins together

with MF59 were very well-tolerated. In HSV-seronegative subjects, this vaccine induced specific neutralizing antibody titers and T-cells at frequencies equal to or higher than those obtained in naturally infected individuals. Also in HSV-seropositive subjects the antibody response induced by vaccination was higher than that acquired with natural infection [32], suggesting a boosting effect of the vaccine on the immune response primed by the natural infection [33]. Tested in Phase III trials in more than 2000 HSV-2-seronegative individuals, this vaccine was shown to reduce the acquisition rate of HSV-2 by 50% during the first 5 months of the trial, as compared with placebo but not on the overall follow-up of the study. In addition, the vaccine did not influence the duration of the first or subsequent HSV-2 genital episodes [34]. In HSV-2-seropositive individuals, immunization with this vaccine did not affect the frequency of the recurrence of the genital lesions but significantly reduced the duration and the severity of the first clinically confirmed herpes lesion [33]. The predominantly Th2 nature of the immune response induced by MF59 suggests that other adjuvants, inducing a Th1 response, might be more suitable to generate an optimal level of protection against HSV-2 [35]

Immunization of children & newborns

The MF59 adjuvant has been tested in toddlers and infants using candidate vaccines against CMV and HIV.

CMV vaccine

Following clinical testing in adult volunteers with a MF59-adjuvanted recombinant CMV vaccine containing the gB protein produced from CHO cells [36–39], the safety and immunogenicity of this vaccine was also tested in seronegative toddlers aged between 12 and 35 months [40]. In this toddler trial, the CMV gB/MF59 vaccine was well-tolerated by all children and no significant systemic reactions occurred after administration of any of the three doses, although transient local reactions were more frequent after the third dose. After three doses (20 µg each), the MF59-adjuvanted gB-based vaccine exhibited a very high immunogenicity. Vaccine-specific antibody titers were greater than 100 times higher than those observed after the first dose, at least six times higher than those normally found in the adults immunized with the same vaccine and higher than those found in adults naturally infected with CMV. Thus, such a vaccine may represent a tool to reduce CMV transmission among infants and to reduce congenital CMV by providing maternal immunity against primary maternal infection.

HIV vaccine

A recombinant HIV vaccine consisting of the gp120 from HIV-SF2 adjuvanted with MF59 was evaluated in adult volunteers. The safety profile of this vaccine was very good both in adult American and Thai volunteers; moderate-to-severe side effects were observed only when MF59 was formulated with MTP–PE [41–44]. When administered at doses ranging between 25 and 100 µg according to a three-dose primary schedule, given over a period of 6 months, followed by one or two booster doses, the MF59-adjuvanted vaccine was very well-tolerated [41–44]. After the primary immunization, all vaccinees developed strain-specific neutralizing antibodies and about two-thirds exhibited antibodies that cross-neutralized other HIV strains (e.g., HIV–MN) [41,42,44]. A strong antigen-specific proliferative response was also observed in these adult subjects.

The recombinant gp120–SF2 protein formulated with MF59 was also administered to neonates born to HIV-1-infected women and was shown to be safe within the first 72 h of immunization when given at doses of 5, 15 or 50 µg at birth and then 4, 12 and 20 weeks later [45] (TABLE 3). In fact, no local or systemic reactions were reported 48 h after the subjects underwent immunization. This vaccination schedule (or even shorter schedules) induced specific antibody responses in 87% of the infants [46]. Interestingly, the MF59-adjuvanted gp120–SF2 vaccine was much stronger than an alum-adjuvanted gp120–MN vaccine in inducing cell proliferative responses when administered at birth [47].

Five-year view & expert opinion

After extensive preclinical and clinical studies, MF59 has been licensed in several European and non-European countries, as part of an influenza vaccine for immunization of elderly; it is therefore one of the few novel vaccine adjuvants approved for human use. Additionally, several other MF59-adjuvanted vaccines, both bacterial and viral, are currently under clinical investigation. Although the adjuvant is currently licensed for use in the elderly, the clvinical results produced in younger age groups (young adults, adolescents, children and infants) using several candidate vaccines have shown that this adjuvant can be safely used for vaccination from elderly to very young children and even neonates. This might facilitate the neonatal use of vaccines for prevention of infections that peculiarly

Table 3. The MF59-adjuvanted HIV vaccine is safe in newborns. Local and systemic reactions after immunization of newborns with an MF59-adjuvanted HIV vaccine are low and similar to those associated with the alum adjuvanted vaccine.

	No. with moderate-to-severe reactions (%)		
	Vaccine + MF59 (n = 77)	Vaccine + alum (n = 77)	Adjuvant control (n = 29)
Within 48 h of immunization			
Local or systemic	0	0	0
Any time during study period§			
Chemistry	3 (4)	2 (3)	1 (3)
Hematology	8 (10)	9 (12)	7 (24)
Signs and symptoms	2 (3)	3 (4)	0

§Not attributed to vaccination.

affect this age (e.g., pertussis) or for prevention of neonatal transmission of pathogens, such as HIV. In conclusion, the experience with MF59 suggests that this adjuvant can play an important role to strengthen and broaden the immune response against several pathogens and can be used, with a very good safety profile, in all age groups.

Key issues

- The poor immunogenicity of recombinant and subunit antigens requires the addition of strong and safe adjuvants to vaccine formulations. MF59, an oil-in-water emulsion containing squalene, proved to be a powerful adjuvant, able to enhance the immunogenicity of viral and bacterial antigens, in several animal models.

- In combination with subunit influenza antigens, MF59 has been extensively evaluated in human clinical trials and demonstrated not only an increased immunogenicity, but also a good safety profile. These clinical data have led to the registration of the MF59-adjuvanted influenza vaccine in several countries. MF59 has been the first novel adjuvant to be licensed after aluminum salts several decades ago.

- The very good safety profile shown in younger age groups, including infants, with other candidate vaccines adjuvanted with MF59 might pave the way for the registration of new adjuvanted pediatric vaccines.

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