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Review

Safety of MF59™ adjuvant

Viola Schultze[a,*], Vicente D'Agosto[b,1], Andreas Wack[c,2], Deborah Novicki[b,3], Juergen Zorn[a,4], Renald Hennig[a,5]

[a] Novartis Vaccines and Diagnostics, Marburg, Germany
[b] Novartis Vaccines and Diagnostics, Siena, Italy
[c] Department of Molecular Immunology, Novartis Vaccines and Diagnostics, Research Center, Siena, Italy

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ABSTRACT

The need to enhance the immunogenicity of purified subunit antigens has prompted the development of new adjuvants. The adjuvant emulsion MF59™ has been tested in animals in combination with different antigens and finally evaluated in humans. It was licensed after the successful outcome of preclinical and clinical testing.

This paper summarizes the main characteristics of the MF59™ adjuvant, including animal testing, clinical experience with various vaccines, and information from current postmarketing surveillance data.

This review supports the hypothesis that MF59™ is a safe adjuvant for human use.

Contents

* Corresponding author at: Novartis Vaccines and Diagnostics, P.O. Box 1630, 35006 Marburg, Germany. Tel.: +49 6421 39 2763; fax: +49 6421 39 3662.
E-mail addresses: viola.schultze@novartis.com (V. Schultze), vicente.dagosto@novartis.com (V. D'Agosto), Andreas.Wack@novartis.com (A. Wack), Deborah.Novicki@novartis.com (D. Novicki), Juergen.Zorn@novartis.com (J. Zorn), Renald.Hennig@novartis.com (R. Hennig).
[1] Tel.: +39 0577 24 3068.
[2] Tel.: +39 0577 24 3469.
[3] Tel.: +39 0577 24 5141.
[4] Tel.: +49 6421 39 4017.
[5] Tel.: +49 6421 39 2025.

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1. Introduction

Most existing vaccines are administered parenterally and are formulated with adjuvants to enhance their potency. Major efforts have been undertaken in the past decades to develop new vaccine adjuvants. Despite all these efforts, aluminum salts, introduced in the 1920s, remain the standard adjuvants for human use worldwide. Safety of the vaccine formulation represents one of the limiting factors in the introduction of new vaccine adjuvants.

The challenge for Novartis (formerly Chiron) was to provide vaccines that would ensure sufficient protection against infectious diseases in elderly and immuno-compromized persons. An additional argument for the development of the MF59™ adjuvant was to enhance the immunogenicity of recombinant and subunit antigens. In such efforts, the safety of the adjuvant is the always the limiting factor. The safety and immunogenicity of MF59™ were investigated in various combinations with a large number of antigens at the preclinical stage. Clinical development of various combinations of MF59™ and antigens clearly demonstrated that MF59™ is not only safe, but also its high immunogenicity allows the amount of antigen in the vaccine to be reduced. The safety of MF59™-adjuvanted vaccines (primarily influenza vaccines) was tested in a large number of randomized and controlled clinical studies (see Section 5.3.2).

In 1997, the MF59™ adjuvant emulsion was the first new adjuvant to be licensed for human use. This was 70 years after the introduction of aluminum salts, and MF59™ was the only adjuvant licensed for human use in addition to alum based adjuvants. MF59™ was licensed after the successful outcome of preclinical experience and intensive clinical testing that yielded a database of more than 20,000 subjects, most of which were immunized with an influenza vaccine. It is licensed as part of the influenza vaccine Fluad® in many countries.

A number of publications include reviews of its adjuvant properties in animal and human studies. An array of *in vitro* and *in vivo* studies showed that MF59™ has ideal adjuvant properties, comprising a strong enhancement of the immune response, as well as a favourable safety profile.

This paper summarizes a range of unpublished investigational data as well as published literature reports on the safety of MF59™ used as adjuvant for human vaccines. The main characteristics of the MF59™ adjuvant are described, including a basic overview on animal testing and details on the clinical experience with various vaccines (licensed and investigational), as well as information on current postmarketing surveillance data [1].

2. History of MF59™

The need to enhance the immunogenicity of purified subunit antigens has prompted the development of new adjuvants. However, several of these new molecules have shown a reactogenicity profile not suitable for inclusion in vaccines for human use. In this context, the adjuvant emulsion MF59™ has been developed and tested in several animal models in combination with different antigens and finally evaluated in humans.

Clinical trials with several MF59™-adjuvanted vaccines have been performed in different age groups (from newborns to elderly) and have shown an increase of immunogenicity of co-administered antigens, associated with a high level of safety and tolerability. MF59™ was initially developed as a vehicle for a muramyl peptide adjuvant, MTP-PE, but was found to possess marked adjuvant properties itself. A wealth of clinical data is now available for elderly and at-risk populations [2–4], for adults [5], and for children [6,7]. Recently, studies with a potentially pandemic H5 influenza strain were carried out in the adult population [8–10]. MF59™ has been the first adjuvant for human use to be licensed since the introduction of aluminum as adjuvant and, as part of an enhanced influenza vaccine (Fluad®) for the elderly, is now commercially available in 23 countries worldwide including 12 EU countries [11].

3. Composition and manufacturing

MF59™ adjuvant (MF59™C.1) is an oil-in-water emulsion (o/w) consisting of small (∼160 nm in diameter), uniform, and stable microvesicles, consisting of a drop of oil surrounded by a monolayer of non-ionic detergents (Table 1). The oil is squalene, which is obtained from shark liver. Squalene is a natural component of cell membranes; it is found in human sebum (a skin surface lipid) and is a naturally occurring hydrocarbon precursor of cholesterol. Squalene droplets are stabilized by addition of 2 non-ionic surfactants, a low hydrophilic–lipophilic balance (HLB) surfactant, Polysorbate 80 (Tween 80), which is widely used as an emulsifier in foods, cosmetics and pharmaceuticals, including parenteral formulations [12], and sorbitan triolate (common name is Span 85).

For the manufacturing of stable and uniform o/w emulsions, it is essential to keep the droplets within the lower nanometer range (10–200 nm). Emulsions tend to be unstable systems (i.e., subject to flocculation and sedimentation during storage). For MF59™, particle size reduction and homogenization is more readily achieved by microfluidization, which enhances the contact between the dispersed oil droplets and the continuous aqueous phase. After sterile filtration of the emulsion through a 0.22-μm membrane, the mean particle size and the composition and pH of MF59™ remain unchanged compared to initial values for at least 3 years at 2–8 °C, providing an excellent stability profile. The very low concentration of large droplets of MF59™ remained stable for the same period of time, another important feature.

A wide variety of vaccine antigens has been formulated with MF59™, either as one-vial (which is more convenient) presentations or as extemporaneous formulations of separate vials (e.g., if different storage conditions are required) and mixed just before use. The use of MF59™ with antigens that display very different

Table 1
Composition of MF59™ [33]

Appearance	Milky white oil-in-water (o/w) emulsion
Composition	0.5% (v/v) Tween 80 0.5% (v/v) Span 85 4.3% (v/v) Squalene Water for injection 10 nM Na–Citrate buffer
Density	0.9963 g/ml
Viscosity	Close to water, easy to inject
Size	160 ± 10 nm

physical–chemical characteristics has proven to be feasible. These antigens range from monomeric (HIV gp120) to particulate (surface antigens from hepatitis B virus [HBV]) in nature, and from soluble (HSV-2 gD2) to insoluble (influenza virus haemagglutinin) in water, indicating the versatility of the MF59™ adjuvant formulation.

4. Mechanisms of adjuvanticity

Although vaccine adjuvants have been used for more than 70 years, little is known about the exact mechanisms responsible for the biological effect. For aluminum salts, it was postulated that a major contribution to adjuvanticity is the depot effect which suggests that antigen adsorbed to the adjuvant is kept for an extended time at the site of injection and thus available at comparably high concentrations for uptake by phagocytic cells [13–15]. However, this effect cannot solely explain all the immunological phenomena triggered by alum itself or other vaccine adjuvants, including activation and recruitment of antigen-presenting cells (APC), which in turn play a key role in the amplification and differentiation of antigen-specific T-cells [16].

The precise mechanism of MF59™ adjuvanticity is still unknown. Studies conducted with fluorescently labelled MF59™ have shown that 4 h after intramuscular administration, a mere 36% of injected adjuvant was still present in the muscle and that the peak of localization in the corresponding lymph nodes was reached 2 days after injection. The presence of adjuvant did not influence the distribution of the co-administered antigen (HSV-2 gD2), which was cleared from the site of injection independently of MF59™. Two days after intramuscular injection, MF59™ localized in the draining lymph node was shown to be partially located in T-cell areas within lymph node-resident cells that had the characteristics of antigen-presenting cells (APC) (Fig. 1).

Administration of MF59™ also induced a significant influx of macrophages at the site of injection, which was significantly suppressed in mice deficient for chemokines receptor 2 (CCR2) [17–19]. Thus, it is conceivable that one of the effects of MF59™ is to trigger the production of chemokines in cells resident at the injection site. Ongoing studies show that a local immuno-stimulating environment is generated and that human immune cells can be directly activated *in vitro* by MF59™. Further studies are underway to fully explore the effects of MF59™ [18,19] on the immune response.

Irrespective of its mechanism(s) of action, the data currently available for young and old mice strongly suggest that MF59™ enhances functional and protective antibody responses [20–22] and/or induces strong T-cell responses [20,23,24] to several different types of antigens including bacterial toxoids (e.g., tetanus toxoid and diphtheria toxoid), outer membrane vesicles (OMVs) (e.g., *Neisseria meningitidis*), polysaccharide conjugates (e.g., meningococcal C conjugate vaccines), recombinant antigens (e.g., hepatitis B surface antigen [HBsAg], meningococcal B, Herpes Simplex virus [HSV]-2 gD2), and viral antigens (e.g., influenza antigens).

The strength of MF59™ compared to conventional adjuvants is the enhanced immunogenicity and the option to decrease the amount of adjuvant. The immunogenic strength of the adjuvant has been published several times in the past and will be published in the future. The scope of this article is to highlight the safety and tolerability of the product.

5. Preclinical, clinical and postmarketing experience

5.1. Methods of safety evaluation

MF59™ containing vaccines are evaluated by the usual methods of preclinical pharmacology and toxicology, clinical pharmacology and pharmacovigilance. Certain specificities are taken into account, such as mechanism of action and repeated administration to healthy subjects. Vaccine safety is assessed during preclinical period in animals, in clinical trials and during the postmarketing period according to the past and current standards as laid down in good clinical practice (GCP), good laboratory practice (GLP), good manufacturing practice (GMP) and good publication practice (GPP).

In all clinical phases 1–4 studies, local and systemic reactions were collected as solicited adverse events (AEs) during the 7 days post-vaccination with standardized patient diary cards and graded by size and clinical intensity (mild, moderate, severe), with the following definitions applying: (1) mild = "transient or mild discomfort. No limitation in normal daily activity"; (2) moderate = "some limitation in normal daily activity"; and (3) severe = "unable to perform normal daily activity".

All other AEs (i.e., unsolicited AEs) from clinical studies were collected for a predefined time period, while serious AEs (SAEs) were collected for the entire study period. The durations of the follow-up periods were between 4 weeks (50% of the studies) and 6 months.

All adverse events were defined as "... any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product at any dose that did not necessarily have a causal relationship with the treatment. An adverse event (AE) could, therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product. This definition included intercurrent illnesses or injuries and exacerbation of pre-existing conditions." All adverse events, regardless of severity, were closely monitored by the Investigator until resolution. All subjects experiencing adverse events – whether considered associated with the use of the study vaccine or not – were monitored until symptoms subsided and any abnormal laboratory values have returned to baseline, or until there was a satisfactory explanation for the changes observed. All findings had to be reported to the company, and were reported as part of the clinical study reports to the health authorities. All adverse events meeting predefined criteria, like death, life threatening, hospitalization or prolonged hospitalization, severe disability or incapacity, congenital anomaly or birth defect) were considered serious [25,26].

In the context of Pharmacovigilance activities during the postmarketing period, ADRs are defined according to Volume 9A of EUDRALEX [27]. The term "Adverse drug experience" was used as determined in the respective US regulations *21 CFR 314.80*, *21 CFR 600.80*. Spontaneous AE reports were recorded according to the applicable guidelines and are coded using the Medical Dictionary for Regulatory Activities (MedDRA), thus ensuring the confirmation of clinical diagnoses, in particular of significant diseases regardless of whether these are serious or not. Special care was taken to ensure consistency of data recording and assessment within and across studies. Although the specificities of each protocol need to be taken into account, the comparability of the recorded data is of utmost importance.

At Novartis Vaccines and Diagnostics (NVD), two different systems for causality assessment of AEs are in use: Pharmacovigilance is using a binary system (event possibly related/not related to product, plus not applicable [N/A]). The clinical department uses the sub-categories "probably related", "possibly related" or "not related", defined as follows:

(1) Probably related: Exposure to the investigational vaccine and AEs are reasonably related in time *and* the investigational vaccine is more likely to be responsible for the AE than other causes, *or* is the most likely cause for the AE.

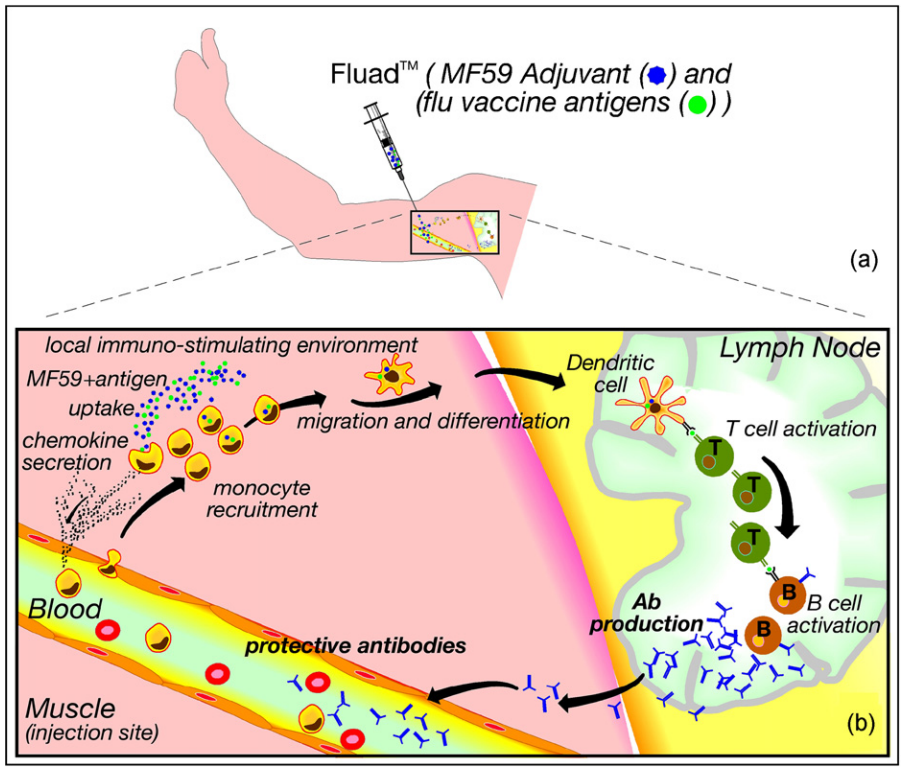

Fig. 1. MF59™ uptake, monocyte recruitment, migration, T-/B-cell activation and differentiation (with permission of Derek O'Hagan).

(2) Possibly related: The administration of the investigational vaccine and AE are considered reasonably related in time *and* the AE could be explained by causes other than exposure to the investigational vaccine.
(3) Not related: The AE is not related if exposure to the investigational vaccine has not occurred, *or* the occurrence of the AE is not reasonably related in time, *or* the AE is considered unlikely to be related to use of the investigational vaccine, i.e., there are no facts (evidence) or arguments to suggest a causal relationship.

Recent efforts to develop case definitions for local and systemic reactions after immunization and for AEs following immunization (AEFI) are ongoing (Brighton Collaboration Working Groups). This will enable standardized assessments and presentation of data collected in clinical trials and in pharmacovigilance systems, and will allow the comparability of data in future. In pharmacovigilance predefined case definitions (representing clinically related MedDRA preferred terms) were used to be able to including or excluding a case into the different clinical symptom groups (see Section 5.4).

All preclinical, clinical and postmarketing data presented and analyzed have not been published elsewhere in this comprehensive manner. Results from "Section 4" will soon be published elsewhere in more detail by the Novartis Research Group. The present assessment of the safety of MF59™ is based primarily on the clinical development programs of the influenza vaccine Fluad® (MF59™-adjuvanted vaccine) and Agrippal (formulation identical to Fluad®, but without MF59™ adjuvant).

5.2. Preclinical experience

During the development of MF59™ various formulations were tested, including a water-based formulation (referred to as MF59™ [water] or MF59W.1). MF59W.1 was later optimized by the addition of citrate buffer to increase stability (MF59C.1). Safety information pertaining to both formulations is relevant. Citrate is a common, well-tolerated excipient and immunogenicity and toxicology studies have not identified any notable difference between the two formulations.

The nonclinical testing of MF59™ consists of research studies performed to explore its mechanism of action, 'adjuvanticity', and ability to enhance protection in challenge models. GLP (Good Laboratory Practice) tolerability and toxicology studies have also been conducted to fulfil regulatory requirements. Several publications describe the enhancement of immunogenicity of a variety of antigens adjuvanted with MF59™ in animals [33,28–30,11], whereas the results of Novartis' GLP toxicology studies performed to fulfil global health authority requirements for clinical testing or product approvals have not been published, to date.

MF59™ administered alone and in combination with a variety of antigens has been tested in several animal models including mice (product release, immunogenicity, challenge, and micronucleus test), rats (reproductive toxicity), Guinea pigs (product release, immunogenicity, and sensitisation), rabbits (immunogenicity, standard toxicology, and reproductive toxicity), dogs (toxicology), goats (immunogenicity) and several non-human primates, including chimpanzees (immunogenicity and efficacy testing). Antigens adjuvanted with MF59™ have included recombinant proteins or glycoproteins from herpes simplex virus 2 (HSV), human immunodeficiency virus (HIV), cytomegalovirus (CMV), hepatitis B virus (HBV), hepatitis C virus (HCV), *E. coli*, parvovirus, human papillomavirus (HPV), and malaria, haemophilus influenza B, Neisseria meningitides, as well as natural glycoproteins from influenza virus. The antigen and MF59™ combinations generated high antigen-specific antibody titres, compared to non-adjuvanted antigens and, where tested, high virus neutralizing titres. Two review articles provide summaries of the research results with these antigens, as well as the primary references for specific antigens [31,32].

The regulatory toxicology studies (internal reports) with MF59™ and MF59™-adjuvanted antigens were designed to meet United States Food and Drug Administration and the European Medicines Evaluation Agency requirements and complied with applicable international guidelines for the nonclinical assessment of vaccines and adjuvants. The pivotal toxicology studies performed with MF59™ adjuvant (alone) included the evaluation of local tolerability, repeat-dose toxicity (one clinical dose administered to rabbits once daily for 14 days), genotoxicity, sensitisation, and embryofetal and developmental toxicity. These studies provided the basis for using MF59™ as an adjuvant platform for combination with many antigens.

Product-specific GLP toxicology studies have been conducted with vaccine formulations composed of antigens combined with MF59™, submitted to health authorities, and enabled clinical testing programs (described in Section 5.3). In some toxicology studies a saline control was used; in others, MF59™ without antigens served as the control. Formulations of antigen plus MF59™ and MF59™ without antigen were well tolerated. Based on the comprehensive in-life and post-mortem parameters evaluated, no treatment-related safety issues were identified. Histopathology findings were generally limited to inflammatory responses at the injection site. These were of low severity and were partially to fully resolved by the end of a 7- to 14-day recovery period. Consistent systemic treatment-related findings in animals treated with antigen plus MF59™ included increases in fibrinogen levels and slight increases in globulin. These findings are consistent with administration of adjuvanted vaccine formulations.

The nonclinical safety program, using several animal species, provides a complete and accurate assessment of the safety of MF59™ for use as an adjuvant. Its use is not associated with any potential for systemic toxicity and it has a low order of local reactogenicity. In repeat-dose rabbit studies, clinical pathology findings of increased fibrinogen, and minor inflammatory and degenerative changes at the injection site are consistent with the effects of intramuscular (i.m.) injections of an immunological adjuvant. These reactions are readily reversible within days to 1–2 weeks. MF59™ is not genotoxic, teratogenic, nor does it cause sensitisation.

When comparing findings with antigens plus MF59™ versus MF59™ alone, no additional notable adverse effects were seen with the antigen–adjuvant combinations. In general, although immunogenicity is enhanced, toxicological findings with MF59™-adjuvanted vaccines are comparable to findings with MF59™ alone [33].

5.3. Clinical experience

5.3.1. Antibodies against squalene

MF59™ adjuvant contains squalene. Previous studies have raised the concern that squalene may induce the production of specific antibodies [34]. This finding was based on semi-quantitative data obtained with a dot-blot assay. However, recent studies show that MF59™-adjuvanted vaccines do not elicit antibodies against squalene.

To evaluate whether antibodies against squalene are produced, an analysis using very sensitive and specific assays, developed at the Walter Reed Army Institute of Research, USA [5,35], were used. Serum samples taken from individuals before and at various times after immunization with MF59™-adjuvanted influenza vaccine or, as a control, with influenza vaccines without adjuvants were tested. The results of these assays show that:

- IgG and IgM antibodies against squalene were detectable at very low levels already before immunization with MF59™-adjuvanted influenza vaccines in the vast majority of young, adult, and elderly individuals.
- Immunization with MF59™-adjuvanted vaccines did not induce any change in the levels of serum anti-squalene antibodies.
- The level of anti-squalene antibodies detectable in the sera of subjects immunized with vaccines adjuvanted or not adjuvanted with MF59™ was similarly low [36].

Data obtained with sera from subjects from different geographic areas were similar (USA, Western and Eastern Europe) [36].

Taken together, these data show that the MF59™ adjuvant squalene is not associated with the production of specific antibodies (Fig. 2). These antibodies may well represent low-avidity antibodies naturally occurring in healthy individuals, and their serum titres are not influenced by immunization with MF59™-adjuvanted vaccines.

5.3.2. Clinical studies with MF59™

Extensive clinical immunogenicity and safety data on various MF59™-adjuvanted vaccine antigens have been generated in clinical trials over the last 15 years. The data show that MF59™-adjuvanted antigens elicit a strong antibody response, and are safe and generally well tolerated [4]. The clinical findings are instrumental in the understanding of the adjuvanticity of MF59™, and more importantly of the safety of this compound.

As an o/w emulsion, the MF59™ adjuvant is very fluid, and is expected to be well tolerated and to induce strong short-term immune responses as the oil content is very low (between 15 and 25%) [37]. Furthermore, the route of administration is important as it influences local reactogenicity and the immune response.

Data have been generated across all age groups, including the elderly, younger adults, adolescents, and also newborn infants. Most experience has been gathered in conjunction with influenza vaccines with more than 14,000 individuals exposed in more than 30 phases 1–4 clinical studies [38,3,39–44]. Before registration in May 1997 Fluad®, had been tested in 28 single- or double-blind, randomized, and controlled studies, 13 of which with a 4- to 6-month follow-up and 12 studies with a 4-week follow-up. 24 of 30 studies enrolled elderly subjects (≥65 years). Fluad® was tested for equivalence of antigenic content against the inactivated subunit comparator vaccine Agrippal® (same antigenic content as Fluad®, but without MF59™ adjuvant), in 20 studies, against Fluogen®/Fluvirin® in one study, against Fluzone® in two studies, against Influvac® in three studies, and against Flushield® in two studies.

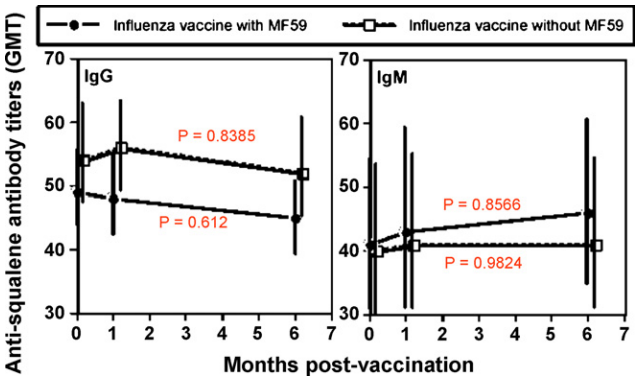

Fig. 2. Anti-squalene IgG and IgM antibodies in serum samples after immunization with subunit influenza vaccine with the MF59™ adjuvant ($n = 48$) or with a plain, split influenza vaccine without the MF59™ adjuvant ($n = 52$) [36]. Vertical lines represent 95% CI. None of the differences (either between vaccines or between time points with one vaccine) were statistically significant (P values ranged between 0.130 [vaccine with the MF59™ adjuvant versus vaccine without, IgG titers 1 month after immunization] and 0.863). There were no trends over time detected as significant for either vaccine or either antibody ($P \geq 0.6212$).

In addition, MF59™ adjuvantation has been evaluated in clinical trials with HSV, HIV, CMV, HBV, HCV, *E. coli*, parvovirus, and HPV vaccine candidates. MF59™ adjuvant has also been tested in several different formulations, including single-container presentation (HSV vaccine, influenza vaccine), dual container without buffer in the MF59™ portion (influenza vaccine), and dual container with citrate buffer in the MF59™ portion (HBV vaccine, influenza vaccine), similar to the current vaccine formulation. To date, more than 20,000 subjects have received i.m. injections of either unbuffered MF59™ or MF59™ containing citrate buffer in clinical trials. Of these, approximately 19,100 subjects have received MF59™ with a vaccine antigen and 1600 received adjuvant alone. The data are summarized in Table 2.

5.3.3. MF59™ as adjuvant to prophylactic vaccines

5.3.3.1. HCV vaccine. Persistent HCV infection affects 170 million people worldwide. Acute HCV infection is often asymptomatic, but many infected individuals develop persistent infection that may lead to development of the end-stage liver disease, including liver cirrhosis and hepatocellular carcinoma. Thus, an HCV vaccine, which significantly lowers the rate of chronic infection, would have a major impact on disease burden. Unfortunately, HCV is a highly mutable virus, and escape mutations can undermine vaccine-induced virus-specific immunity. Furthermore, HCV exists in multiple genotypes, and hence genotype-specific vaccines might be required in order to achieve broad protection. Additionally, HCV vaccine development has been hampered by the lack of small animal models and cell culture systems, although these are currently in development. Despite these obstacles, several vaccine candidates tested in the chimpanzee HCV model have shown some encouraging results. Some of these vaccine candidates are now in phase 1 clinical studies, including two (HCVE2 and HCVE1E2) with MF59™ utilized as adjuvant, as summarized in Table 2.

MF59™ in combination with either the HCVE2 or HCVE1E2 antigen has been administered to approximately 168 volunteers. The HCVE2/MF59™ vaccine has been tested in a phase 1 randomized, controlled, observer-blind 6-month follow-up safety study in 48 healthy adults, and the HCVE1E2/MF59™ vaccine has been evaluated in a phase 1 program that includes 2 controlled studies (60 healthy volunteers in each study) for the prophylactic indication. So far, at the end of the second phase 1 study, one unrelated SAE has been reported (papillary carcinoma of the thyroid, stage 1, in the HCVE2/MF59™ study) during the entire program. The most common systemic post-immunization reactions noted in the small randomized, double-blind, controlled HCVE2/MF59™ study in the low dose group (10 μg vs. 50 μg antigen) were headache (12/18 subjects), myalgia (8/18 subjects), and malaise (5/18 subjects). Systemic reactions did not show a trend toward increasing frequency with increasing numbers of immunizations. The most common AE that occurred was injection-site pain, experienced in (1/18 subjects) with a maximum duration ≤8 days.

All other clinical data from these phase 1 studies, some of these are still preliminary and blinded, show that the HCVE2/MF59™ and HCVE1E2/MF59™ vaccines are safe and well tolerated [45].

5.3.3.2. HBV vaccine. More than 30 years after the discovery of human HBV, this virus remains one of the major global health problems. A total of 5–10% of infected adolescents or adults become chronic carriers, whereas up to 90% of infected neonates develop chronicity. It is estimated that approximately 370 million people are chronic carriers of HBV worldwide. In many regions of the world, chronic HBV infection is still the major cause of liver cirrhosis and hepatocellular carcinoma.

MF59™ has been tested as an adjuvant in a candidate vaccine in 156 HBV-seronegative individuals who received the vaccine in a prophylactic setting in three phase 1 studies [46,47]. No SAEs related to HBV/MF59™ were reported [46].

5.3.3.3. HSV vaccine. Effective vaccination is still considered the best method for preventing the spread of HSV. The HSV candidate vaccines tested to date have mostly been purified subunit vaccines and/or recombinant envelope glycoproteins (such as gB and gD).

MF59™ has been evaluated in clinical trials of HSV-2 in combination with truncated versions of gD2 and gB2 antigens (Table 2). The gB2dTM antigen (a modified gB2 delta transmembrane antigen) has also been tested in phases 1–3, randomized, blind, controlled studies in combination with MF59™ adjuvant. These vaccines were studied for the prevention or limitation of genital HSV-2 acquisition. Overall, the vaccine formulations were well tolerated. A higher incidence of local and systemic reactions was

Table 2
Clinical experience with MF59™ adjuvanted to different vaccine antigens [33]

Antigen(s)	Route of immunization	Population	Size database (*N*)	Indication[a]
Influenza + MF59™	Intranasally	Healthy adults	31	P
Influenza + MF59™	IM	Adults	460	P
Influenza + MF59™	IM	Elderly	11,462	
Influenza vaccine + MF59™	IM	Children; adolescents	116	P
HCVE2 + MF59™	IM	Healthy adults	36	P
Controls: MF59™ + buffer	IM	Healthy adults	12	P
HCVE1E2MF59™	IM	Healthy adults	48	P
Controls: saline	IM	Healthy adults	12	P
HCVE1E2MF59™ + CpGβ	IM	Healthy adults	48	P
Controls: HCVE1E2MF59™	IM	Healthy adults	12	P
HBV + MF59™	IM	Healthy adults	156	P
HSV-2MF59™ (HSVMF59™ + MTP-PE adjuvant)	IM	HSV-seronegative and -seropositive subjects	2,422 (104)	P
CMV antigens + MF59™	IM	Seronegative volunteers: Incl. 15/500 toddlers 30/500 seropositive volunteers	500	P
Influenza + MF59™	Intranasally	Healthy adults	31	P
Influenza + MF59™	IM	Adults	460	P
Influenza + MF59™	IM	Elderly	11,462	P
Influenza vaccine + MF59™	IM	Children; adolescents	116	P
HBV + MF59™	IM	HBV-infected subjects	159	Th
HBV + MF59™ + Lamivudine	IM	HBV-infected subjects	120	Th
Controls: MF59™ + buffer	IM	HBV-infected subjects	99	Th
HCVE1E2MF59™ ± pegylated Interferon + Ribavirin	IM	HCV-infected patients	48	Th

[a] P = prophylactic; Th = immunotherapeutic.

reported in HSV-2 seropositive subjects. These reactions were generally self-limited and moderate, and were considered clinically acceptable. Among HSV vaccine trial participants [total $N = 3719$ (2422 HSV vaccine and 1297 control subjects)] 4 subjects were reported with SAEs considered possibly related to vaccine (3 in the vaccine group and 1 in the placebo group). The possibly related SAEs included myofasciitis, asthmatic bronchitis, ulcerative colitis, and anaphylactoid reaction. A total of 345 healthy adults were enrolled in another phase 3, randomized, double-blind HSV trial, assessing the lot consistency of 3 different production lots versus a reference lot. One adult woman developed neuritis of the right upper arm (injection site), upper back, and neck associated with deep injection-site inflammation, with an onset on the day of a third immunization and a duration of 6 days; the event was considered serious and definitely related to the experimental vaccine.

During the conduct of phases 1–3 HSV vaccine studies, 4 cases of possible fibromyalgia and 20 cases of atypical pain syndromes (defined as myalgias, arthralgias, and/or paresthesias persisting longer than 14 days were reported; 14/20 [70%] cases considered possibly related to the vaccine) were reported among all subjects receiving MF59™ with or without HSV antigens, with 3 of 4 subjects who experienced these syndromes being female. One of the 4 subjects was among 104 recipients of the adjuvant MTP-PE in addition to MF59™. The prevalence of fibromyalgia has been reported in the general population older than 18 years as 0.9–7.4% among women, and 0.1–1.2% among men. Symptoms typically appear between the ages of 20 and 55 years. The predominant symptom of fibromyalgia is widespread musculoskeletal pain. The overall relationship of these cases to the vaccine remains uncertain. No cases of atypical pain syndromes have been identified in vaccine programs using MF59™ adjuvant with other antigens [45].

5.3.3.4. HIV vaccine. The demand for an affordable, safe and effective HIV vaccine has never been greater. As the immunogenicity of all the vaccine vectors currently being evaluated in human populations is limited, novel vaccine strategies are needed to stimulate the innate immune system to have the appropriate immune response.

Clinical trials have been conducted with MF59™ combined with genetically engineered HIV antigens, p24, Env 2-3, gp120, and gp120 "Thai E" [48–51]. A total of 1351 HIV-seronegative and 113 HIV-seropositive individuals received one of the vaccines, and an additional 168 seronegative and 69 seropositive subjects received placebo with MF59™ (Table 2). No HIV vaccine-related SAEs have been reported. The results of these trials indicate that HIV vaccine formulations with MF59™ are safe and well tolerated.

An additional phase 1 clinical study is being conducted in collaboration with the HIV Vaccine Trials Network to evaluate the safety and immunogenicity of 3 injections of the Clade B gag DNA/PLG and env DNA/PLG microparticles boosted by 2 injections of Clade B HIV o-gp140 and MF59™ vaccine in healthy, HIV-1 uninfected adults. The study was amended to include a fifth group to receive either 3 injections of Clade B HIV o-gp140 and MF59™ vaccine or placebo. So far, 61 subjects have received at least one dose of either Clade B o-gp140 and MF59™ vaccine (or placebo) as a boost or as primary immunization. The study is still ongoing, but preliminary blinded safety data indicate that the regimen is generally safe and well tolerated in healthy adult recipients. No vaccine-related SAEs have been reported [45].

5.3.3.5. CMV vaccine. Because the CMV glycoprotein B (gB; gpUL55) is the major target for CMV neutralizing antibody, it is a prime candidate for a CMV vaccine. More than 500 CMV-seronegative (including 15 toddlers) and 30 CMV-seropositive volunteers have received a CMV vaccine composed of CMV gB antigen with MF59™ adjuvant in Novartis sponsored blinded, randomized, controlled trials [52–54]. The study participants were actively monitored as described in "Section 5.1". The gB/MF59™ reactogenicity profile was favourable and was independent of the gB dose. This profile was similar to the reactogenicity profile of gB combined with aluminum [53], and differed from the placebo reactogenicity profile in that episodes of local pain and malaise were more frequent. According to the judgement of the investigators, these symptoms were mild in most cases, although in some instances they were described as moderate or severe. Among more than 500 subjects in Novartis-sponsored CMV trials, one adult subject had an immediate increase in blood pressure to 164/120 mmHg on the day of immunization. The subject recovered following treatment, but was withdrawn from the study. The blood pressure rise was considered as an SAE that was at least possibly related to the immunization. No other vaccine-related SAEs were reported. In summary, the vaccine was well tolerated [45].

5.3.3.6. Influenza vaccine. The US Advisory Committee on Immunization Practices produces a regularly updated rationale for vaccination against influenza [55]. The current version identifies 12 categories of patients at high risk of complications from influenza. Annual vaccination against influenza is recommended for: (1) all persons, including school-aged children, who want to reduce the risk of becoming ill with influenza or of transmitting influenza to others; (2) all children aged 6–59 months (i.e., 6 months–4 years); (3) all persons aged >50 years; (4) children and adolescents (aged 6 months–18 years) receiving long-term aspirin therapy who therefore might be at-risk for experiencing Reye syndrome after influenza virus infection; (5) women who will be pregnant during the influenza season; (6) adults and children who have chronic pulmonary (including asthma), cardiovascular (except hypertension), renal, hepatic, haematological or metabolic disorders (including diabetes mellitus); (7) adults and children who have immunosuppression (including immunosuppression caused by medications or by human immunodeficiency virus; (8) adults and children who have any condition (e.g., cognitive dysfunction, spinal cord injuries, seizure disorders, or other neuromuscular disorders) that can compromise respiratory function or the handling of respiratory secretions or that can increase the risk for aspiration; (9) residents of nursing homes and other chronic-care facilities; (10) health-care personnel; (11) healthy household contacts (including children) and caregivers of children aged <5 years and adults aged >50 years, with particular emphasis on vaccinating contacts of children aged <6 months; and (12) healthy household contacts (including children) and caregivers of persons with medical conditions that put them at higher risk for severe complications from influenza.

There is general agreement on the need to enhance the immunogenicity and efficacy of influenza vaccines, especially in adults approaching elderly age and in the elderly themselves, when the ability of the immune system to mount a strong and efficacious response decreases. MF59™ has been proven to strongly enhance the immunogenicity in mice, and other small animals, inducing antibody titres 5 to >100 times higher than those obtained in the absence of adjuvants [56]. Importantly, the enhancement of the immune response to an influenza subunit vaccine, mixed with MF59™, was not affected by pre-existing immunity to the virus [57]. This observation is particularly important because immunization against influenza is routinely carried out every year and pre-existing immunity can negatively affect the efficacy of subsequent immunizations. In preclinical studies, MF59™ adjuvant offered improved protection against influenza virus challenge and significantly reduced the viral load in the lungs of challenged mice [58]. Clinical trials of the MF59™-adjuvanted influenza vaccine are summarized in Table 2.

5.3.4. Special populations

5.3.4.1. Adults (non-elderly). The overall database consists of 460 subjects aged 18–64 years who have received at least one immunization with Fluad®; 104 of these subjects also received a second immunization. The pooled analysis includes data from 460 subjects vaccinated with Fluad® and 453 subjects vaccinated with comparator vaccines (Agrippal®, Fluzone®). Overall, these data show that post-immunization reactions, particularly local reactions, were more frequent in non-elderly adults than in elderly subjects. Compared to unadjuvanted vaccines, Fluad® induced more local reactions, most of which, however, were mild and of short duration. In the Fluad® group after the first injection pain was rated mild to moderate in 87%, 10% had none and 3% of subjects had severe pain; after the second injection pain was rated mild to moderate in 83%, 16% had no pain, and 1% had severe pain. A statistically significant increase in the incidence of injection site—warmth, chills, myalgia, and analgesic/antipyretic use, occurred in the Fluad® group after the first injection, but not after the second. No longer lasting reactions were noted [38].

5.3.4.2. Elderly subjects. The overall database for safety of Fluad® and Fluad®-like vaccines consists of 11,462 elderly subjects (≥65 years of age) who received at least one immunization with Fluad®. The comparator vaccine group includes 6216 subjects who have received at least one dose of licensed comparator vaccine (e.g., Flushield®, Fluvirin®, Influvac®, Vaxigrip®).

Immunization against influenza is normally administered every year due to the antigenic variability of the viruses responsible for seasonal epidemics. Therefore, the evaluation of a potential increased reactogenicity associated with repeated immunizations was part of this clinical program. This aspect is even more relevant for Fluad®, since it contains MF59™ inducing a higher incidence of mild and transient local reactions compared to unadjuvanted vaccines.

For this reason, several trials of this clinical program were 'extended' to the following influenza seasons to evaluate the safety of a second and a third immunization with Fluad®.

These data indicate that multiple immunizations (up to three) with Fluad® were well tolerated (Table 3). Subjects enrolled in second and third immunization trials were predominantly those who did not experience local reactions to the first immunization. An ad hoc analysis showed that demographic characteristics and incidence of reactions in subjects withdrawing from the first trial were not different from those of subjects included in the extension trials.

One phase 4, single-blind, randomized study to evaluate the safety and effectiveness of Fluad® versus a licensed influenza vaccine (Influvac®) administered to elderly (≥65 years of age) subjects enrolled a total of 9194 subjects to receive Fluad® vaccine, and 4550 subjects enrolled to receive the control vaccine (Influvac®). A total of 750 serious adverse events were observed in the Fluad® group (i.e., 8.2%) versus 386 in the control (Influvac®) group (i.e., 8.5%). The rates of adverse events requiring a physician visit with onset between days 0 and 6 and of serious adverse events during the study period (October 1997 to April 1998) were low and similar to the non-adjuvanted control vaccine Influvac®. Hospitalizations and deaths during local influenza season period (24 December 1997 to 4 May 1998) were similar to the control vaccine. One SAE was considered possibly related to Fluad®. From this finding it can be derived that for Fluad® the incidence rate of such AEs (i.e., both serious and possibly related) in the entire population does not exceed 0.05% (or 1 in 1933 subjects).

In the entire clinical database, only 3 SAEs were considered by the investigator to be related (exudative erythema multiforme, Herpes Zoster, pancreatitis and cholangitis) to immunization [33].

5.3.5. Other routes of administration of influenza vaccine

The MF59™-adjuvanted influenza vaccine was given intranasally to 31 subjects in a phase 1 study of healthy adults. The safety and immunogenicity was compared to unadjuvanted influenza vaccine (Agrippal® and placebo). Neither local (sneezing, unpleasant taste, bloody nasal discharge) nor systemic reactions differed significantly between the treatment groups. No SAEs occurred in this study [59].

5.3.6. Pediatric immunization

Until recently, influenza vaccination in children was recommended only for individuals with medical conditions that could put them at higher risk from influenza infection, such as bronchial asthma. The Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention recommended use of trivalent inactivated influenza vaccine in all children 6–23 months old, including healthy children with no chronic medical condition, beginning in the winter season of 2004–2005 based on increasing evidence of high morbidity from influenza infection in young children [60]. An increasing number of children in the United States are being vaccinated [61]. To achieve the goal of universal influenza immunization coverage for healthy children, health care professionals will need a greater understanding of the severity of influenza illness in this age group, coupled with an increased knowledge of indications for vaccine administration.

One pediatric trial has compared the safety and immunogenicity of Fluad® and unadjuvanted vaccines (Fluogen®, Flushield®), in children and adolescents. In this study, performed in the USA, Fluad® was compared to two other influenza vaccines licensed in the USA. A total of 116 subjects (9- to 17-year-olds) were vaccinated with Fluad® and 100 subjects were vaccinated with comparator vaccines. The safety profile emerging from this trial was similar to that of older age groups with a moderately increased rate of pain, chills, malaise and headache in the Fluad® group.

A second randomized, observer-blind pediatric trial in children recently conducted in Finland [62] compared safety and immunogenicity of Fluad® to a conventional Influenza split vaccine. A total of 130 healthy children (6–59 months of age) received 2 doses of Fluad®. As with other clinical trials, local and systemic reactions were recorded for 7 days after each immunization, and all other AEs recorded throughout the entire study period with a follow-up period of 6 months. Both vaccines were equally well tolerated except for injection site swelling which was higher in the Fluad® recipients.

A third clinical study in children is ongoing to investigate safety and immunogenicity of a monovalent influenza vaccine, containing H5N1 antigen with MF59™ adjuvant [33].

However, these clinical data are too limited to draw final conclusions on the pediatric indication of Fluad®, which will require more extensive clinical investigation.

5.3.7. MF59™ adjuvanted to therapeutic vaccines

5.3.7.1. HBV vaccine. MF59™ has been evaluated as an adjuvant in a candidate immunotherapeutic HBV vaccine in 158 patients with chronic HBV infection in phases 1 and 2 studies (Table 2). An additional 99 patients with chronic HBV infection received MF59™ with vehicle buffer without antigen. The first SAEs possibly related to the vaccine were reported in the phase 2, proof of concept trial (total enrolment of 219 HBV-infected subjects of whom 120 received HBV/MF59™ plus lamivudine). One death occurred in a subject receiving HBV/MF59™, resulting from an intercurrent infection that was unrelated to study treatment. The only possibly or probably related SAE was a grade 2 hepatitis flare. The rates of hepatitis flares and other AEs or SAEs were comparable in all treatment

Table 3
Clinical experience: selected local and systemic reactions after first, second and third immunization in elderly subjects—meta-analysis results [33]

Reaction	Vaccine	First immunization Fluad® $N = 2112$ (comparator $N = 1437$), % (95% CI)	Second immunization Fluad® $N = 492$ (comparator $N = 330$), % (95% CI)	Third immunization FLUAD® $N = 150$ (comparator $N = 87$), % (95% CI)
Pain	FLUAD®	32 (30–34)	27 (23–31)	28 (21–36)
	Comparator	14 (12–16)	21 (17–26)	16 (9–26)
Erythema	FLUAD®	18 (16–19)	22 (18–26)	22 (16–29)
	Comparator	13 (11–15)	19 (15–23)	9 (4–17)
Induration	FLUAD®	15 (13–17)	11 (8–14)	13 (8–19)
	Comparator	10 (8–11)	8 (6–12)	6 (2–13)
Malaise	FLUAD®	6 (5–7)	8 (6–11)	7 (3–12)
	Comparator	4 (3–5)	7 (4–10)	3 (1–10)
Headache	FLUAD®	6 (5–7)	8 (6–11)	7 (1–9)
	Comparator	4 (3–5)	7 (3–8)	3 (1–10)
Myalgia	FLUAD®	8 (7–9)	3 (2–5)	1 (0–5)
	Comparator	3 (2–4)	2 (1–4)	2 (0–8)
Fever (≥38 °C)	FLUAD®	1 (0–1)	1 (0–3)	1 (0–4)
	Comparator	<1 (0–1)	1 (0–3)	0 (0–4)

groups, and were at least as high in the placebo and vehicle/MF59™ group as in the active-treatment group [45].

5.3.7.2. HCV vaccine. MF59™ in combination with the HCVE1E2 antigen has been administered in a randomized, controlled study, testing the potential use as a therapeutic vaccine (in combination with pegylated Interferon plus Ribavirin) in 72 chronically infected HCV patients.

By the end of the 6-month follow-up period (2006), no SAE had occurred; therefore HCVE1E2/MF59™ vaccine may be considered safe in HCV-infected subjects (Table 2), given the limitations of an ongoing study [42].

5.4. Postmarketing experience

The favourable safety data demonstrated in clinical trials are supported by postmarketing pharmacovigilance since first registration of Fluad® in September 1997.

Meanwhile, more than 27 million patients have received Fluad® (or Fluad®-like vaccines sold with other brand names such as Adiugrip®, Addigrip®, Prodigrip®, Influpozzi® Adiuvato, Gripguard®, Chiromas®) during the past 9 years, most of which were administered to the elderly.

Fluad® is only licensed in the elderly, and a separate safety evaluation in children will be required, once the target population of immunization can be extended to children.

Reports on suspected spontaneous ADRs after immunization with Fluad® are collected on an ongoing basis.

The Fluad® pharmacovigilance database involves all individual ADR case reports, regardless of their causality. All reports from September 1997 (when Fluad® was first marketed) to August 2006 (before start of the 2006 influenza season) were included in this analysis. In order to ensure coherence and analysis of reported events, all were classified according to MedDRA.

All spontaneous reports are continually evaluated by Pharmacovigilance and reported according to international regulatory requirements, independent of causality assessment. The ADR reports are processed by means of a sequential system of reception, validation, collection of additional data, checking for duplicates, coding, database recording, technical and scientific analysis including causality assessment, issue detection and generation of possible safety signals.

Not all reported AEs are actually caused by the vaccine. Appropriate vaccine safety monitoring includes (but is not limited to) careful review of all case reports using a structured methodology to determine causality, and using these data to initiate appropriate follow-up actions.

Methods of Causality assessment: "Possibly related" is any reaction, which is assessed as at least possibly related (hence including "probably related" and "very likely/certain" as per World Health Organization [WHO] definition [63]). "Not related": The AE is not related if exposure to product has not occurred, *or* the occurrence of the AE is not reasonably related in time, *or* the AE is considered unlikely to be related to use of product, i.e., there are no facts (evidence) or arguments to suggest a causal relationship. "Not applicable" (N/A): Causality assessment not applicable (e.g., in the cases without AE occurrence, such as "medication errors").

Except for the category "N/A", Pharmacovigilance is using a binary system for causality assessment of adverse events following immunization. This system of classification mirrors regulatory reporting requirements to regulatory authorities, as either an individual case report which represents a serious, unexpected event from a clinical trial has to be notified to the authorities or not, dependent on the relatedness to the administered drug/vaccine product. The Federal Institute for Drugs and Medical Devices in Germany (BfArM) and the Council for International Organizations of Medical Sciences (CIOMS) also recommend a binary system.

The usual duration of follow-up is 8 weeks, although the length may be extended to longer periods of time, whenever necessary (e.g., outcome of unintended "Exposure during pregnancy"-cases).

During the period of evaluation, Novartis Vaccines Pharmacovigilance received a total of 387 case reports (representing approximately 1400 single AEs, i.e., an average of 3.5 events/symptoms per case report) (Fig. 3). A total of 107 case reports fulfilled at least one seriousness criterion[6] regardless of their severity and causality.

[6] Resulting in any of the following outcomes: death, life-threatening event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening or require hospitalization may be considered to be serious drug experiences when, based upon medical judgement, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (21CFR§312.32(a)).

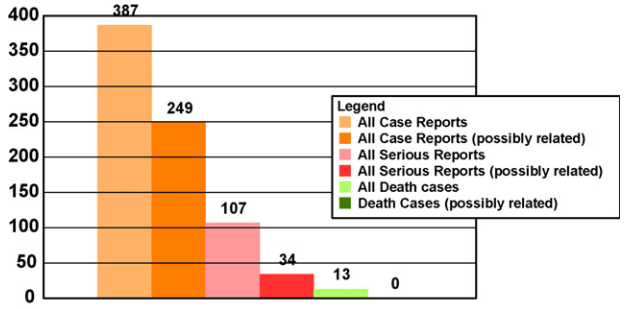

Fig. 3. Number of reported adverse events for Fluad® or Fluad®-like vaccines per 27,374,412 doses sold. Reporting period: September 1997 to August 2006.

A total of 57% of case reports were from elderly vaccine recipients who were 65 years or older, which is the target group for immunization (Fig. 4). Another 34% of cases were from adults (18–64 years), 1% of cases were from adolescents and children, and 8% were cases of unreported age.

The median age of the patients reporting at least one event was 67.5 years (range: 7–102 years) with 65% female and 35% male subjects.

We were interested in acute, short-term reactions to the vaccine (up to 1 day risk period), such as allergic reactions, in medium-term reactions (1 week risk period), such as local and systemic reactions, also rash, seizures, and unspecified adverse events after vaccination, and long-term reactions (1 week to 1 month period and greater than 1 months risk windows), such as possible delayed reactions to immunization that might occur via immune-mediated mechanisms. In particular, given the rare association of Guillain-Barré syndrome (GBS) with some formulations of trivalent inactivated influenza vaccines in the past [59], we screened for neurological diagnoses, including GBS, neuropathies, and demyelinating disease.

The median time between the date of immunization and the onset date of all adverse events (AEs) was ≤1 day (range: seconds to 5 months). Most AEs were experienced within the first days following the immunization. Late onset of AEs was rare (only 2% of all cases had an onset latency ≥1 month).

Cases were classified into six different clinical symptom groups. The number of cases per symptom group was 132 for "injection-site reactions", 117 for "systemic reactions", 41 for "allergic reactions", 51 for "neurological disorders", 9 for "vascular disorders", and 64 for "others" (Fig. 5). A case might have been allocated to more than one symptom group, e.g., a case with an injection-site reaction in addition to a systemic reaction.

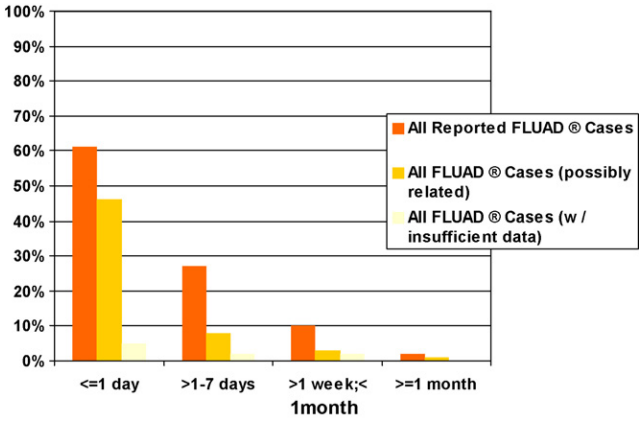

Fig. 4. Percentage of all cases ($n = 387$) entered between September 1997 and August 2006 into the Pharmacovigilance database with time between Fluad® immunization and the onset of adverse events.

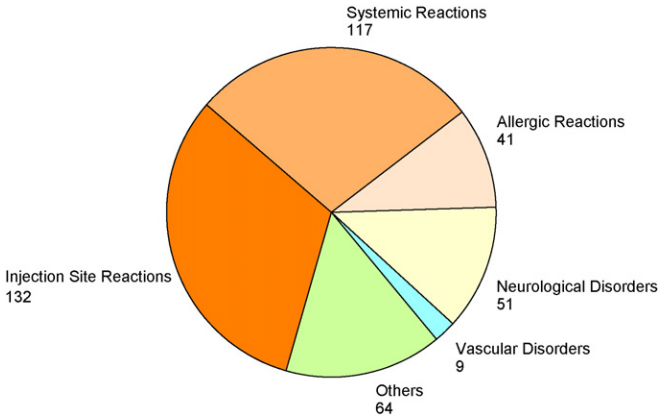

Fig. 5. Number of Cases per 27,374,412 Fluad® doses sold from September 1997 to August 2006, sorted by clinical symptom groups, regardless of causality.

Based on approximately 27 million sold doses, presumed to represent the approximate number of administered doses, a total reporting rate of 1.4 cases per 100,000 sold doses was estimated.

5.4.1. Serious cases

In total, 107 cases met at least one seriousness criterion, most often (64.8%) "hospitalization", resulting in a reporting rate of 0.39 serious cases per 100,000 doses sold. Of these, 34 cases were considered possibly related due to temporal or biological plausibility, or both, according to the company internal causality assessment. The AEs reported in these serious cases belonged to a variety of clinical entities. Most frequently reported AEs were injection-site reactions, skin reactions and subcutaneous tissue disorders, neurological disorders (myelitis ($n = 1$), transverse myelitis ($n = 1$), Guillain-Barré syndrome (GBS) ($n = 6$), Personage Turner Syndrome ($n = 1$)), and respiratory symptoms. No new or uncommon trend of AEs (compared with company internal data on other influenza vaccines, like Agrippal®, Begrivac®, and Fluvirin®) or any signs of increased frequency of listed AEs have been identified.

5.4.2. Fatal cases

During the evaluation period, 13 cases of death in elderly patients, 68–91 years of age, were reported after use of Fluad®. None of the death cases occurred in persons below 65 years of age. One woman was reported to have died caused by a severe hemorrhage; however, her age was not specified. None of the deaths were considered to be causally related to the administration of the vaccine by the reporting health care professional (9 cases were considered unrelated and in 2 cases only insufficient data were available). The most frequently reported causes of death were related to the cardiovascular system, including ischemic cardiopathy, cardio-respiratory arrest, cardiac insufficiency, dyspnea and acute pulmonary edema. Other causes of deaths were cancer of the prostatic gland, renal insufficiency, unspecified fever and coma, unspecified sudden death, severe hemorrhage and respiratory diseases including, bronchitis and bronchopneumonia. There were 5 deaths reported within 1 week post-immunization (severe hemorrhage, fever/coma, lung edema, sudden death, and bronchopneumonia). No clusters in time, for a certain batch or for a certain geographical region of fatal outcome have been identified. "Signal detection conferences" discuss predefined datasets – without prior statistical hypothesis – exploring the hypothesis of a causal link, in the light of associated factors/background factors.

Table 4
Incidence of reported adverse events following influenza immunization from September 1997 to August 2006 [33]

Adverse events	Reported cases (*N*)	Number of cases assessed as possibly related	Reporting rate per 100,000 doses
All reported events[a]	387	249	1.4
Serious cases	107	34	0.39
Fatal cases	13	0	0.05
Vaccine failures	4	4	0.01
Allergic reactions	39	34	0.14
Neurological disorders	51	21	0.18
ADEM, encephalitis, myelitis	8	2	0.02
GBS	9	7	0.03
Parsonage-Turner Syndrome	3	2	0.01
Blood and vascular disorders	9	2	0.03

[a] Sold doses of Fluad® or Fluad®-like vaccine. *N* = 27,374,412.

5.4.3. Vaccine failures

A high degree of efficacy has been observed with Fluad® and Fluad®-like vaccines. Several clinical trials have shown that the efficacy of Fluad® is markedly enhanced compared to similar influenza vaccines without MF59™ [40,42,43].

During the period of evaluation, 4 cases of influenza A or B infections were reported after 3 or 5 months despite previous immunization, a total of 0.01 cases per 100,000 doses.

5.4.4. Incidence of reported adverse events after immunization

The numbers of ADR cases after use of MF59™-adjuvanted influenza immunization are summarized in Table 4.

5.4.5. Adverse events of special interest

5.4.5.1. Allergic reactions. We classified severe immediate allergic reactions observed within 24 h after immunization into: (a) skin reactions, such as urticaria, pruritic rash/flush, and (b) reactions such as generalized or facial edema, bronchospasm, and larynx edema. A total of 39 cases of allergic reactions were reported, resulting in an estimated 0.14 cases per 100,000 doses. A total of 7 cases were assessed as serious, none of which had a fatal outcome (Table 5). A total of 34 cases were considered possibly related to influenza immunization. One case of anaphylactic shock occurred in a 71-year-old female vaccine recipient. The low reporting rates of immediate type allergic reactions in vaccine recipients confirms that the vaccine is safe for use in elderly patients.

5.4.5.2. Neurological disorders. A variety of neurological syndromes have been occasionally reported after use of influenza vaccines, such as acute disseminated encephalomyelitis (ADEM), GBS, Parsonage-Turner Syndrome (PTS), neuritis, and other neurological conditions. Due to the clinical significance of these entities, a discussion is ongoing about the causal relationship between vaccines and miscellaneous demyelinating neurological diseases.

A total of 51 cases of neurological disorders have been reported after immunization with Fluad® and Fluad®-like vaccines, an estimated 0.18 cases per 100,000 vaccine recipients. This includes mild adverse reactions, such as headache, nervousness and paresthesias. A total of 21 cases were considered possibly related to vaccine, 14 of which met at least one seriousness criterion. The cases of serious neurological disorders are listed in Table 5.

5.4.5.2.1. Encephalitis and myelitis. Acute disseminated encephalomyelitis can follow viral and some bacterial infections and have been reported in the literature following the administration of vaccines. A total of 8 cases reported after the administration of Fluad® were under review by pharmacovigilance. There have been 2 reports of acute disseminated encephalitis, 4 additional reports of unspecified inflammatory conditions of the brain, one case of myelitis and one case of transverse myelitis. Two cases were assessed as related to the vaccine. The estimated incidence was 0.02 cases per 100,000 vaccine doses. The spontaneous incidence rate of this disorder is unknown. A recent study conducted in San Diego, CA, USA, estimated a mean annual incidence of 0.4 per 100,000 in persons less than 20 years of age [64,65]. For the period of 1994–2004 the Japanese Kitasato Institute [66] reported a total of 4 cases of encephalitis, aseptic meningitis, acute disseminated encephalitis and acute cerebellar ataxia in 38.02 million influenza doses, reflecting a reporting rate of 0.01 cases in 100,000 doses.

5.4.5.2.2. Guillain-Barré syndrome (GBS). During the evaluation period 9 cases of GBS were reported, irrespective of causality assessment, resulting in an estimated 0.03 cases in 100,000 recipients of vaccine, or one report of GBS for every 3 million sold doses of Fluad® and Fluad®-like vaccines. Six cases have been assessed as possibly related. The mean onset latency was 12 days, within a range of 7–17 days after immunization. Four events occurred in male subjects and two events occurred in female subjects. The mean age was 71 years (range 53–82). The spontaneous incidence of the disease is approximately 0.6–4 cases per 100,000 persons per year [67]. It is estimated that GBS occurs in 0.07–0.46 cases per 100,000 within 6 weeks after immunizations [68,69]. The average vaccine adverse event reporting system (VAERS) reporting rates from 1990 to 2003 of GBS after influenza immunization in adults ranged from 0.17 per 100,000 vaccinees in 1993–1994 to 0.04 per 100,000 vaccinees in 2002–2003 [70]. Hence, the reporting rate of cases of GBS following immunization with Fluad® is significantly lower than the spontaneous incidence rate of GBS and, more importantly, within the range of reporting rates of GBS following immunization with conventional, non-adjuvanted vaccines.

Table 5
Cases of serious immediate allergic reactions and serious neurological disorders, assessed to be possibly related to the use of the vaccine by manufacturer—September 1997 to August 2006 [33]

Serious immediate allergic reactions	*N* = 7
Skin reaction	
Urticaria (generalized)	2
Drug exanthema	1
Respiratory system	
Bronchospasm, laryngeal edema, laryngospasm, dyspnea	4
Serious neurological disorders	***N* = 14**
Central nervous system disorders	
Grand mal convulsion	1
Myelitis	1
Transverse myelitis	1
Syncope, vasovagal	1
Peripheral nervous system disorders	
Neuritis, neuralgia	2
Guillain-Barré syndrome	6
Facial paresis	1

5.4.5.2.3. Parsonage-Turner syndrome (PTS). During the period of evaluation, 3 cases involving multiple nerves of the brachial plexus were reported. Two of the cases were considered possibly related, with the diagnosis of brachial neuritis or PTS, irrespective of causality assessment, resulting in a reporting rate of 0.01 cases per 100,000 doses. This is much lower than the spontaneous incidence of the disease (approximately 1–2 cases per 100,000 per year). All age groups may be affected. PTS is often confused with more frequent clinical disorders like cervical spondylosis, rotator cuff tear, shoulder impingement syndrome and acute calcific tendonitis, which are associated with acute and chronic shoulder pain) [71–73].

5.4.5.3. Blood and vascular disorders. Influenza immunization has been associated with very rare reports on serious adverse events affecting the blood system, such as thrombocytopenia and vasculitis [74–78].

A total of 9 cases with blood or vascular disorders were reported after immunization with Fluad® and Fluad®-like vaccines resulting in a reporting rate of 0.03 cases per 100,000 doses. Among these were 4 cases of thrombocytopenia, one case of leucocytosis, 3 cases of vasculitis, and one case of unspecified purpura and microhematuria. In 2 cases a causal relationship could not be excluded, because of clinical course and biological plausibility. However, in one of these 2 cases concomitant medication (captopril) might also have caused thrombocytopenia.

5.5. Summary of preclinical and clinical safety experience

A series of preclinical safety investigations were comprised of single-dose parenteral studies in mouse and other species, repeat-dose parenteral toxicity studies and local tolerance studies. No signs of toxicity or genotoxicity were seen. Repeat-dose toxicity studies showed expected immuno-stimulatory effects; local site reactions were observed and were confirmed in local tolerance studies.

In 1997, by the time of Fluad® licensure, several thousand subjects (~13,000; only study subjects) had been exposed to MF59™-adjuvanted vaccines. Thus, only those SAEs related to the administration of Fluad® at a frequency of greater than 1 in 500–1000 had been identified at that time.

Findings from a range of clinical studies using various vaccines containing MF59™ reflected the lack of toxicity as already observed in animal studies, and showed strong evidence of enhanced immuno-stimulatory activity. Local injection-site reactions and systemic reactions (a common response to any form of immunization) in these studies were similar to those of other vaccines. The lack of findings of toxicological concern found during this review supports the hypothesis that MF59™ is a safe adjuvant for human use.

The overall clinical database available for safety evaluation confirms that Fluad® (MF59™-adjuvanted influenza vaccine) has a good safety profile and induces higher antibody levels than non-adjuvanted vaccines in elderly subjects, including those with chronic diseases who have developed decreased antibody responses to conventional inactivated influenza vaccines. The safety and adjuvanticity of MF59™ has been widely tested in children, adolescents and adults with different antigens.

Furthermore, in the population as a whole (children, adult and elderly populations), the clinical benefit of a MF59™-adjuvanted influenza vaccine is likely to be greatest when new or antigenically variant strains are circulating in the community and the proportion of the population susceptible to disease is high. Given the benefits of Fluad® and the large clinical database available to support the safety of this vaccine, the benefit–risk ratio of Fluad® is positive.

The safety profile of the MF59™ emulsion adjuvant establishes that: (1) MF59™ does not elicit squalene antibodies. These antibodies, of both IgG and IgM isotype, are frequently detected in the serum of healthy, unvaccinated adult individuals, and their titres are not influenced by immunization with either adjuvanted or unadjuvanted vaccines [33]; (2) shows that in the comparison of studies with influenza vaccines with MF59™ to a conventional influenza vaccine (without MF59™ as adjuvant) in 15/31 studies a moderately increased reactogenicity was observed. Fluad® was associated with increased pain at the injection site after immunization; this conforms to the consistently higher immunogenicity in the majority of all clinical trials on Fluad® [79,53,40,80,38].

Currently available data suggest that MF59™ combined with other experimental vaccines for the prevention of infections or manifestations of diseases caused by CMV, HIV, HCV, HBV given to children and adults is safe and well tolerated.

5.6. Summary of postmarketing experience

All spontaneous reporting systems are influenced by underreporting. However, as one major objective of the spontaneous reporting system is the generation of early signals, this objective might be more affected by report quality, including variability in the completeness and accuracy of information provided, despite follow-up efforts, than by underreporting. The number of mild reactions may well be underreported; however, cases of serious reactions requiring medical treatment or hospitalization are less likely to be underreported.

In conclusion, the analysis of postmarketing data show that no fatal case was reported considered possibly related to Fluad® immunization. Several serious ADRs have been reported, though reporting rates are very low (1.4 cases per 100,000 doses). Most of the reactions were transient. The highest reporting rate for serious cases was noted for neurological disorders and for allergic reactions, regardless of causality.

The frequency of reported serious ADRs is not greater than the expected spontaneous incidence in the general population, or beyond the reporting rates known for ADRs after influenza immunizations in other regions of the world (e.g., in Australia 1.8–2.1 in 100,000 doses) [81–83]; hence no signals and no increase in frequency or severity of known ADRs have been found. This postmarketing surveillance safety profile confirms that Fluad® and Fluad®-like vaccines adjuvanted with MF59™ are generally safe and well tolerated.

6. Conclusions and future direction

MF59™ is a safe and potent adjuvant for use with human vaccines, with more than 27 million doses already administered in humans. It has been shown both in animal models and humans to be a potent stimulator of the cellular and humoral immune response to a variety of antigens. Toxicology studies in animal models and phases 1–4 studies in humans have demonstrated the safety with different vaccines. In an extensive set of preclinical studies, clinical studies and postmarketing data, the oil-emulsion MF59™ has been found to be a safe and potent vaccine adjuvant, resulting in the licensure of an MF59™-adjuvanted influenza vaccine in more than 20 countries. In addition, the highly promising clinical data generated using an influenza pandemic vaccine adjuvanted with MF59™ have shown that this adjuvant represents an attractive option for the development of an effective vaccine against a potential pandemic influenza.

In addition, the safety and immunogenicity profile of MF59™ suggests that this adjuvant may also be appropriate for use in pae-

diatric populations. Recently, a study in infants and small children has shown the added benefit of an MF59™-adjuvanted seasonal vaccine also in this population. Trials with pandemic vaccines in children are currently ongoing. The stronger adjuvanticity for MF59™ as compared to alum in newborn infants receiving HIV vaccines might set the stage for further development of MF59™-adjuvanted vaccines in this vulnerable population.

Finally, the large set of preclinical data available to date shows the versatility of MF59™, which can be successfully combined with immunopotentiators, if required, to enable the successful development of more complex vaccines, e.g., against HCV and/or HIV, which may also require the use of a prime with DNA or viral vector.

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