Vaccines and Related Biological Products Advisory Committee Meeting

September 15, 2015

FDA Briefing Document

Influenza Vaccine, Adjuvanted

Proposed Trade Name: FLUAD

Applicant:

Novartis Vaccines and Diagnostics, Inc.

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1.0 General Information

1.1 Seasonal Trivalent Influenza Vaccine, Adjuvanted with MF59C.1 (Fluad)

Product name:	Proper name: Influenza Vaccine, Adjuvanted Proposed Trade name: FLUAD
Description:	Inactivated, trivalent subunit influenza virus antigens and oil- in-water emulsion adjuvant (MF59C.1). The antigen is manufactured in (b) (4), Italy according to the Agriflu seasonal influenza vaccine manufacturing process licensed in the U.S. The adjuvant MF59C.1 contains a biodegradable oil, squalene, mixed with an aqueous phase consisting of sodium citrate dihydrate and citric acid monohydrate with surfactants, polysorbate 80 and sorbitan trioleate, added to stabilize and emulsify the oil/water interfaces. MF59C.1 is manufactured in (b) (4) and combined with the antigen in (b) (4), Italy.
Product composition:	Each 0.5 mL dose contains: Antigen 45 micrograms (mcg) hemagglutinin (HA) – 15 mcg of each influenza virus subtype (A/H1N1, A/H3N2, and B) Adjuvant 9.75 mg squalene 1.175 mg polysorbate 80 1.175 mg sorbitan trioleate 0.66 mg sodium citrate dihydrate 0.0425 mg citric acid monohydrate Each 0.5 mL dose may also contain residual amounts of neomycin (≤ 0.02 mcg), kanamycin (≤ 0.03 mcg), barium (< 0.5 mcg), egg proteins (≤ 0.4 mcg), formaldehyde (≤ 10 mcg) and hexadecyltrimethylammonium bromide (CTAB) (≤ 12 mcg) from the antigen manufacturing process.
Applicant:	Novartis Vaccines and Diagnostics, Inc. (NVD)
Proposed Indication:	For active immunization of adults 65 years of age and older against influenza disease caused by influenza subtypes A and type B contained in the vaccine.
Dosage Form and Route of Administration:	Emulsion for intramuscular injection supplied as 0.5 mL single-dose, pre-filled syringes.

1.2 Unadjuvanted Seasonal Trivalent Influenza Virus Vaccine (Agriflu)

Agriflu, the comparator product in the main supportive trial (V70_27) submitted to the Fluad BLA, is a trivalent inactivated influenza virus vaccine licensed in the U.S., that is produced using the same manufacturing process as that used for the HA antigens in Fluad and formulated without adjuvant.

2.0 Executive Summary

A Biologics License Application (BLA) was submitted by Novartis Vaccines and Diagnostics (NVD) to the Food and Drug Administration (FDA) for an adjuvanted seasonal trivalent influenza virus vaccine (Fluad). The candidate vaccine includes a proprietary adjuvant, MF59C.1 and influenza antigens (total dose of 45 micrograms HA) that are produced in eggs using the Agriflu manufacturing process.

The BLA includes immunogenicity and safety data from one phase 3 clinical trial conducted in adults $\geq$ 65 years of age (V70_27), which was designed to provide data to support licensure under the accelerated approval pathway, as outlined in the May 2007 Guidance for Industry: "Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines" [1]. Under the accelerated approval regulations (21 CFR§601.41), licensure is based on a surrogate marker that is reasonably likely to predict clinical benefit. For the evaluation of Fluad, the surrogate immune marker is the 21 day post-vaccination antibody response, as measured by a hemagglutination-inhibition (HAI) assay. Under the accelerated approval regulations, a confirmatory study is required to verify and describe the clinical benefit of Fluad. NVD will conduct an efficacy trial to verify and describe the clinical benefit of Fluad. Of note, the requirement for demonstration of safety under the accelerated approval regulations is the same as for "traditional" approval.

With regard to inclusion of an adjuvant in the vaccine formulation, from a regulatory perspective, adjuvants are not active ingredients as defined in 21 CFR§210.3 (b) (7) and adjuvants added to preventive vaccines are not licensed separately. The Code of Federal Regulations defines adjuvants as constituents materials (21CFR§610.15). These regulations state, "*All ingredients…shall meet generally accepted standards of purity and quality*" and state further, "*An adjuvant shall not be introduced into a product unless there is satisfactory evidence that it does not adversely affect the safety or potency of the product*." Therefore, while there is a regulatory requirement for the adjuvanted vaccine formulation, as for any vaccine, to be demonstrated as both safe and effective, with a favorable benefit-risk evaluation, there is no explicit requirement for demonstrating the safety and effectiveness of the adjuvanted vaccine formulation in comparative clinical trials using adjuvanted and unadjuvanted vaccine formulations. However, such trials may be requested by the Agency on a case-by-case basis; for example, if an applicant is planning to make a claim of superiority of their adjuvanted vaccine over their unadjuvanted vaccine in the package insert. In this case, NVD is not planning to make such a superiority claim.

Trial V70_27 was a randomized, active-controlled, observer-blind, multicenter clinical trial that compared the safety and immunogenicity of Fluad to Agriflu (an unadjuvanted trivalent inactivated influenza subunit vaccine licensed in the U.S.) in subjects $\geq$ 65 years of age. Subjects were randomly allocated in a 1:1:1:3 ratio to receive one of three lots of Fluad (N = 3552) or Agriflu (N= 3552). The primary immunogenicity objectives to be analyzed in a stepwise fashion were lot-to-lot consistency, noninferiority, and then superiority of Fluad over Agriflu for homologous strains. Criteria for equivalence had to be met in order for the data from the 3 lots to be pooled for the non-inferiority analyses. Non-inferiority criteria were met if the lower bound of the 95% confidence interval for the

difference in seroconversion rates (Fluad-Agriflu) and geometric mean titers (GMT) ratios (Fluad: Agriflu) were < -10% and > 0.67, respectively. Superiority criteria were met if the lower bound of the 95% confidence interval for the difference in seroconversion rates (Fluad-Agriflu) and GMT ratios (Fluad: Agriflu) were >10% and > 1.5, respectively for at least two of the three strains. The pre-specified criteria for demonstration of equivalency of three lots of Fluad and noninferiority relative to Agriflu were met. Immunologic superiority of Fluad compared to Agriflu was demonstrated for one of the three influenza vaccine strains (H3N2).

Fluad was associated with increased solicited local and systemic reactogenicity compared to Agriflu within the 7 day post-vaccination period (43% versus 33%, respectively), but rates of severe AEs were balanced across groups for both solicited local and systemic reactions and comprised $\leq$ 1% of subjects across all categories. The percentage of unsolicited adverse events (AEs) through Day 21 postvaccination was 16% in both groups; 4% in Fluad versus 5% in Agriflu were considered by the investigator to be related. Four serious adverse events (SAEs) were assessed to be possibly or probably related to the study vaccination: 1 SAE (bronchitis, presented day 8) in the Fluad group and 3 SAEs (asthmatic crisis [presented day 13], chronic obstructive pulmonary disease [presented day 63], and Guillain-Barré syndrome [presented day 227]) in the Agriflu group. There were no deaths within 21 days of vaccine administration. Deaths occurring during the 1 year study duration were reported in similar proportions in both the Fluad and Agriflu groups: 1.5% and 1.3%, respectively. One death was considered related to the study vaccination, a death attributed to Guillain-Barré Syndrome in a subject who received Agriflu.

Data from an additional 49 supportive studies, conducted in adults $\geq$ 65 years of age between 1992 and 2013 (N=27,787) were submitted to the BLA. These studies evaluated 4 different formulations of a MF59 adjuvanted product and were small and highly varied in design (e.g., uncontrolled, open-label, non-randomized, and/or using comparators that were not licensed in the US). Thus, the purpose of submitting these data was to provide a larger safety database. NVD and the Center for Biologics Evaluation and Research (CBER) agreed prior to submission of the BLA that immunogenicity data from these studies would not be reviewed or included in labeling because antibody response may vary by strains included in the vaccine, there were differences in the assays used and the laboratory conducting the assays, and the assays were not adequately validated. Review of pooled safety analyses from these studies showed a similar safety profile to that observed in the above referenced pivotal trial V70_27 and did not reveal safety concerns.

This Vaccines and Related Biological Products Advisory Committee (VRBPAC) meeting is being convened to review and discuss the safety and immunogenicity data derived from studies conducted with Fluad and submitted in the BLA. The committee will be asked to vote on whether the available data support the safety and effectiveness of Fluad for the proposed indication via the accelerated approval pathway.

3.0 Introduction and Background

3.1 Seasonal Influenza

Influenza illness and its complications follow infection with influenza viruses. Global surveillance of influenza identifies yearly antigenic variants. Since 1977, antigenic variants of influenza A (H1N1 and H3N2) viruses and influenza B viruses have been in global circulation. Specific titers of HAI antibody induced by vaccination with inactivated influenza virus vaccines have not been absolutely correlated with protection from influenza illness. However, in some human studies, HAI antibody titers of 1:40 or greater have been associated with protection from influenza illness in up to 50% of subjects [2, 3].

Antibody against one influenza virus type or subtype may confer limited or possibly no protection against another. Furthermore, antibody to one antigenic variant of influenza virus may not protect against a different antigenic variant of the same type or subtype. Frequent development of antigenic variants through antigenic drift is the virologic basis for seasonal epidemics and the reason for the usual change of one or more strains in each year's influenza vaccine. Therefore, influenza strains are selected to represent the influenza viruses anticipated to be circulating in the United States during the following winter, typically containing two type A and one or two type B strains for trivalent and quadrivalent vaccines, respectively.

Annual influenza vaccination is recommended by the Advisory Committee on Immunization Practices (ACIP) because immunity declines during the year after vaccination, and because circulating strains of influenza virus change from year to year [4].

3.2 MF59C.1 Adjuvant

The MF59 adjuvant is an oil-in-water emulsion, which is manufactured to generate uniform (165 nm in diameter) squalene oil droplets stabilized by the addition of two non-ionic surfactants [5]. The squalene oil is a biosynthetic precursor of cholesterol and steroid hormones, and is fully biodegradable.

It is thought that the MF59 functions as a delivery system that activates local cells to take up the co-injected HA antigens that are present in the aqueous phase of the emulsion [6]. A depot effect has not been shown to be involved in the mechanism of action of MF59, since both antigen and adjuvant are cleared rapidly, but with independent kinetics from the injection site [7]. In human immune cells, MF59 has been shown to directly increase phagocytosis and pinocytosis and promote antigen uptake by antigen presenting cells (APCs) [8]. Monocytes, macrophages and granulocytes have been found to be specifically targeted by MF59 induced cytokines, and this in turn may lead to an enhanced rate of migration of antigen positive cells from the muscle to the draining lymph nodes [9].

3.3 Seasonal Influenza Vaccines approved for Use in Persons 65 Years of Age and Older

Currently, nine seasonal trivalent influenza vaccines are licensed in the U.S. for use in adults, including those 65 years of age and older: Fluzone®, Fluzone High-Dose (HD)®, Flucelvax®, Fluvirin®, FluLaval®, Fluarix®, Afluria®, Agriflu® and FluBlok© (all contain a total of 45 mcg of HA per dose, except FluBlok with a total of 135 mcg of HA per dose and Fluzone HD, which is approved exclusively for use in adults 65 years of age and older, with a total of 180 mcg HA per dose). In addition, three quadrivalent inactivated influenza vaccines (containing a total of 60 mcg of HA per dose) are available for use in adults, including those 65 years of age and older: Fluarix Quadrivalent®, FluLaval Quadrivalent® and Fluzone Quadrivalent®.

4.0 Overview of Clinical Trials Evaluating Fluad in Persons 65 Years of Age and Older

Trial V70-27 (summarized in Table 1 and described in Section 5) conducted under IND provides the pivotal safety and immunogenicity data for the Fluad BLA.

Table 1. Overview of Trial V70_27

Trial	Design	Control	Total # Subjects	Age (years)	Countries
V70_27	Randomized, observer-blind, multi-center	Agriflu	7104 (3552 in each group)	65 years of age and older	United States (21 sites), Philippines (11 sites), Colombia (4 sites), Panama (2 sites)

Additional safety information was provided with the submission of data from 49 clinical studies conducted between 1992 and 2013 evaluating 27,787 subjects who received either MF59C.1 adjuvanted or unadjuvanted trivalent influenza vaccine (TIV) (described in Section 6). However, as noted in the executive summary, data from these studies were only provided to assess safety, but were not used to support immunogenicity due to limitations in study design and variations in vaccine formulations and testing.

5.0 Clinical Immunogenicity and Safety Trial V70_27

5.1 Trial Design

Trial V70_27 is a randomized, observer-blind, multi-center, active-controlled trial to evaluate the immunogenicity and safety of one dose of Fluad administered on Day 1 intramuscularly (IM) to healthy adults ≥ 65 years of age. The trial was designed to evaluate lot-to-lot consistency, immunogenicity and safety of Fluad as compared to Agriflu (NVD's unadjuvanted influenza vaccine). The trial was conducted at 38 sites across four countries which included the U.S. (21 sites), Colombia (4 sites), Panama (2 sites), and the Philippines (11 sites).

5.2 Selected Trial Objectives and Endpoints

The co-primary objectives evaluated lot-to-lot consistency and immunogenicity of Fluad compared to Agriflu, and were evaluated in a step-wise fashion in the order listed below. After demonstration of lot consistency (primary objective 1), data from the 3 lots of Fluad were pooled for analyses of noninferiority (primary objective 2a) and then superiority (primary objective 2b).

Immunogenicity Objectives

Primary:

1. To demonstrate immunologic equivalence of 3 consecutive production lots of Fluad as measured by HAI geometric mean titers (GMTs) at day 22 for each virus strain (lot-to-lot consistency).

2a. To demonstrate immunologic noninferiority of Fluad compared to Agriflu with regards to all homologous strains in adults ≥ 65 years of age as measured by GMT ratios and seroconversion rate differences at day 22.

2b. To demonstrate immunologic superiority of Fluad compared to Agriflu with regards to at least 2 homologous strains in adults ≥ 65 years of age as measured by GMT ratios and

seroconversion rate differences at day 22.

Selected Secondary:

1a. To demonstrate noninferiority of Fluad compared to Agriflu with regards to all homologous strains in high-risk subjects ≥ 65 years of age with predefined comorbidities[1] as measured by GMT ratios and seroconversion rate differences at day 22.

1b. To demonstrate superiority of Fluad compared to Agriflu with regards to at least 2 homologous strains in high risk subjects ≥ 65 years of age with predefined comorbidities[1] as measured by GMT ratios and seroconversion rate differences at day 22.

2a. To demonstrate noninferiority of Fluad compared to Agriflu with regards to three selected heterologous strains in adults ≥ 65 years of age as measured by GMT ratios and seroconversion rate differences at day 22.

2b. To demonstrate superiority of Fluad compared to Agriflu with regards to at least 2 heterologous strains in adults ≥ 65 years of age as measured by GMT ratios and seroconversion rate differences at day 22.

3. To assess the difference between Fluad and Agriflu with regards to homologous and heterologous strains in subjects ≥ 65 years of age included in the antibody persistence group as measured by GMT ratios and seroconversion rate differences at day 181 and day 366.

Of note, homologous strains were defined by the sponsor as those strains included in the Fluad and Agriflu vaccines. Heterologous strains were selected which were "generally regarded as antigenically distinct from those included in the vaccine."

[1]High-risk subjects had 1 or more of the following predefined comorbidities: congestive heart failure, chronic obstructive pulmonary disease, asthma, hepatic diseases, renal insufficiency (4% to 5%), and the most commonly reported neurological/neuromuscular or metabolic conditions including diabetes mellitus.

Safety Objectives

1. To describe safety and tolerability of Fluad compared to Agriflu in all subjects through day 8 following vaccination and all AEs and SAEs through day 22.

2. To describe SAEs, new onset of chronic diseases, AEs resulting in withdrawal from the trial, and other adverse events of significance through day 366.

The criteria used to determine whether selected primary and secondary objectives listed above are met are described below:

Primary Endpoints and Criteria for Success:

1. Lot-to-lot consistency (Per Protocol Set [PPS, defined in section 5.5.1]): For each of the three influenza strains (A/H1N1, A/H3N2, and B) equivalence (consistency) between each pair of lots (i.e. Group A and B, Group B and C, Group A and C) was demonstrated if the two-sided 95% CI of the ratios of Day 22 GMTs between each pair of Fluad vaccine lots fell within the equivalence range (0.67 to 1.50).

2a. Non-inferiority (PPS): For each of the three influenza strains, non-inferiority was achieved if the lower limit of the 95% CI for Day 22 post vaccination ratio for GMTs (Fluad / Agriflu) was greater than 0.667, and if the lower limit of the 95% CI for Day 22 post vaccination difference in percentages of seroconversion (Fluad minus Agriflu) was greater than -10%. (Tables 4 and 5)

2b. Superiority (Full Analysis Set [FAS, defined in section 5.5.1]): For each of the three influenza strains, superiority was achieved if the lower limit of the 95% CI for Day 22 post vaccination ratio for GMTs (Fluad: Agriflu) was greater than 1.5, and if the lower limit of the 95% CI for Day 22 post vaccination difference in percentages of seroconversion (Fluad minus Agriflu) was greater than 10%. (Tables 6 and 7)

Selected Secondary Endpoints (Table 8) and Analyses:
Superiority analyses for the below endpoints 1 and 2 were evaluated using the same criteria as described for the primary endpoints.

1. Superiority of Fluad vs. Agriflu for heterologous strains was assessed in the total FAS population as well as restricted to the subjects with high-risk pre-defined underlying chronic conditions based on GMTs and seroconversion rates using the same criteria as above.

2. Superiority of Fluad vs. Agriflu for homologous strains in subjects with comorbid conditions (FAS) was based on GMTs and seroconversion rates using the same criteria as above.

3. To assess the difference between Fluad and Agriflu with regards to homologous strains in subjects included in antibody persistence group as measured by GMT ratios and seroconversion rate differences at day 181 and day 366.

Selected Safety Endpoints:
1. Local and systemic reactions occurring within 1 week after administration of the trial vaccine, assessed at 30 minutes post-vaccination and for the intervals 6 hours through day 3, days 4 through 7, and 6 hours through day 7 post-vaccination. (Table 9, 10)

2. All unsolicited adverse reactions through day 22

3. All SAEs including death, new-onset chronic disease and other AEs of significance through day 366

5.3 Population

Subjects eligible for the study were males or females $\geq$ 65 years of age at the time of vaccination. Subjects were in good general or stable health and had not received vaccination against seasonal influenza within the previous 6 months.

5.4 Subject Demographics and Disposition

Table 2 outlines subject disposition by treatment arm with definitions for each population below. The sample size calculation was based on a completion rate of at least 90%. The analysis populations for the primary objectives were the PPS and the FAS (PPS and FAS populations defined in sections 5.5.1), for which 94% and 92% of trial subjects were included, respectively.

Table 2. Subject disposition

Disposition	Fluad	Agriflu	Total
Enrolled	n/a	n/a	7109
Randomized	3552	3552	7104
Vaccinated[1,2]	3541 (100%)	3541 (100%)	7082 (100%)
Full analysis set (FAS)[3]	3479 (98%)	3482 (98%)	6961 (98%)
Per protocol set (PPS)[3]	3227 (91%)	3259 (92%)	6486 (92%)
Safety Set	3545 (>99%)	3537 (>99%)	7082(100%)
Premature withdrawals (total)	191 (5%)	196 (6%)	392 (6%)
missing reason	1 (<1%)	0	1 (<1%)
Death	51 (1%)[4]	46 (1%)	97 (1%)
AE	3 (<1%)	2 (<1%)	5 (<1%)
Withdrew consent	52 (1%)	43 (<1%)	95 (1%)
Lost to follow-up	73 (2%)	91 (3%)	164 (2%)
Inappropriate enrollment	5 (<1%)	4 (<1%)	9 (<1%)
Administrative reason	1 (<1%)	1 (<1%)	2 (<1%)
Protocol deviation	2 (<1%)	2 (<1%)	4 (<1%)
Unable to classify	3 (<1%)	7 (<1%)	10 (<1%)

Adapted from 125510/0.11: Clinical Study Report Tables 10.1-1, 11.1-1, 12.2.

[1]Seven subjects randomized to Agriflu received Fluad and 3 subjects randomized to Fluad received Agriflu. Safety analyses were based on vaccine received, not randomization group.

[2]Total number of subjects who received any vaccine (whether or not it was correct) is defined as 100%

[3]FAS and PPS defined in section 5.5.1

[4]One subject withdrew after developing an AE (lung neoplasm) that subsequently led to death; the death is not included in this table because the AE, rather than death, was the cause of the withdrawal.

Table 3 outlines subject demographics and baseline characteristics. Of note, only 28% of subjects were over the age of 75.

Table 3. Subject Demographics and Baseline Characteristics for Subjects at Day 22 for Trial V70_27 (FAS[1])

	Fluad N[2]=3479	Agriflu N=3482
Age (mean ± SD; years)	71.9±5.3	71.8±5.3
Gender		
Male	1252 (36%)	1178 (34%)
Female	2227 (64%)	2304 (66%)
Age cohorts		
65-75 years	2504 (72%)	2531 (73%)
>75 years	975 (28%)	951 (27%)
Country		
Colombia	503 (14%)	495 (14%)
Panama	108 (3%)	102 (3%)
Philippines	1832 (53%)	1830 (53%)
United States	1036 (30%)	1055 (30%)
Ethnic Origin		
Asian	1837 (53%)	1840 (53%)
Black	44 (1%)	39 (1%)
Caucasian	969 (28%)	971 (28%)
Hispanic	616 (18%)	613 (18%)
Other	11 (<1%)	16 (<1%)
Native American/Alaskan	1 (<1%)	3 (<1%)
Pacific Islander/Hawaiian	1 (<1%)	0

Adapted from 125510/0.11: Clinical Study Report Table 11.2-4

[1] FAS: full analysis set

[2]N: number of subjects

5.5 Results of Trial V70_27

5.5.1 Definition of Populations Used for Analysis

Safety and immunogenicity analyses were performed on the below described subsets depending on the analysis. With regard to the primary immunogenicity objectives, lot-to-lot consistency and non-inferiority were evaluated in the per-protocol set (PPS) whereas superiority was evaluated on the full-analysis set (FAS). These primary analysis populations were pre-specified. Additional analyses using the alternate population (PPS or FAS) yielded comparable results.

Per Protocol Set (PPS), Immunogenicity Day 22:

All subjects in the FAS who received the correct vaccine, provided evaluable serum samples on both day 1 and day 22, and had no major protocol deviation prior to unblinding. A major deviation was defined as a deviation from per protocol procedures likely to significantly impact the day 22 immunogenicity results for that subject. Protocol deviations were to be identified prior to unblinding and analysis of the data. Major deviations include:

- Subjects outside age cutoff (i.e., **<** 65 years)
- Subject enrolled who did not meet trial entry criteria
- Subjects who did not attend the scheduled visits for blood draws:
    - Day 1 (prior to vaccination)

- Inside of the day 22 visit window (days 22 through 25)
- Subjects who did not receive the correct trial vaccine
- Subjects randomized in the wrong age cohort
- Subjects who did not attend visits within the allowed window (see above)
- Subjects who prematurely withdrew from the trial (e.g., lost to follow up or withdrew consent)
- Subjects who developed withdrawal criteria during the trial, but were not withdrawn
- Subjects missing antibody data due to technical problems
- Subjects who received a concomitant medication not permitted by the protocol (in the judgment of the cluster physician)
- Deviations identified through monitoring listings might be considered as well

Full Analysis Set (FAS), Immunogenicity Day 22:
All randomized subjects who received a trial vaccination and provided evaluable serum samples both at day 1 (baseline) and at day 22. In the event that the administered vaccine was not assigned according to randomization, subjects were to be analyzed as randomized in the FAS. In the event that subjects were randomized in the wrong age cohort, subjects were to be analyzed in the age cohort they were randomized to in the trial.

Safety Set:
All randomized subjects who received a trial vaccination and provided post-vaccination safety data.

5.5.2 Immunogenicity Results

Primary Immunogenicity Analyses:
Lot consistency was demonstrated (data not shown). Therefore, for subsequent immunogenicity analyses, data from these three lots were pooled. Non-inferiority of Fluad compared to Agriflu, (per criteria outlined in Section 5.2), was demonstrated for GMTs and seroconversion rates to all three homologous (vaccine) strains (Tables 4 and 5).

Table 4. Non-Inferiority[1] Comparison of Post-Vaccination Geometric Mean Day 22 Hemagglutinin Inhibition Antibody Titers by Influenza Strain for Trial V70_27 (PPS[2])

Strain	Fluad GMT[3] (95% CI[4]) N[5]=3227	Agriflu GMT (95% CI) N=3259	Ratio[6] Fluad:Agriflu (95% CI)
A H1N1 California /2009	99 (93, 106)	70 (66, 75)	**1.40 (1.32, 1.49)**
A H3N2 Perth/2009	272 (257-288)	169 (159, 179)	**1.61 (1.52, 1.70)**
Influenza B Brisbane/2008	28 (26-29)	24 (23-26)	**1.15 (1.08, 1.21)**

Adapted from 125510/0.11: Clinical Study Report Table 11.4.1.1-2
[1]Non-inferiority (GMTs): lower bound of 95%CI for ratio of Fluad: Agriflu > 0.67
[2]PPS: per protocol set
[3]GMT: geometric mean titers
[4]CI: confidence interval
[5]N: number of subjects
[6]Day 22 Ratio is adjusted for baseline titer, country, and age cohort

Table 5. Non-Inferiority[1] Comparison for Seroconversion[2] Rates Against Homologous Strains at Day 22 for Trial V70_27 (PPS[3])

Strain	Fluad % (95% CI[4]) N[5]=3227	Agriflu % (95% CI) N=3259	Difference[6]: Fluad-Agriflu (95%CI)
A H1N1 California /2009	69 (67, 70)	58 (57, 60)	**9.8 (7.5, 12.1)**
A H3N2 Perth/2009	73 (71, 74)	58 (56, 60	**13.9 (11.7-16.1)**
Influenza B Brisbane/2008	33 (31, 35)	29 (28, 31)	**3.2 (1.1, 5.3)**

Adapted from 125510/0.11: Clinical Study Report Table 11.2.1.1-3

[1]Non-inferiority (% seroconversion): lower bound of 95% CI for difference of Fluad-Agriflu >-10%

[2]Seroconversion defined as a prevaccination HI titer <10 and postvaccination HI titer ≥ 40, or at least a 4-fold increase in HI titer from prevaccination titer ≥ 10

[3]PPS: per protocol set

[4]CI: confidence interval

[5]N: number of subjects

[6]Day 22 differences are adjusted for baseline titer, country and age cohort

The criteria for immunologic superiority of GMT and seroconversion rates were met for one of three homologous strains, H3N2. (Tables 6 and 7).

Table 6. Superiority[1] Comparison of Post-Vaccination Geometric Mean Day 22 Hemagglutinin Inhibition Antibody Titers by Influenza Strain for Trial V70_27 (FAS[2])

Strain	Fluad GMT[3] (95% CI[4]) N[5]=3479	Agriflu GMT (95% CI) N=3482	Ratio[6] Fluad:Agriflu (95% CI)
A H1N1 California /2009	98 (92, 104)	71 (67, 76)	1.37 (1.29, 1.46)
A H3N2 Perth/2009	267 (253, 282)	167 (158, 176)	**1.60 (1.51, 1.68)**
Influenza B Brisbane/2008	27 (26, 29)	24 (23, 25)	1.14 (1.08, 1.20)

Adapted from 125510/0.11: Clinical Study Report Table 11.4.1.1-4

[1]Superiority (GMTs): lower bound of 95%CI for ratio of Fluad: Agriflu > 1.5

[2]FAS: full analysis set

[3]GMT: geometric mean titers

[4]CI: confidence interval

[5]N: number of subjects

[6]Day 22 ratio is adjusted for baseline titer, country and age cohort

Table 7. Superiority Comparison of Percentage of Subjects with Seroconversion[2] in Hemagglutinin Inhibition Antibody Titers Against Homologous Strains at Day 22 for Trial V70_27 (FAS[3])

Strain	Fluad % (95% CI[4]) N[5]=3479	Agriflu % (95% CI) N=3482	Difference[6]: Fluad-Agriflu (95%CI)
A H1N1 California /2009	68 (67, 70)	59 (57, 60)	9.6 (7.4, 11.8)
A H3N2 Perth/2009	72 (71, 74)	58 (56, 60)	**13.8 (11.7-16.0)**
Influenza B Brisbane/2008	33 (31, 34)	30 (28, 31)	3.0 (1.0, 5.0)

Adapted from 125510/0.11: Clinical Trial Report Table 14.2.1.1.5

[1]Superiority (% seroconversion): lower bound of 95% CI for difference of Fluad-Agriflu >10%

[2]Seroconversion defined as a prevaccination HI titer <10 and postvaccination HI titer ≥ 40, or at least a 4-fold increase in HI titer from prevaccination titer ≥ 10

[3]FAS: Full Analysis Set

[4]CI: confidence interval

[5]N: number of subjects

[6]Day 22 differences are adjusted for baseline titer, country and age cohort

Selected Secondary Analyses:

Selected secondary endpoints and analyses are summarized below in Table 8. Of note, these analyses do not provide the basis for licensure which was achieved by demonstration of adequate safety and immunological noninferiority to Agriflu.

Table 8. Summary of Selected Secondary Analyses[1]

Endpoints and Analyses	Criteria	Results: point estimates (95% CI)
Superiority of GMT[2] and seroconversion rate for homologous strains , high-risk subjects (FAS)[5]	LL[3] of 95% CI[4] day 22 GMT ratios (Fluad: Agriflu) > 1.5	H1N1: 1.32 (1.2, 1.45) H3N2: 1.54 (1.42, 1.68) B: 1.11 (1.03, 1.21)
	LL of 95% CI day 22 percent seroconversion[6] difference rates (Fluad-Agriflu) > 10%	H1N1: 10.2 (6.5, 13.9) H3N2: 13.3 (9.7, 16.9) B: 1.7 (-1.4, 4.8)
Superiority of GMT and seroconversion rate for heterologous strains (FAS)	LL of 95% CI day 22 GMT ratios (Fluad: Agriflu) > 1.5	A/Brisbane/10/2007-like (H3N2): 1.49 (1.33, 1.67) A/Wisconsin/67/2005-like (H3N2): 1.38 (1.25, 1.52) B/Malaysia/2506/2004-like: 1.09 (0.99, 1.21)
	LL of 95% CI day 22 difference in % seroconversion rates (Fluad-Agriflu) > 10%	A/Brisbane/10/2007-like (H3N2): 12.8 (8.4, 17.2) A/Wisconsin/67/2005-like (H3N2): 12.5 (8.1, 17) B/Malaysia/2506/2004-like: 4.2 (0, 8.4)
Antibody persistence for homologous strains by both GMT and seroconversion rate(FAS)	GMTs at days 181 and 366, LL of 95% CI (Fluad: Agriflu) presented	H1N1: Day 181: 1.05 (0.82, 1.33) Day 366: 0.94 (0.73, 1.22) H3N2: Day 181: 1.35 (1.06, 1.71) Day 366: 1.3 (1.01, 1.67) B: Day 181: 1.12 (0.9, 1.39) Day 366: 1.3 (0.83, 1.27)
	Percent seroconversion rate differences (Fluad - Agriflu) at days 181 and 366	H1N1: Day 181: 7.1 (-1.2, 15.4) Day 366: 0 (-7.1, 7.4) H3N2: Day 181: 11.9 (3.1, 20.6) Day 366: 3.8 (-2.6, 10.2) B: Day 181: -1 (-3.2, 2.2) Day 366 -2.6 (-4.9, 0)

Adapted from 125510/0.11: Clinical Trial Study Report Tables 11.4.1.2-3, 11.4.1.2-4, 11.4.1.2-9, 11.4.1.2-10, 11.4.1.2-23, 11.4.1.2-24

[1]Full endpoint criteria described in section 5.2

[3]LL: lower limit

[4]CI: confidence interval

[5]FAS: Full Analysis Set

[6]Seroconversion defined as a prevaccination HI titer <10 and postvaccination HI titer ≥ 40, or at least a 4-fold increase in HI titer from prevaccination titer ≥ 10

5.5.3 Safety Results

Almost all (>99%) enrolled subjects received 1 of 2 trial vaccines; 27 subjects did not receive trial vaccine (Table 2). In general, AEs were balanced between groups with the exception of mild pain and tenderness which was reported at a greater rate in Fluad recipients (Table 9). Slightly higher rates of solicited systemic AEs (mild) were seen across most categories among Fluad recipients (Table 10).

Immediate AEs

Ten percent of subjects receiving Fluad and 8% of those receiving Agriflu had a solicited AE within 30 minutes of vaccination. The most common AE was pain with 5% and 4% for Fluad and Agriflu, respectively. The majority of AEs, including injection site pain, tenderness, erythema and induration as well as fever, chills, myalgia, arthralgia, headache, nausea, vomiting and diarrhea were mild with $\leq$ 1% reported as moderate severity. Grade 3 (severe) AEs in the Fluad group included pain (2 subjects), headache (1 subject), myalgia (1 subject), and diarrhea (1 subject). One subject in the Agriflu group reported grade 3 myalgia. There were no anaphylactic episodes reported.

Solicited AEs within 7 days of vaccination

In the time frame of 6 hours through 7 days after receiving either Fluad or Agriflu, 46% versus 33% had at least one reactogenicity sign, respectively. Local reactions were reported by 32% and 17% of recipients, respectively (Table 9), and systemic reactions by 32% and 26% of subjects, respectively (Table 10). Severe reactions were balanced for both solicited local and systemic reactions and comprised $\leq$ 1% of subjects in each group across all categories.

Table 9. Solicited Local AEs by Type and Maximum Severity within 7 Days of Vaccination for Trial V70_27

Subjects experiencing at least one local AE[1] by maximum intensity	Fluad n[2]/% (N[3]=3505)	Agriflu n/% (N=3495)
Overall	1137 (32%)	593 (17%)
Pain: Total	875 (25%)	425 (12%)
Mild (easily tolerated)	726 (21%)	351 (10%)
Moderate (interferes with normal behavior or activities)	138 (4%)	66 (2%)
Severe (incapacitating; can't perform usual activities)	11 (<1%)	8 (<1%)
Tenderness: Total	739 (21%)	391 (11%)
Mild (easily tolerated)	628 (18%)	349 (10%)
Moderate (interferes with normal behavior or activities)	106 (3%)	36 (1%)
Severe (incapacitating; can't perform usual activities)	5 (<1%)	6 (<1%)
Erythema: Total	43 (1%)	18 (1%)
25mm ≤ 50mm	37 (1%)	17 (<1%)
51mm ≤ 100mm	6 (<1%)	1 (<1%)
> 100mm	0	0
Swelling: Total	43 (1%)	15 (<1%)
25mm ≤ 50mm	35 (1%)	14 (<1%)
51mm ≤ 100mm	7 (<1%)	1 (<1%)
> 100mm	1 (<1%)	0
Induration: Total	45 (1%)	17 (<1%)
25mm ≤ 50mm	35 (1%)	17 (<1%)
51mm ≤ 100mm	10 (<1%)	0
> 100mm	0	0

Source: Adapted from BLA 125510/0.0 Clinical Study Report Tables 12.2.1.1-1 and 12.2.3.1-1

[1]AE: adverse event

[2]n: number experiencing a particular event

[3]N: total number of subjects

Table 10. Solicited Systemic AEs by Type and Maximum Severity within 7 Days of Vaccination for Trial V70_27

Subjects experiencing at least one systemic AE[1] by maximum intensity	Fluad n[2]/% (N[3]=3505)	Agriflu n/% (N=3495)
Overall	1120(32%)	902 (26%)
Temperature ≥ 38.0 °C: Total	122 (4%)	116 (3%)
≥ 40.0 °C	3 (<1%)	0
Chills: Total	235 (7%)	163 (5%)
Mild (easily tolerated)	169 (5%)	111 (3%)
Moderate (interferes with normal behavior or activities)	53 (2%)	43 (1%)
Severe (incapacitating; can't perform usual activities)	13 (<1%)[4]	9 (<1%)
Myalgia: Total	515 (15%)	339 (10%)
Mild (easily tolerated)	414 (12%)	251 (7%)
Moderate (interferes with normal behavior or activities)	91 (3%)	63 (2%)
Severe (incapacitating; can't perform usual activities)	10 (<1%)	25 (1%)
Arthralgia: Total	296 (8%)	272 (8%)
Mild (easily tolerated)	232 (7%)	196 (6%)
Moderate (interferes with normal behavior or activities)	57 (2%)	56 (2%)
Severe (incapacitating; can't perform usual activities)	7 (<1%)	20 (1%)
Headache: Total	463 (13%)	391 (11%)
Mild (easily tolerated)	343 (10%)	281 (8%)
Moderate (interferes with normal behavior or activities)	105 (3%)	89 (3%)
Severe (incapacitating; can't perform usual activities)	15 (<1%)	21 (1%)[4]
Fatigue: Total	466 (13%)	361 (10%)
Mild (easily tolerated)	344 (10%)	254 (7%)
Moderate (interferes with normal behavior or activities)	109 (3%)	85 (2%)
Severe (incapacitating; can't perform usual activities)	13 (<1%)	22 (1%)[4]
Nausea: Total	101 (3%)	98 (3%)
Mild (easily tolerated)	81 (2%)	72 (2%)
Moderate (interferes with normal behavior or activities)	14 (<1%)	21 (1%)
Severe (incapacitating; can't perform usual activities)	6 (<1%)	5 (<1%)
Vomiting: Total	48 (1%)	59 (2%)
Mild (easily tolerated)	33 (1%)	38 (1%)
Moderate (interferes with normal behavior or activities)	13 (<1%)	17 (<1%)
Severe (incapacitating; can't perform usual activities)	2 (<1%)[4]	4 (<1%)
Diarrhea: Total	168 (5%)	158 (5%)
Mild (easily tolerated)	111 (3%)	119 (3%)
Moderate (interferes with normal behavior or activities)	44 (1%)	30 (1%)
Severe (incapacitating; can't perform usual activities)	13 (<1%)[4]	9 (<1%)[4]

Source: Adapted from BLA 125510/0.0 Clinical Study Report Tables 12.2.1.1-1 and 12.2.3.2.1-2

[1]AE: adverse event

[2]n: number experiencing a particular event

[3]N: total number of subjects

[4]in these groups one each of the grade 3 episodes was identified as "potentially life threatening"

Unsolicited AEs

During the period from day 1 through day 21, 16% of subjects in each of the vaccine groups reported at least 1 unsolicited AE. Unsolicited AEs considered by the investigator to be possibly or probably related to the study vaccination were reported by 4% and 5% of subjects in Fluad and Agriflu groups respectively. Possibly or probably related AEs were balanced between groups by category with the most common events reported being nasopharyngitis (38 subjects [1%] in the Fluad group versus 28 subjects [1%] in the Agriflu group); headache (12 subjects [<1%] in the Fluad group versus 17 subjects [<1%] in the Agriflu group); and cough (8 subjects [<1%] in the Fluad group versus 12 subjects [<1%] in the Agriflu group).

SAEs, Deaths, AE's Leading to Study Withdrawal, New-Onset Chronic Disease, and Other Significant Adverse Events

No deaths occurred within 21 days of vaccination. Review of SAEs, deaths, and AEs leading to study withdrawal, did not reveal imbalances by system organ class or evidence of relatedness (Table 11). The applicant captured new-onset chronic diseases and other AEs of special interest for one year following vaccine administration whether or not they met criteria for an SAE; review and analyses of these events did not reveal any new signals or imbalances.

Table 11. Overview of SAEs, Deaths, AEs Leading to Trial Withdrawal, or New-Onset Chronic Disease for Trial V70_27

Parameter	Fluad N[1] (%)	Agriflu N (%)
SAEs (total)	264 (7%)	243 (7%)
SAEs (day 1-21)	19 (1%)	20 (1%)
Deaths (day 1-21)	0	0
Deaths (total)	52 (1.5%)	46 (1.3%)
AEs leading to study withdrawal (total)	52 (1%)	49 (1%)
AEs leading to study withdrawal (day 1-21)	4 (<1%)	2 (<1%)
New Onset Chronic Disease (total)	227 (6%)	223 (6%)
New Onset Chronic Disease (day 1-21)	18 (1%)	17 (<1%)

Source: Adapted from BLA 125510/0.0 Clinical Study Report Tables

[1]N: number of subjects

6.0 Supportive Safety Studies

Forty-nine clinical studies conducted from 1992 through 2013 were submitted to this BLA for evaluation of safety with a focus on SAEs and adverse events of special interest (N= 27,787). It was agreed that data would be pooled in 8 groups, with some studies included in multiple groups, based on age, type of study design and vaccine formulation (Table 12). The duration of follow-up ranged from 3 weeks to 1 year post-vaccination. Thirty-six of these studies evaluated subjects who were > 65 years of age receiving their first-dose of Fluad; 8 studied subjects > 65 years of age; and 5 were extension studies (evaluating repeat annual dosing). Of the 36 studies in adults 65 years of age and older, 15 were randomized controlled trials, 17 were uncontrolled studies and 4 were bridging or stability studies. Over this time the vaccine formulation underwent changes from water to citrate buffer and from thimerosal-containing, to trace thimerosal-containing, and ultimately to thimerosal free. Thus, pooling group 5 included only studies that contained the citrate formulation, as is used in the current product under review for this BLA.

Table 12. Overview of Fluad Studies included in pooled safety analyses

Pooling group	Trial characteristics	Parameters evaluated	Number of trials included	Number of subjects Fluad / TIV
1	First-dose[1] trials conducted in elderly[2] subjects	Solicited and unsolicited AEs, SAE[3]s and AESI[4]s	36	7532/ 5198
2	First-dose RCT[5]s conducted in elderly subjects (a subset of pooling group 1)	Solicited and unsolicited AEs, SAE[3]s and AESI[4]s	15	5754 / 5198
3	First-dose RCTs conducted in elderly subjects with $\geq$ 180 days follow up (a subset of pooling group 2)	SAEs and AESIs	10	4758 / 4690
4	RCT extension studies in elderly subjects received additional doses of seasonal influenza vaccine (5 studies from pooling group 2 were extended for an additional 2 or 3 seasons)	Solicited AEs after first vaccination versus revaccination	5 (3 trials extended 1 season and 2 extended 2 seasons)	492 / 330

Pooling group	Trial characteristics	Parameters evaluated	Number of trials included	Number of subjects Fluad / TIV
5	First-dose RCTs conducted in elderly subjects in which the citrate-buffered formulations of MF59 (with or without thimerosal) were the same as the product being evaluated in the current BLA (another subset of pooling group 2)	Safety and subgroup analyses the same as that of pooling group 1 except for exclusion of immediate post-vaccination events	7	4544 / 4375
6	Uncontrolled studies in elderly (a subset of pooling group 1)	Evaluation of SAEs and AESIs	17	1005 (Fluad alone)
7	Phase 4 study in elderly subjects	SAE, AESI, immediate post-vaccination events and hospitalizations	1	9204 / 4557
8	Studies including healthy subjects < 65 years of age	SAEs, AESIs, and immediate post-vaccination events	8	744 / 552

Source: Adapted from BLA 125510/0.0; 5.3.5.3 Statistical Analysis Plan Version 5.0 tables 4.1

[1]First-dose: subjects receiving a first dose of Fluad, e.g. not those enrolled in extension studies evaluating repeat annual dosing.

[2]Elderly includes those $\geq$ 65 years of age

[3]SAE: serious adverse events

[4]AESI: adverse events of special interest, defined below in this section under heading, "SAEs, Deaths, AE's Leading to Study Withdrawal, and Adverse Events of Special Interest"

[5]RCT: randomized controlled trial

Solicited AEs (pooled analyses)

Review of solicited local and systemic events reported within seven days in first-dose randomized controlled trials (RCTs) (pooling group 2, defined in Table 12) showed similar rates across all categories reported for the phase 3 trial V70_27 (Tables 9 and 10). Similar to trial V70_27, rates in the RCTs were modestly higher in the Fluad group, but the majority of events were mild in severity; rates for moderate and severe AEs were balanced between groups.

Unsolicited AEs (pooled analyses)

Unsolicited events observed in the first-dose elderly RCTs within 30 days of vaccination were balanced (24.8% and 26.7% for Fluad and unadjuvanted influenza vaccine, respectively) and uncommon (< 2% for any given system organ class). Re-vaccination did not show any increase in reactogenicity across

treatment arms. However, bias might have been introduced in these trials by virtue of the fact that not all subjects returned for repeat dosing.

SAEs, Deaths, AE's Leading to Study Withdrawal, and Adverse Events of Special Interest

SAEs, deaths, AEs leading to study withdrawal, and adverse events of special interest (AESI) were evaluated across pooling groups evaluating subjects ≥ 65 years of age (Table 12). Of note, AESI's were defined retrospectively using MedDRA (Medical Dictionary for Regulatory Activities) preferred terms to search for events of potential immune etiology such as neuroinflammatory disorders (including narcolepsy by both a narrow and broad definition), rheumatological disorders, inflammatory bowel disease, thyroid disorders, inflammatory skin disorders, autoimmune hematologic disorders, and vasculitis. No imbalances between MF59 adjuvanted and unadjuvanted vaccine formulations or other safety signals were identified.

7.0 Marketing Experience Outside of the U.S.

Fluad was first registered in Italy in 1997 and is currently authorized in 38 countries including Canada, and 15 European countries through individualized regulatory authorities, but not through the centralized European Medicines Agency (EMA). It is indicated for active immunization against influenza in adults 65 years of age and older, with the exception of the Philippines, South Africa and Canada, where it is indicated for use in individuals ≥ 60 years of age, individuals ≥ 12 years of age and children 6 months to < 2 years of age, respectively. It is estimated that approximately 75.7 million doses have been administered. The most recent periodic safety report from the period between September 1, 2013 through April 30, 2014 summarized the cumulative experience since post marketing data collection began on May 15, 1997. No safety signals have emerged.

On November 27, 2014, the Italian national regulatory agency (AIFA) suspended two batches of Fluad as a precautionary measure when a small number of deaths occurred in elderly patients who had received Fluad. On December 12, 2014, a press release issues by the European Medicines Agency (EMA) indicated that testing of the batches and review of the case reports by the EMA and the AIFA did not reveal a causal link between the fatal events and Fluad administration [10], and the suspension was lifted.

8.0 Post-Marketing Trial to Verify the Clinical Benefit of Fluad Vaccine

Under the accelerated approval regulations, a confirmatory study is required to verify and describe the clinical benefit of Fluad. NVD will conduct an efficacy trial to support traditional approval of Fluad.

9.0 Pharmacovigilance Plan

According to the risk management plan, Novartis will perform passive surveillance by monitoring for important identified risks (anaphylactic reactions, extensive limb swelling), important potential risks (convulsion, neuritis, encephalitis, vasculitis, Guillain-Barre Syndrome, demyelination, Bell's Palsy, idiopathic thrombocytopenic purpura, hemolytic anemia and vaccination failure), and other safety concerns (medication error and off-label use). Novartis has proposed to perform active surveillance using data from the Canadian annual safety surveillance system using 2014/2015 as the pilot season. In addition, Novartis is proposing to consider a prospective active surveillance study in Italy that is similar to the Canadian surveillance system.

Of note, although the pharmacovigilance plan proposed by NVD includes anaphylactic reactions and extensive limb swelling as "important identified risks", there were no cases of anaphylaxis identified in the phase 3 trial V70_27 or in the pooled data evaluating all elderly subjects who received a single dose of an MF59 adjuvanted product (table 12, pooling group 1). However, there was one case out of 492 subjects (0.2%) of anaphylaxis in an elderly subject who received a second annual dose of Fluad and 3 cases out of 330 subjects (0.9%) who received an unadjuvanted vaccine (table 12, pooling group 4). Similarly, there were no cases reported of extensive limb swelling in any of these analyses.

10.0 Focus of Questions to the Committee

The committee will be asked if the data support the safety of Fluad for the proposed indication (i.e., active immunization for the prevention of influenza disease caused by influenza subtypes A and type B contained in the vaccine in persons 65 years of age).

The committee will be asked if the immunogenicity data support the effectiveness of Fluad for the proposed indication under the accelerated approval regulations.

11.0 References

1. Guidance for Industry: Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines, 2007. http://www.worldcat.org/title/guidance-for-industry-clinical-data-needed-to-support-the-licensure-of-seasonal-inactivated-influenza-vaccines/oclc/146205023
2. Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *Journal of Hygiene* (Camb). 70 (1972), pp. 767-777.
3. Ohmit SE, Petrie JG, Cross RT, Johnson E, Monto AS. Influenza hemagglutination inhibition antibody titer as a correlate of vaccine-induced protection*. J. Infect. Dis.* 204(12) (2011), pp. 1879–1885.
4. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices (ACIP) — United States, 2014–15 Influenza Season. 63(32) (August 15, 2014), pp. 691-697
5. D.T. O'Hagan. MF59 is a safe and potent vaccine adjuvant that enhances protection against influenza virus infection. *Expert Rev Vaccines* (October 5, 2007), pp.699–710

6. Ott G, Barchfeld GL, Chernoff D, Radhakrishnan R, van Hoogevest P, Van Nest G. MF59. Design and evaluation of a safe and potent adjuvant for human vaccines. *Pharm Biotechnol*, 6 (1995), pp. 277–296
7. Dupuis M, McDonald DM, Ott G. Distribution of adjuvant MF59 and antigen gD2 after intramuscular injection in mice. *Vaccine*, 18(5–6) (1999), pp. 434–439
8. Dupuis M, et al. Dendritic cells internalize vaccine adjuvant after intramuscular injection. *Cell Immunol*, 186(1) (1998), pp. 18–27
9. Seubert A, Monaci E, Pizza M, O'Hagan DT, Wack A. The adjuvants aluminum hydroxide and MF59 induce monocyte and granulocyte chemoattractants and enhance monocyte differentiation toward dendritic cells. *J Immunol*, 180(8) (2008), pp. 5402–5412.
10. http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2014/12/news_detail_002228.jsp&mid=WC0b01ac058004d5c1