EMA/639703/2010
Committee for Medicinal Products for Human Use (CHMP)

Assessment Report

AFLUNOV

Common Name: Prepandemic influenza vaccine (H5N1) (surface antigen, inactivated, adjuvanted)

Procedure No. EMEA/H/C/002094

Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.

7 Westferry Circus • Canary Wharf • London E14 4HB • United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7523 7455
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

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1. Background information on the procedure

1.1. Submission of the dossier

The applicant Novartis Vaccines and Diagnostics S.r.l. submitted on 2 December 2009 an application for Marketing Authorisation to the European Medicines Agency (EMA) for AFLUNOV, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA on 27 July 2006.

The applicant applied for the following indication: "Active immunisation against H5N1 subtype of Influenza A virus. This indication is based on immunogenicity data from healthy subjects from the age of 18 years onwards following administration of two doses of the vaccine containing A/Vietnam/1194/2004 (H5N1)-like strain".

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or studies.

Information on Paediatric requirements

Pursuant to Article 8 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/150/2009for the following condition(s):

- Prevention of influenza

on the agreement of a paediatric investigation plan (PIP)
The PIP is not yet completed.

Scientific advice:

The applicant received Scientific Advice from the CHMP on 18 October 2006. The Scientific Advice pertained to pre-clinical and clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:

Rapporteur: Daniela Melchiorri Co-Rapporteur: Christian Schneider

- The application was received by the Agency on 2 December 2009.
- The procedure started on 23 December 2009.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 15 March 2010. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 18 March 2010.
- During the meeting on 19-22 April 2010, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 28 April 2010.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 18 May 2010.
- The summary report of the inspection carried out at the following sites, Tampere University (Finland between 12-16 April 2010), Section of International Medicine & Public Health Dept. of Infect. Disease and Tropical Medicine (Germany between and 26-30 April 2010), Novartis Vaccines and Diagnostics GmbH (Germany between 25-28 May 2010), was issued on 23 July 2010.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 7 July 2010.
- The Rapporteurs circulated the updated Joint Assessment Report to all CHMP members on 16 July 2010. Further updated Joint Assessment Report was circulated to all CHMP members on 19 July 2010.
- During the CHMP meeting on 19-22 July 2010, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 23 August 2010.
- The Rapporteur circulated the Preliminary Joint Assessment Report on the Applicant's responses to the CHMP List of Outstanding Issues to all CHMP members on 7 September 2010.
- The Rapporteur circulated the final Joint Assessment Report on the Applicant's responses to the CHMP List of Outstanding Issues to all CHMP members on 13 September 2010.
- The applicant submitted the responses to the final Joint Assessment Report on 15 September 2010.
- The Rapporteur circulated the updated final Joint Assessment Report on the Applicant's responses to the CHMP List of Outstanding Issues to all CHMP members on 22 September 2010.
- During the meeting on 20-23 September 2010, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to AFLUNOV. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled on 23 September 2010.

2. Scientific discussion

2.1. Introduction

Novel influenza viruses may emerge following the reassortment of two co-circulating viral strains (Belshe, 2005) or from a series of genetic mutations in one strain (Taubenberger et al., 2005; Tumpey et al., 2005). Pandemic influenza outbreaks occur when a new virus strain is able to infect humans and to transmit efficiently from person to person in a previously unexposed population. A pandemic outbreak is expected to spread quickly and cause substantial global morbidity and mortality (Tumpey et al., 2005; Johnson and Muller, 2002). Due to the anticipated difficulty in producing sufficient quantities of the specific pandemic vaccine against the emerging strain within such a short time after identification and confirmation of the pandemic influenza viral strain, the production during the interpandemic period of a vaccine such as AFLUNOV against a potential influenza pandemic viral strain (i.e., a pre-pandemic vaccine) may:

i) Permit early vaccination at the start of a pandemic (World Health Organization [WHO] phase 6) when the "fast track pandemic" vaccine is not yet available.

ii) Prime during pre-pandemic stages (WHO phases 3 to 5) to reduce mortality against a closely matched pandemic strain in those countries where infections are occurring.

iii) Reduce the chance of the emergence of a reassortant pandemic strain by vaccinating those (e.g., veterinarians, poultry workers, operators involved in the manufacturing of vaccines with pandemic-like strains, laboratory workers) at high risk of infection from both avian and human viruses.

Indeed, even a vaccine of limited efficacy, such a pre-pandemic vaccine, is expected to be able to mitigate a pandemic (Ferguson et al., 2006).

The H5N1 strain is still considered as a likely candidate from which pandemic influenza may evolve. Differently from the recent swine influenza pandemic caused by the A/H1N1 (A/California/7/2009) strain, which seems to be easily transmitted between humans but has a lower mortality rate, with avian origin H5N1 strains causing influenza outbreaks transmission within the human population is rare but the mortality rate in infected humans can be high. From the start of the H5N1 outbreaks in mid-2003 until 24 September 2009, 442 individuals have been infected with laboratory-confirmed avian H5N1 influenza, 262 of whom died (http://www.who.int/csr/disease/avian_influenza/country/cases_table_2009 _09_24/en/index.html).

This MAA took into account the "Guideline on dossier structure and content of MAA for influenza vaccines derived from strains with a pandemic potential and intended for use before the pandemic is declared" (EMEA/CHMP/VWP/263499/2006). The pre-pandemic vaccine AFLUNOV is identical to the H5N1 pandemic mock-up vaccine, previously identified as Focetria, already approved by EMA (May 2007, Commission decision) for pandemic use. Currently the name Focetria identifies the actual H1N1 pandemic vaccine and the name Foclivia has been attributed to the H5N1 mock-up vaccine. With the exception of antigen composition and dose, AFLUNOV is identical to authorized inter-pandemic seasonal vaccine Fluad. Both vaccines are egg-derived, surface antigen, inactivated, adjuvanted with MF59C.1 (MF59), and are produced following the same manufacturing process.

AFLUNOV has been approved for the active immunisation against H5N1 subtype of Influenza A virus in adults and elderly (18 years of age and over), outside of the context of a mock-up core dossier, that is, for prophylaxis before the pandemic is declared.

2.2. Quality aspects

2.2.1. Introduction

AFLUNOV is an egg-derived, monovalent vaccine, containing surface antigens from an H5N1 (A/Vietnam/1194/2004) influenza virus.

The formulation proposed for AFLUNOV, selected basing on the Clinical Trials performed using pandemic strains, contains 7.5 µg HA of antigen/dose It is 6-folds lower that the total amount of HA present in conventional trivalent seasonal influenza vaccine, that is 15 µg HA per strain (i.e. 45 µg HA/dose).

The vaccine is presented as a suspension for injection in an emulsion in a pre-filled syringe (single dose).

With the exception of antigen composition and dose, the candidate vaccine against avian A/H5N1 influenza is identical to authorised interpandemic seasonal Fluad. This egg-derived, surface-antigen, inactivated, influenza vaccine, adjuvanted with MF59C.1 (MF59) is produced by an identical manufacturing process.

2.2.2. Active Substance

The Active Substance (Monovalent Pooled Harvest, MPH) is a buffered sterile suspension containing predominantly the purified outer membrane proteins, Haemagglutinin (HA) and Neuraminidase (NA), of the H5N1 avian influenza virus strain.

Manufacture

The influenza avian vaccine of the present application has been developed from Fluad, a Novartis seasonal, trivalent influenza vaccine and is produced by the same manufacturing process.

The manufacturing process of Monovalent Pool Harvest (MPH) involves the cultivation of the pandemic influenza virus strain in embryonated chicken eggs, harvesting of allantoic fluid, concentration by ultrafiltration and formaldehyde inactivation, followed by whole virus purification using sucrose gradient centrifugation and diafiltration.

The HA and NA antigens from the surface of the purified whole virus are solubilised by treatment with a detergent (CTAB). The solubilised antigens are then separated from the non-solubilised components of the virus by centrifugation. The resultant supernatants are treated with a polystyrene based resin to remove CTAB. The polystyrene resin is removed by filtration and the resulting MPH is filter sterilised.

Embryonated Eggs

The Influenza Virus Strain is propagated in embryonated chicken eggs. While Master and Working seed lots are grown in specific pathogen-free (SPF) eggs the Monovalent Pooled Harvest is produced using Production eggs. The SPF status of the flock is compliant with the chapter entitled "Chicken Flocks free from Specified Pathogens for the Production and Quality Control of Vaccines" of Ph.Eur. monograph on Vaccina ad Usum Veterinarium, chapter.

Preparation and control of Virus Seeds

The reference virus (H5N1 NIBRG-14) was manufactured by Reverse Genetics (RG) technology and provided by NIBSC, UK, an authorized WHO reference laboratory.

According to Ph.Eur. the Master Seed (MS) is obtained after no more than 15 passages from the approved reference virus. H5N1 MS was obtained after one passage from the reference virus received from the WHO reference laboratory. Aliquots of MS are aseptically filled into sterile sealed vials, and stored in a freezer at a temperature lower than -60°C.

The Working Seed (WS) is obtained after only one passage from the MS using SPF eggs. WS is QC tested and the vials are stored at a temperature lower than -60°C. WS is tested for HA and NA identity, absence of mycoplasma, sterility, infectivity, HA titre and egg infectivity (spot HA test) to assure identity and microbial quality.

Virus cultivation

Pre-incubated, candled, fertile hens eggs are disinfected by fumigation and are held until inoculation at a controlled temperature. The virus inoculum is prepared from the WS at a dilution calculated to ensure total egg infection and maximum virus yield and is injected into the allantoic cavity of each production egg. After inoculation, the eggs are incubated at an optimum temperature and time for maximum virus yield. After incubation the eggs are cooled to 2-8°C and thereafter fed directly into the Harvesting room.

Harvesting of Allantoic Fluid

The allantoic fluid (AF) is collected into the harvesting vessel. The resultant fluid is then clarified by centrifugation, collected in a refrigerated tank, which is then connected to an ultrafiltration system to concentrate the allantoic fluid.

Inactivation

An aqueous solution of formaldehyde is added to the clarified concentrated allantoic fluid . The content of the tank is then transferred to a sanitised and temperature controlled inactivation vessel and stirred throughout the inactivation period. The inactivation cycle depends upon the characteristics of specific virus strains. The inactivation temperature is selected in order not to compromise antigenicity.

Purification

The virus is removed from the inactivated allantoic fluid using continuous flow isopycnic ultracentrifugation and a sucrose density gradient, which concentrates and purifies the virus. The different fractions which contain the purified virus are collected and tested.

The fractions are pooled together, and stored under controlled temperature conditions. The Pool is diluted with PBS and then diafiltered. The Pool is sampled and tested . After the sampling a clarifying filtration takes place with pre-filters and filters of different pore sizes. The filtered product represents the Whole Virus Concentrate, and it is sampled for testing. A Polysorbate 80 solution is added to the Whole Virus Concentrate and the product is stored waiting for the Split test results.

Haemagglutinin and Neuraminidase solubilisation

Following the split test results, CTAB Solution is added to the Whole Virus Concentrate to solubilise the HA and NA antigens. The product is then centrifuged under continuous flow and the supernatant collected.

A polystyrene resin, suspended in PBS, is added to the Subunit Supernatant Pool to absorb the CTAB. Afterwards, the product undergoes a clarifying filtration to remove the resin, and a stabilizer solution is added and the product is then filtered into a closed sterile stainless steel tank.

Filling, storage and transportation

After the filtration described above, the monovalent pool is transferred to Rosia, where it is sampled for Bioburden, and sterile filtered into a sterile container. The Monovalent Pooled Harvest is sterile filtered. An Integrity Test of the sterilising filter is performed before and after filtration. The filtered Monovalent Pooled Harvest (i.e. the Active Substance) is sampled for release testing and stored at 2-8°C in a stainless steel tank.

Process Validation and/or Evaluation

Consistency of production was demonstrated by data provided on three H5N1 full-scale batches and further supported by batch analysis results of the inter-pandemic vaccine production campaigns of previous years.

MPHs are manufactured in compliance with GMP and according to requirements of the Ph.Eur. Studies have been carried out to evaluate the effectiveness of the antigen production process to inactivate potential viral, bacterial and mycoplasma contamination in addition to influenza viruses.

The formaldehyde inactivation step has been evaluated for three consecutive production egg harvests for H5N1.

The optimum quantities of polysorbate 80 and of CTAB to allow complete splitting of the virus vaccine strain is determined in the QC laboratory, prior to application to production lots. The optimal conditions are determined on the basis of the electrophoresis patterns, the haemagglutinin and the neuraminidase activity identified in the lots. Tests are performed to determine the levels of potential impurities, which may arise in Monovalent Pooled Harvest. Limits are applied to these impurities.

Transportation between Novartis Siena and Rosia sites is carried out using validated procedures.

Characterisation

The Active Substance complies with Ph.Eur. monograph for "Influenza Vaccine, Surface Antigen, Inactivated". It is a sterile suspension containing predominantly the purified outer membranes proteins: HA and NA of the influenza virus strain. The crystal structure of HA has been determined to atomic resolution for the native HA, for the HA bound to a number of different receptor analogues, for proteolytic fragments of HA which have gone through the conformational changes required for mediating membrane fusion, and for HA complexed with neutralizing antibody.

Influenza virus NA structure has been determined with structural studies of NA in complex with specific monoclonal antibodies, by electron microscopy, X-ray crystallography amino acid sequencing and gene sequencing.

The concentrations of potentially contaminant substances (formaldehyde, barium sulphate, sodium citrate, and CTAB) are controlled during the process or in the MPH. Limits are applied. Polysorbate 80 is also used as an excipient of MF59C.1 adjuvant and is not considered as a residual of production but is nevertheless tested on the MPH. It is concluded that the impurities in the Active Substance are sufficiently controlled.

Specifications

The MPH complies with the Ph.Eur. monograph 01/2006:0869 on Influenza Vaccine (Surface Antigen, Inactivated).

The MPH is tested for release for Haemagglutinin Identity and Content (SRID), Neuraminidase Identity (ELISA), Viral inactivation, Purity (SDS-PAGE), Sterility, CTAB, Polysorbate 80, Barium, Citrates, Endotoxin, Formaldehyde, Ovalbumin Content and Appearance. Specifications have been selected to be

as much as possible in accordance to the Ph.Eur. monograph for the influenza vaccine (surface antigen inactivated).

Specification for Appearance, Haemagglutinin identity and content, Neuraminidase Identity, Viral Inactivation, Purity, Sterility comply with Ph.Eur. for the Monovalent Bulk. A limit for Endotoxin and Ovalbumin is set up on the Active Substance to ensure that the Ph.Eur. specification for the Final Lot (i.e. monovalent at 7.5 µg HA/dose) is met.

Due to the presence of the adjuvant in the Finished Product, the test for Formaldehyde is performed on the Monovalent Pooled Harvest. Ovalbumin is also controlled on the Active Substance. The acceptance limits have been set to ensure that the Ph.Eur. limits for the Finished Product (i.e. monovalent at 7.5 µg HA/dose) are not exceeded. The concentrations of the other substances used during manufacture of the vaccine (i.e. CTAB, Barium, Citrates, Polysorbate 80) are controlled in the Active Substance. The limit for the Citrates is calculated considering the content of HA on the Final Lot. It has to be noted that Polysorbate 80 is also used as an excipient of MF59C.1 adjuvant. Test for Barium may be omitted in case the barium treatment is not performed during production.

All the relevant analytical methods have been validated or qualified for the Active Substance. It is acceptable that some analytical validations have been performed on the inter-pandemic strains, as the methods are not strain specific.

Influenza reference antigens and reagents for strain characterization and vaccine standardization are provided by WHO Collaborating Centres, usually National Institute for Biological Standards and Control (NIBSC), UK. The reference antigen and antiserum reagents are used to calibrate the haemagglutinin content of inactivated influenza vaccines by the single radial immunodiffusion (SRID) Test.

Stability

The applicant has provided stability data up to 36 months for three full-scale batches of NIBRG-14 H5N1 Monovalent Pooled harvest. The key stability-indicating parameter are the HA content and purity, measured with the same methods and acceptance limits used at release. The data are consistent with the proposed shelf-life of 24 months for the active substance when stored at 2-8°C.

In accordance with EU GMP guidelines [1], any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

2.2.3. Finished Medicinal Product

AFLUNOV is an influenza vaccine for human use (surface antigen, inactivated, adjuvanted with MF59C.1) for pre-pandemic use.

The Finished Medicinal Product is a combination of MPH, MF59C.1 adjuvant bulk and buffer solutions. The vaccine application is based on the H5N1 Reverse Genetics Strain NIBRG 14, which is derived from the highly pathogenic avian influenza strain A/Vietnam/1194/2004.

The MF59C.1 adjuvant is an oil-in-water emulsion, composed mainly of squalene that is an intermediate metabolite in the synthesis of cholesterol. Squalene is a commercially available natural product distilled from shark liver oil. It is then redistilled and supplied by qualified manufacturers.

[1] *6.32 of Vol. 4 Part I of the Rules Governing Medicinal products in the European Union*

The process for the Final Bulk preparation consists is a simple mixing operation. The formulated suspension is filled into syringes. The potency of the vaccine is expressed as the concentration of the HA protein.

The vaccine is presented as a suspension for injection in pre-filled syringe.

Description and Composition of the Finished Product

Each 0.5 ml dose of vaccine has the following composition:

Ingredients	Quantity per dose		
Active Ingredient			
Influenza virus surface antigen (HA and NA), H5N1.	≥ 7.5 μg HA		
Adjuvant			
Squalene	9.75 mg		
Polysorbate 80	1.175 mg		
Sorbitan trioleate	1.175 mg		
Sodium citrate dihydrate			
Citric acid monohydrate			
Other Ingredients			
Sodium chloride			
Potassium chloride			
Potassium dihydrogen phosphate			
Disodium phosphate dihydrate			
Magnesium chloride hexahydrate			
Calcium chloride dihydrate			
Water for injections			

Pharmaceutical Development

AFLUNOV contains the same adjuvant and is manufactured with the same process used for Fluad. Fluad a surface antigen, trivalent inactivated, inter-pandemic influenza vaccine, adjuvanted with MF59C.1, is currently the only influenza vaccine with an adjuvant on the market, and has been approved in 2000 through a Mutual Recognition Procedure in 12 EU countries. Fluad is also licensed and marketed in other European countries and outside Europe.

Formulation Development

The Finished Product is a combination of MPH, MF59C.1 adjuvant bulk and buffer solutions. The present core pandemic dossier describes the H5N1 mock-up vaccine.

The MF59 adjuvant has been used in pre-clinical and clinical studies for a range of different vaccines.

Since 1999, Fluad has been formulated using adjuvant containing citrate buffer to improve adjuvant stability, designated as MF59C.1. This formulation has been the one used for AFLUNOV.

Manufacturing Process Development

The first production of the Finished Product with the H5N3 strain was in 1999. The manufacturing process was the same of the seasonal influenza vaccine Fluad. Successively, the Finished Product was produced with the H9N2 strain in 2004 and with the H5N1 strain in 2005, with the same manufacturing process approved for Fluad in 2005.

From 2000, some changes in formulation and manufacturing process were introduced for Fluad and approved through the relevant MRP variations. However these differences, as already demonstrated for the variations submitted for Fluad, do not affect the quality, safety and efficacy of the product.

Thiomersal was previously used in the production of the Active Substance (as reagent during the process and as preservative at the final stage) and of the Finished Product (as preservative). Thiomersal was removed in two steps: in the first one it was removed from the active substance and finished product as preservative (remaining as traces); in the second one it was removed completely (in 2003).

Clinical trials performed to compare Fluad formulations (with and without preservative) and of the stability studies confirmed that the presence of Thiomersal, as a preservative in Fluad, does not have any impact on the quality, immunogenicity and safety of the product. For that reason, the current licensed Fluad in pre-filled syringe is a thiomersal-free product.

Fluad with H5N3 strain was produced before 2003 with the preservative, while Fluad with H9N2 and H5N1 strains were produced in 2004 and 2005 without any preservative.

Adventitious Agents

In addition to inactivation of influenza virus, the Ph.Eur. requires that the formaldehyde inactivation process be shown to be capable of inactivating avian leucosis viruses and mycoplasma. Studies have been carried out to evaluate the effectiveness of the antigen production process to inactivate potential viral, bacterial and mycoplasma contamination in addition to influenza viruses.

CTAB, as detergent, could contribute to virus inactivation. Its capacity to inactivate mycoplasma has been validated.

Sucrose gradient centrifugation could contribute to virus removal as well as the centrifugation steps, which follow the Polysorbate 80/CTAB treatment.

With respect to the transmission of TSE, the only animal derived starting materials are eggs (used in production of the Drug Substance) and squalene (used in the MF59C. 1 adjuvant). There is no scientific evidence to suggest that eggs are likely to present any risk of contamination from TSE-agents. Copy of the Declaration of compliance with the annex to Directive 75/ 318/ EEC, as amended by directive 1999/ 82/ EEC relating to TSE was submitted.

Regarding squalene, derived from shark liver, it does not present any risk of potential contamination from TSE agents as well.

With regards to the use of reverse genetics strains, which may be prepared using materials from ruminant origin (foetal calf serum, bovine trypsin, Vero Cells), the TSE compliance was performed by NIBSC.

Manufacture of the Product

Description of Manufacturing Process and Process Controls

Final Bulk Vaccine Process

The components are added to the Final Bulk container in a defined sequence. Dedicated sterile vessels are used for the preparation of the Final Bulk.

After the additions are completed, the bulk is stirred to allow adequate mixing. The pH of the Final Bulk is checked. Samples are taken for Final Bulk release control testing. The Final Bulk is then aliquoted by aseptic transfer into sterile containers. Each container is aseptically sampled and tested for sterility. The containers are stored at 2-8°C, until released for filling.

The adjuvant MF59C.1 is produced in Germany and transported to Italy, where it is filtered and used to formulate the Final Bulk. Bioburden is controlled before the sterilizing filtration, while sterility and other in-process parameters are monitored after filtration and before the addition into the Final Bulk mixing tank.

Filling and packaging Process (Final lot syringes)

Filling operations are carried out at the Rosia manufacturing site. Syringes are filled by a validated, aseptic procedure which is also registered for Fluad. The packed product is stored at 2-8°C until released.

Product Specification

The release tests are the same approved for the inter-pandemic Fluad vaccine (except for the haemagglutinin content - 7.5μg/dose vs. 15 μg for each strain/dose) and fulfil the Ph. Eur. requirements for surface antigen influenza vaccine.

The specifications might differ from the Ph.Eur. monograph for the influenza vaccine (surface antigen inactivated) due to the presence of the MF59C.1 that could interfere with some analytical methods.

The specification for Haemagglutinin identity and content complies with Ph.Eur. for the Final Lot. This test is also performed in the Final Bulk. In case the quantity of relevant materials to carry out the assay is insufficient (a real possibility in a Pandemic emergency), this test will be performed only on the Final Bulk and will not be repeated in the final lot. The specifications for squalene content and identity and for particle size distribution are specific to control the MF59C.1 adjuvant into the vaccine.

The Test for free formaldehyde is performed earlier in the process, on the Monovalent Pooled Harvest, rather than on the Final Bulk vaccine or the Final Lot, as required by Ph.Eur. The MF59C.1 adjuvant in the Drug Product interferes with the performance of this test.

All the relevant analytical procedures have been validated or qualified for the drug product.

All excipients used during production and in the formulation of Monovalent Pooled Harvest, MF59C.1 adjuvant and Final bulk Vaccine comply with Ph.Eur., except for squalene (in-house specifications).

Batch analysis

Batch analysis results of three H5N1 full production scale lots show consistent production and are consistent with results obtained for the seasonal Fluad vaccine.

Stability of the Product

The reported stability data, for three full-scale batches of AFLUNOV (Fluad H5N1) produced in 2005 and 2006 shows complete stability up to 36 months when the product is stored at the recommended temperature of 2°-8°C, protected from light. The key stability-indicating parameter is the potency as determined by the Haemagglutinin (HA) content assay.

Stability data of the Final Bulk of Fluad H5N1 demonstrates stability up to three months of storage at 2-8 °C in the bulk tank.

The proposed shelf life for the adjuvant MF59C.1 of 3 years when stored at 2-8 °C is sufficiently supported by data.

In general, the results support the shelf life and storage conditions as defined in the SPC.

In accordance with EU GMP guidelines[1], any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product have been presented in a satisfactory manner. The results of tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.3. Non-clinical aspects

2.3.1. Introduction

The manufacturing processes for AFLUNOV and Fluad are the same and the formulation of AFLUNOV is the same as Fluad with the exception of lower antigen content.

Overall, the non-clinical support for AFLUNOV is based on pharmacology and toxicology studies performed with Fluad®, three ferret challenge studies with AFLUNOV and the reproductive toxicity study with AFLUNOV in rabbits. Module 4 reported the same Non Clinical studies already assessed in the previous submission. Only new data for the pharmacological section (two challenge studies in ferrets, see section 3.3.2) were reported in the current application.

The studies with MF59 adjuvant alone and MF59 adjuvant in combination with a wide variety of non-influenza antigens also contribute to the overall non-clinical assessment. During the development of MF59 adjuvant and Fluad®, various formulations were tested.

2.3.2. Pharmacology

Primary pharmacodynamic studies

The program of immunogenicity and challenge studies performed with AFLUNOV is reported in the Table 2.

Table 2: Non-clinical evaluation of AFLUNOV immunogenicity and efficacy

Study number	Study type	Species	Vaccine (strain)
NIH Mouse Study	Challenge with homologous and heterologous wild-type virus	Mouse	AFLUNOV (Vietnam)
UBA00021	Reproductive and developmental toxicity	Rabbit	AFLUNOV (Vietnam)
765-N106857	Challenge with wild-type virus homologous and heterologous to the vaccine strain	Ferret	AFLUNOV (Vietnam) and AFLUNOV (Turkey)
673-N106850	Challenge with homologous wild-type virus	Ferret	AFLUNOV (Vietnam)
CBI-PCS-008 & VIV-PCS-001	Challenge with homologous reverse genetics virus	Ferret	AFLUNOV (Vietnam)

Studies in mice

A series of cross-protection/cross-reactivity experiments was conducted to assess the immune response and protection from homologous and heterologous viral challenge in mice following vaccination with AFLUNOV and non-adjuvanted H5N1 antigens.

In these experiments cross-protection was defined as the ability of a vaccine (e.g. a Clade 1 vaccine) to protect animals from challenge with a heterologous (e.g. Clade 2) viral strain. Instead, cross-reactivity was defined as the ability of a vaccine to induce antibodies *in vivo* that are able to recognize heterologous virus in *in vitro* assays. These experiments demonstrated that AFLUNOV is more immunogenic than non-adjuvanted H5N1 antigens. The antibodies induced by AFLUNOV cross-react with at least one heterologous strain of H5N1 virus. Furthermore, AFLUNOV induced protection from homologous and heterologous viral challenge in mice. The vaccine was effective in preventing clinical signs of illness/death and viral replication in brain, lung and spleen of mice challenged with homologous or heterologous virus.

Studies in Rabbits

Immunogenicity in rabbits was evaluated as part of the Study NoUBA0002: Intramuscular reproductive and developmental toxicity of Fluad H5N1 in rabbits, including a postnatal evaluation. The study design is summarized in the toxicology section.

Analysis of serum samples taken throughout the study confirmed the presence of antibodies using the hemagglutinin inhibition assay (HAI). The results of this study are important to confirm AFLUNOV immunogenicity. The vaccine was immunogenic in maternal rabbits, developing foetuses and F1 pups. In particular HI titres were measurable beginning on day 15 of the study in all animals treated, and subsequently increased or remained sufficiently elevated to demonstrate continued immune response to the vaccine. Interestingly, anti-influenza antibodies at the time of C-sectioning were detected in all foetal pooled samples at levels comparable to those of the respective maternal samples. The antibodies persist through the first 4 weeks of life in F1 offspring.

Studies in Ferrets

Three challenge studies in ferrets have been performed with AFLUNOV in order to study the protecting effectiveness of AFLUNOV in the most suitable animal model for human infection. These studies are briefly reported below.

Study No. CBI-PCS-008: A study to determine the efficacy of an H5N1 influenza vaccine adjuvanted with MF59 in the ferret experimental challenge model.

Because of the need for priming infection with a heterologous strain in order to enhance responses in the ferret model, as previously demonstrated for Focetria, the priming was included in this study design. From the results it can be concluded that AFLUNOV containing either 7.5 µg or 15 µg of A/NIBRG-14 (H5N1) antigen was immunogenic and decreased viral titres in ferrets challenged with the homologous reverse genetics strain. The 15 µg dose was slightly more immunogenic in ferrets but in humans the two doses appear equivalent (see clinical sections). Moreover, additional analyses were reported on the activity of neutralizing and hemagglutinin-specific antibodies against a heterologous virus strain (Influenza A NIBRG-23 (H5N1) reference virus, a reassortant prepared by reverse genetics from A/turkey/Turkey/1/2005 (H5N1) virus and A/PR/8/34). The vaccine did induce antibodies that were able to recognize (HI) and neutralize (MN) the heterologous virus NIBRG-23.

Although this study has been well performed, some doubts on the interpretation of statistical data were raised. Therefore in the second submission the Applicant provided two new challenge studies in ferret as follows.

Study No. 673-N106850: Evaluation of the protective efficacy of Fluad H5N1 vaccine in ferrets challenged with HPAI.

This study provided additional information in order to demonstrate non-clinical cross-protection and cross-reactivity. In this study, ferrets were vaccinated with AFLUNOV prior to challenge with a highly pathogenic avian influenza (HPAI) virus, A/Vietnam/1203/04.

Study N°765-N106857: Evaluation of the protective and cross-protective efficacy of AFLUNOV Turkey and AFLUNOV Vietnam vaccines in ferrets challenged with highly pathogenic avian influenza virus

The objective of this study was to evaluate the protective and cross-protective efficacy of AFLUNOV vaccine formulations in ferrets given a lethal challenge of highly pathogenic avian influenza (HPAI) strain A/Vietnam/1203/04. The vaccines used contained either hemagglutinin from clade 1 A/Vietnam/1194/2004 (AFLUNOV Vietnam) or hemagglutinin from clade 2.2 A/turkey/Turkey/1/2005 (AFLUNOV Turkey). The immune response was monitored using hemagglutination inhibition (HI) and microneutralisation (MN) assays. Survival, body weights and temperatures, clinical signs, virus shedding, and clinical pathology were the parameters used to assess vaccine efficacy as well as haematological and clinical chemistry.

Overall the protective efficacy data from the studies 673-N106850 and 765-N106857 were more persuasive than the earliest ferret study and indicated the protective and cross-protective efficacy of AFLUNOV vaccine formulations in ferrets given a lethal challenge of highly pathogenic avian influenza virus (HPAI).

Studies with Fluad in mice

Since responses to influenza vaccine can be significantly impaired in the young and elderly, a series of studies were conducted to investigate the immune response in young (3 months) and old (18 months) mice after subcutaneous or intramuscular administration of Agrippal® vaccine either alone or combined with MF59 (water formulation).

These data showed evidence of immunogenicity both in old and young mice. More specifically, the addition of MF59 to the vaccine formulation allowed a reduction of the amount of HA antigen needed to yield antibody titres similar to non-adjuvanted vaccine and resulted in complete protection of mice against a lethal challenge.

MF59 Adjuvant

MF59C.1 is an oil-in-water emulsion with a squalene internal oil phase and a citrate buffer external aqueous phase. The surfactants, sorbitan trioleate and polysorbate 80, serve to stabilize the emulsion. The components of MF59 adjuvant are described in the quality section of this assessment report.

MF59C.1 was optimized by the addition of citrate buffer to provide increased stability. Citrate is a common and a well-tolerated excipient and immunogenicity and toxicology studies have identified no notable differences between the two formulations. The Applicant studied various MF59-adjuvanted vaccines in various animal species, including hamsters, guinea pigs, rabbits, dogs, goats, and primates.

Ratio of Antigen to Adjuvant

Based on observations in mice, the clinical development with MF59 at a ratio of 1:1 (v/v) with antigen was used in the MF59-adjuvanted subunit inter-pandemic influenza vaccine Fluad®. Following the clinical experience with Fluad®, the formulation for a 'pre-pandemic' or 'pandemic' vaccine was not changed (pandemic-like strains H5N3 and H9N2). Clinical data with these vaccines, confirmed that the immunogenicity was significantly enhanced, as compared with a non-adjuvanted vaccine, using the 1:1 ratio of MF59:antigen.

Mechanism of Action of MF59

One of the effects of MF59 is to generate a local immunostimulatory environment at the injection site, which activates local immune cells. This results in maturation of resident dendritic cells (DCs), further recruitment and activation of immune cells into the injection site, enhanced antigen uptake, enhanced maturation of DCs and enhanced migration of APCs to the local draining lymph nodes. Recently, the effects of MF59 on human immune cells were assessed directly in vitro and it was found that following immunization, MF59 enhances the immune response at a range of points, including the induction of chemokines to increase recruitment of immune cells to the injection site, enhanced antigen uptake by monocytes at the injection site, and enhanced differentiation of monocytes into DCs, which represent the gold-standard cell type for priming naïve T cells. A particularly important feature of MF59 may be that it facilitates DCs migration into draining lymph nodes where they can trigger the adaptive immune response specific to the vaccine. Nevertheless, further studies are necessary to better define the mechanism of action of MF59.

So far there appears to be an impressive consistency between data obtained in vitro with human cells, and the in vivo data from mouse. These observations suggest that MF59 induces a local pro-inflammatory environment within the muscle, which promotes the induction of potent immune responses to co-administered vaccines. Overall, the justification for the use of MF59 as adjuvant is sufficiently supported, also in light of new studies reported in this submission.

Secondary pharmacodynamic studies

Because of the nature of the product, *in vivo* secondary pharmacodynamic studies were not performed. This is in accordance with the relevant guidelines, note for guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95) and the guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application, CPMP/VEG/4717/03.

The potential for undesirable pharmacological activities of MF59 was assessed as a component of repeated-dose toxicology studies in dogs (see below).

Safety pharmacology programme

Standard safety pharmacology studies were not performed with AFLUNOV in accordance with the relevant guidelines, CPMP/SWP/465/95 and CPMP/VEG/4717/03.

MF59C.1 adjuvant

On request of the CHMP within the Focetria procedure, the applicant further justified the approach not to carry out dedicate safety pharmacology study. The applicant's approach was approved to be adequate and sufficient and it was included in the AFLUNOV file. This is in line with the WHO Guideline on Preclinical testing of Vaccines indicating that safety pharmacology should be considered in case of bacterial vaccines associated with specific toxins.

During the early development of MF59C.1 adjuvant, two repeat-dose studies in dogs were conducted to evaluate the toxicity of vaccine formulations containing antigens that are not related to this dossier. However, both studies had an MF59C.1 group and a saline/buffer control group, and included the evaluation of cardiovascular and neurological parameters. This information provides supportive secondary or safety pharmacological data for AFLUNOV, showing no pharmacological effects following administration of vaccine with MF59.

Pharmacodynamic drug interactions

Because of the nature of the vaccine, the guideline for non-clinical testing of vaccines (CPMP/SWP/465/95) and the guideline on adjuvants (EMEA/CHMP/VEG/134716/2004) state that pharmacokinetic studies are not needed. This concept can be extended also to drug-drug pharmacokinetic or pharmacodynamic interaction studies. Such non-clinical studies would not provide clinically-relevant information.

2.3.3. Pharmacokinetics

Pharmacokinetic or classic absorption, distribution, metabolism and excretion (ADME) studies with Fluad H5N1 or Fluad are not required according to CPMP/SWP/465/95 and EMEA/CHMP/VEG/134716/2004 because they are considered not relevant for a vaccine. However, distribution and clearance studies on the MF59C.1 adjuvant constituent (squalene) were considered, since it may play an active role in metabolism pathways or possess other pharmacological actions. Squalene is an intermediate in the biosynthesis of cholesterol and is a constituent in dietary product (vegetable and fish oil). Clearance studies performed in rabbits injected intramuscularly with labelled squalene (^{125}I) demonstrated that it is rapidly cleared and 5% remains at the injection site for approximately 5 days after injection. Overall, considering the data available on squalene, the low amount required and the route and frequency of administration in humans, the use of squalene should not constitute a risk factor in clinical use.

2.3.4. Toxicology

A stand-alone toxicology program was conducted for MF59 adjuvant. These studies were performed with the various formulations of adjuvant alone and in combination with different antigens.

Toxicology studies were performed with AFLUNOV and with the formulations equivalent to Fluad (Agrippal + MF59). The toxicology profile for AFLUNOV could also be extrapolated from the studies conducted in mouse and ferret. The vaccine was well tolerated and effective in these species. These studies are described in pharmacological section of this AR.

Single dose toxicity

No formal single dose toxicity studies have been conducted with AFLUNOV. However single-dose studies were performed as Quality Control batch release tests suggesting absence of systemic toxicity both in rabbits and in mice, no pyrogenicity in rabbits and good local tolerability.

Other available information on single-dose toxicity and local tolerability of Agrippal administered with and without MF59W.1 (equivalent to MF59C.1 but without citrate buffer) are those obtained in the repeated-dose toxicity study (Experiment N° 940292) by considering the effects of the first dose only. The available information is considered adequate to show a lack of systemic toxicity and a moderate reaction at the injection site for Fluad (Agrippal + MF59) after single dose treatment.

Repeat dose toxicity (Study 940292)

In a pivotal repeat-dose study three groups of rabbits received 0.5 ml of Agrippal® alone, MF59W.1 adjuvant alone (equivalent to MF59C.1 but without citrate buffer) or Agrippal® formulated together with MF59W.1 (equivalent to Fluad) by intramuscular route on day 1 and day 15 of the study. Each 0.5 ml injection contained the full single human dose of trivalent vaccine (15 µg HA from each virus strain). In this study, conducted with a vaccine formulation comparable to Fluad®, no systemic toxicity was evident at a dose equal to six times the anticipated pandemic clinical dose.

The only adverse effects were limited transient local reactivity at the injection sites more evident after the second injection with adjuvanted vaccine, but overall it was well tolerated. The results showed absence of systemic adverse effects.

Genotoxicity

Genotoxicity studies were not performed, according to the relevant guidelines, CPMP/SWP/465/95 and CPMP/VEG/4717/03 and WHO guidelines.

MF59 contains a natural product with inherent contaminants (squalene). Therefore, MF59 adjuvant formulations (MF59 W.1 and MF 59C.1) were evaluated in a mutagenicity testing programme, using the mouse micronucleus cytogenetic assay and the bacterial reverse mutation assay (Ames Test). These studies were performed under GLP and showed that MF59 adjuvant formulations have no genotoxicity potential.

Carcinogenicity

Carcinogenicity studies were not performed, in accordance to the relevant vaccines guidelines, CPMP/SWP/465/95 and CPMP/VEG/4717/03 and WHO. The final product does not contain any known component or impurity at levels that would be expected to be of concern.

Reproduction Toxicity

The pivotal repeat-dose toxicity study 940292 in rabbits with Fluad (Agrippal + MF59W.1) showed no test-article related toxic effects on sex organs (ovaries, testes and epididymis) after two intramuscular injections. Moreover the embryofoetal and developmental toxicity of MF59 was evaluated in rats and

rabbits. These pivotal studies were of adequate quality and performed under GLP. They indicated that MF59 is devoid of teratogenic or fetotoxic effect.

The reproductive toxicity of AFLUNOV was evaluated in a GLP study in rabbits (Study n. UBA00021: Intramuscular reproductive and developmental toxicity of Fluad H5N1 in rabbits, including a postnatal evaluation). This study was planned according to the EMA guideline on pre-pandemic influenza vaccines (EMEA/CHMP/VWP/263499/2006). The object of this study was to assess the potential effects of AFLUNOV on reproductive and developmental toxicity in female rabbits and their foetuses or pups when administered by intramuscular injection twice the clinical dose for humans of 7.5 ug, before mating and during gestation. The study was well design to assess both objectives. AFLUNOV did not induce maternal/embryofoetal toxicity or teratogenic and post-natal development effects, resulting well tolerated. Additionally, the vaccine is immunogenic in maternal rabbits, in foetuses and in F1 pups for the first 4 weeks of life.

Local Tolerance

No local tolerance studies with AFLUNOV are required according to CPMP/VEG/4717/03. An evaluation of local tolerability was reported in the pivotal repeat-dose toxicity study with Fluad (Agrippal + MF59W.1) done in rabbits (Study N° 940292). This study demonstrated good local tolerability (a low order of local reactivity) for all formulations. This conclusion is further supported by clinical data in humans.

Other toxicity studies

In order to investigate the delayed contact hypersensitivity potential of Agrippal + MF59C.1 (Fluad) or MF59 alone, two GLP studies were conducted in Guinea pigs. These studies showed that Fluad (Agrippal + MF59C.1), MF59 and MF59C.1, apart from injection site reactions, did not cause sensitisation in Guinea pig.

2.3.5. Ecotoxicity/environmental risk assessment

AFLUNOV is an inactivated viral product and active substance is constituted by highly purified virus surface antigen proteins. The Drug Substance and the Drug Product do not contain nor consist of GMOs therefore there is no environmental risk for the product itself.

2.3.6. Discussion on non-clinical aspects

In general, non clinical data were well organized to assess immunogenicity, systemic toxicity and local tolerability both in single and in repeated administration of various formulations of adjuvanted and non adjuvanted vaccines. Moreover, the Applicant provided three studies on protective activity using the ferret model with adequate disease markers. The earliest study is a GLP ferret challenge study with A/NIBRG-14(H5N1) antigens adjuvanted with MF59 (AFLUNOV). This study showed that the formulation of vaccine containing either 7.5 µg or 15 µg of A/NIBRG-14 (H5N1) antigen per dose is both immunogenic and efficacious in reducing the viral load and viral shedding. Two most recent studies highlight the protective and cross-protective efficacy of AFLUNOV vaccine formulations in ferrets given a lethal challenge of highly pathogenic avian influenza (HPAI).

No data were produced regarding secondary pharmacology (safety pharmacology) with respect to potential effects on cardiovascular, respiratory and CNS systems. However there were sufficient clinical

tolerability data available in humans with Fluad and Agrippal to override any potential concern. Moreover two repeat-dose studies in dogs were performed during the early development of MF59C.1 to evaluate its toxicity (with antigens unrelated to this dossier). These studies did not show secondary or safety pharmacological effects.

The toxicology program was designed based on EMEA/CHMP/VWP/263499/2006, CPMP/VEG/4717/03 and appropriate global regulatory requirements for the non-clinical testing of vaccines and adjuvants. The toxicology aspects of AFLUNOV were derived from the collection of non-clinical and clinical data generated on selected vaccine licensed formulations equivalent to AFLUNOV (Agrippal and Fluad) and a "Reproductive toxicity study in rabbits" performed with AFLUNOV. This study demonstrated vaccine' immunogenicity in maternal rabbits and developing foetuses; antibodies persisted through the first 4 weeks of life in F1. Moreover a stand-alone toxicology program was conducted for MF59 adjuvant.

All these studies fulfil regulatory requirements for the non-clinical testing of vaccines and adjuvants, as well as the guidelines on the development of pandemic vaccines derived from licensed manufacturing process. Finally, the release testing of AFLUNOV will assure the safety of individual clinical lots, and selected vaccine lots that are equivalent to those tested in toxicology studies.

2.3.7. Conclusion on non-clinical aspects

Having considered that:

- the process to manufacture antigens for AFLUNOV is the same process as used for Agrippal® and Fluad® vaccines and the MF59 adjuvant is identical to that used in Fluad®;
- the release testing of AFLUNOV will assure the safety of individual clinical lots and selected vaccine lots that are equivalent to AFLUNOV were tested in toxicology studies;
- AFLUNOV will contain lower doses of antigen than were tested in toxicology studies with seasonal vaccines, and the standard amount (0.25 mL) of MF59C.1;

it could be concluded that the submitted data on various antigens, alone or in combination with MF59C.1, were sufficiently informative for the characterization of the pre-clinical toxicological profile of AFLUNOV. From the release testing program for AFLUNOV finished vaccine batches and the clinical experience with AFLUNOV, Fluad® and Agrippal®, the safety and tolerability profiles for AFLUNOV are predicted. Finally, considering that this submission is supported by the same Non-clinical documentation presented for Focetria®, that no major outstanding issues were raised for Non-clinical parts of the first AFLUNOV submission (Centralized Procedure N° EMEA/H/C/804/0000) and that additional information included with the present application further supports the efficacy and safety of the proposed vaccine, no other preclinical studies were requested.

2.4. Clinical aspects

2.4.1. Introduction

Two clinical studies (V7P37 and V7P37E1) have been conducted with H5N3 strain to investigate regimen, adjuvant and antigen amount. Based on the results of these studies, the subsequent studies have been conducted using Fluad formulated with H5N1 (see tabular overview below). Immunogenicity and safety data on the first and second (i.e., primary) AFLUNOV administrations have been analyzed and were presented in this dossier in addition to evaluation of a third (booster) vaccination

administered 6 months afterwards. The two H5N3 studies and all the H5N1 studies provide data for the 18-60 year olds. Two large AFLUNOV phase 2 and 3 studies (V87P1, V87P13) additionally provide data in adults above 60 years (see table 3).

GCP

The studies presented were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines, with approval from the Ethics Committee/Institutional Review Board.

The Clinical trials were performed in accordance with GCP as claimed by the applicant. The first MAA for AFLUNOV was withdrawn by the Applicant in June 2008, based on the results of an EMA inspection that showed non-GCP compliance for the pivotal study V87P4. The negative outcome of the GCP inspection in 2008 triggered another GCP inspection of the new pivotal study (V87P13) that replaced V87P4. According to the Integrated Inspection Report on V87P13 (released on the 9th of August 2010), the data presented in the clinical study report were accurately described and hence the data was deemed acceptable for evaluation.

The applicant has provided a statement to the effect that no clinical studies conducted outside the European Union were included in this application (with reference to the ethical standards of Directive 2001/20/EC).

Table 3: Tabular overview of clinical studies

Study Year (Fluad-H5N1 Lot number)	**Test Product(s); Dosage Regimen; Route of Administration**	**Subjects Exposed: Test Comparator**	**Individual Subject's Participation**	**Subjects**	**Study objectives**	**Study Design**
Studies with Fluad-H5N3						
V7P37 1999	Fluad-H5N3 2 x 7.5 µg, 2 x 15µg or 2 x 30µg, 3 weeks apart, IM	32 Fluad-H5N3 33 Non-adjuvanted[a]	6 weeks	Healthy 18-40 year olds	Dose response study to evaluate safety and immunogenicity of adj and non-adj H5N3 vaccine	Phase 1 Observer-Blind, Randomized
V7P37E1 2000	Fluad-H5N3 1 x 7.5 µg, 1 x 15 µg or 1 x 30µg, 3 weeks apart, IM	17 Fluad-H5N1 11 Non-adjuvanted [a]	3 weeks	Healthy 18-40 year olds	Extension of previous study with booster administration to test non-inferiority of the 7.5ug versus the 15ug dose and adj versus non-adj formulations	Phase 1, Observer-blind Extension
Studies with Fluad-H5N1						

Study Year (Fluad-H5N1 Lot number)	Test Product(s); Dosage Regimen; Route of Administration	Subjects Exposed: Test Comparator	Individual Subject's Participation	Subjects	Study objectives	Study Design
V87P1 2006/2007 (15µg:W52P06H1; 7.5µg:W52P07H1)	Fluad-H5N1 2 x 7.5µg or 2 x 15µg, 3 weeks apart, IM; 6 months booster	485 Fluad-H5N1 NA	approximately 13 months	Healthy 18–60 and >60 year olds	Immunogenicity, persistence of immune response and safety of 2 or 3 injections of 7.5-15ug HA AFLUNOV. Non-inferiority of 7.5 to 15 ug HA	Phase 2 Observer-Blind, Randomized
V87P2 2006/2008 (15µg:W51P85H1; 7.5µg:W52P07H1A)	Fluad-H5N1 2 x 7.5µg or 2 x 15µg, 3 weeks apart, IM; 6 months booster	27 Fluad-H5N1 13 Non-adjuvanted[b]	approximately 13 months	Healthy 18–60 year olds	Immunogenicity, CMI and safety of 2 injections and booster of 7.5-15ug HA H5N1 compared to non-adj vaccine	Phase 2 Observer-Blind, randomized
V87P3* 2007/2008 (W52P07H1B)	Fluad-H5N1 2 x 7.5µg 3 weeks apart, IM;	58 Fluad-H5N1 NA	approximately 7 months	Healthy 18–60 year olds	Immunogenicity and safety of 2 injections of AFLUNOV in adults unprimed and primed with MF59 adj or non-adj H5N3 vaccines.	Phase 1 Controlled Open-label
V87P12** 2008/2009 (070701A)	Fluad-H5N1 2 x 7.5µg 1-, 2-, 3-, 6- weeks apart, IM;	240 Fluad-H5N1 NA	approximately 7 months	Healthy 18–60 year olds	Immunogenicity and safety of 2 injections of 7.5ug AFLUNOV administered using 4 different vaccinations schedules (1-2-3-6 weeks apart)	Phase 3 Open-label Randomized
V87P13 2008/ongoing[c] (070701C;070701D; 070701A; 070701B)	Fluad-H5N1 2 x 7.5µg, 3 weeks apart, 3 weeks after one of seasonal Agrippal, IM	2828[d] Fluad-H5N1 712 Fluad[e]	approximately 7 months	Healthy 18–60 and >60 year olds	Immunogenicity and safety of 2 injections of 7.5ug HA H5N1 after one injection of Agrippal compared with 2 injections of seasonal Fluad after one of placebo	Phase 3 Observer-Blind, Controlled Randomized

Study Year (Fluad-H5N1 Lot number)	Test Product(s); Dosage Regimen; Route of Administration	Subjects Exposed: Test Comparator	Individual Subject's Participation	Subjects	Study objectives	Study Design
V101P1 2007/2008 (W52P16H1D)	Fluad-H5N1 2 x 7.5µg, the 1st concomitantly with seasonal Agrippal, IM	600 Fluad-H5N1[f] NA	approximately 7 months	Healthy 18–60 and >60[g] year olds	Immunogenicity and safety of concomitant use of 7.5ug HA H5N1 with seasonal influenza vaccine	Phase 2 Observer-blind, Placebo-Controlled, Randomized
V87P6 2007/2009 (060601A, 070701) (078402)	Fluad-H5N1 2 x 7.5µg, 3 weeks apart, IM; 12 months booster; MF59 seasonal 2 x 0.25/0.5mL, 3 weeks apart, IM	334 Fluad-H5N1; 137 MF59 seasonal	approximately 20 months	Healthy 6 months-<18 years of age	Immunogenicity, safety and tolerability of 2 doses of AFLUNOV 3 weeks apart in children 6m-17y of age.	Phase 2 Observer-blind, seasonal comparator-Controlled, Randomized

a: 7.5µg, 15µg, or 30µg of the same vaccine but without MF59; **b**: 15µg H5N1 without MF59; **c**: in the 6 month safety follow up period; **d**: two subjects randomized to receive Agrippal followed by two vaccinations of Fluad-H5N1 received wrong vaccination and were excluded from the Fluad-H5N1 pooled safety analysis since they did not receive Fluad-H5N1; **e**: subjects received placebo followed by two vaccinations with seasonal Fluad; **f**: 7.5µg Fluad-H5N1 was administered either i) before a tetravalent influenza vaccine (pre-formulated with interpandemic seasonal influenza strains + the pandemic H5N1 strain), ii) after the tetravalent influenza vaccine; or iii) after a concomitant administration, at different injection sites, of 7.5µg Fluad-H5N1 and seasonal Agrippal. Only results for the latter group are presented in this document, however the total number of subjects exposed to 7.5µg Fluad-H5N1 either as first or second vaccination is in the table; **g**: only 4 subjects aged >60 years were enrolled; **IM** = intramuscular. ***** heterologous booster study; ****** schedule finding study.

Moreover, additional pediatric studies are planned in accordance with the PIP.

2.4.2. Pharmacokinetics

According to the EMEA Note for Guidance on Clinical Evaluation of New Vaccines, pharmacokinetic studies are generally not required for injectable vaccines, and kinetic properties of vaccines do not provide information useful for establishing adequate dosing recommendations. The rationale for dose selection and dose schedule is discussed in sections 3.5.1 and 3.5.2 of this AR.

2.4.3. Pharmacodynamics

Day 21 post vaccination was selected as the time point for assessing the immune response (IgG) by hemagglutination inhibition (HI) and single radial hemolysis (SRH) assays, as routine for influenza vaccines. Twenty one days post vaccination is also the established time point to assess the immune response by antibody microneutralisation (MN).

For the criteria used for assessing immune response and more details on the assays mentioned above see Methods in section 3.5.3.

2.5. Clinical efficacy

A total of six studies have been conducted using Fluad formulated with H5N1 (AFLUNOV) and were assessed in the present application. Moreover two other clinical studies (V7P37 and V7P37E1) have been conducted with H5N3 strain (Fluad H5N3) to investigate regimen, adjuvant, and antigen amount.

2.5.1. Dose response studies with H5N3 strain (Fluad H5N3)

Study V7P37

Study V7P37 was an observer blind, randomised, comparative dose ranging study to evaluate safety/reactogenicity and immunogenicity of an adjuvanted influenza vaccine containing the mock-up strain H5N3 as compared to a non-adjuvanted split influenza vaccine containing H5N3.

Fifty-five healthy adults, between 18-40 years old were enrolled in the study. Subjects were randomised to receive adjuvanted H5N3 vaccine (7.5 µg, 15 µg or 30 µg HA per dose) or the same dose of a non-adjuvanted comparator vaccine according to a 2-dose schedule (day 0, day 21). Immunogenicity was assessed by microneutralisation assay (MN), haemagglutination inhibition assay (HI), and single radial haemolysis (SRH). Blood samples were drawn at day 0, 21, and 42. Antibody cross-reactivity was assessed against heterovariant H5N1 influenza strains.

Results: Before immunization all participants had serum HI titres of less than 1:10. As previously shown also for other H5N1 vaccines, HI assays read-outs are prone to extreme variances caused by a number of factors which are difficult to standardize and to control (especially as regards choice of erythrocytes). It is therefore accepted to only consider SRH results in combination with MN results. Overall, higher seroprotection and seroconversion rates as well as geometric mean titers (GMTs) clearly correlate with the use of MF59 adjuvant and were not dose-related. Interestingly, the lowest antigen dose (7.5 µg, HA antigen, adjuvanted) was more immunogenic compared to the higher antigen doses (15 µg and 30 µg). With the candidate vaccine (adjuvanted) seroconversion rates between 18-60% were achieved.

Study V7P37E1

In the extension study V7P37E1, 28 subjects who completed study V7P37 were revaccinated approximately 17 months after primary vaccination in order to evaluate the immunogenicity of an additional vaccine dose as previously formulated, with and without MF59C.1 adjuvant. Of the 28 subjects, 26 were included in the immunogenicity analyses; 15 of these individuals were previously vaccinated with the adjuvanted formulations, while the remaining 11 had received the comparator vaccine. Immunogenicity was assessed by MN, HI and SRH. Serum samples were collected before and 3 weeks after revaccination.

Results: At baseline none of the subjects had detectable antibodies as tested by HI and/or MN. By SRH, subjects immunised with any dose of adjuvanted candidate vaccine still had detectable antibodies against both A/H5N3 and A/H5N1. Non-adjuvanted vaccine recipients had detectable antibodies only in the group immunised with the 15 µg dose, and against A/H5N3 only. At re-vaccination with adjuvanted candidate vaccine H5N3, GMTs increased significantly when compared to non-adjuvanted vaccine. MF59C.1-adjuvanted H5N3 vaccine induced antibodies that cross-protected against not only the H5N1 strains from 1997-1998, but also against the more recent and virulent strains isolated in 2003 and 2004 in Vietnam and Hong Kong, which exhibit some antigenic drift as compared to the original strains.

In conclusion, this study was powered to test the non-inferiority of the 7.5 µg compared with the 15 µg dose. By all serological assays, the same or more CHMP criteria were met by the 7.5 µg than the 15 µg dose after the first and second vaccination, both for non-elderly adults and elderly subjects. These two doses were therefore selected for the pivotal study V87P1. Results from booster study V7P37E1 confirmed superiority of adjuvanted versus non-adjuvanted vaccine formulation. The effect of the booster immunization was only observable in groups given the various adjuvanted vaccine formulations, suggesting that non-adjuvanted vaccine was insufficiently or not at all able to prime. Like in the primary vaccination study V7P37, no dose dependency was seen in this booster study (the lowest antigen dose was the most effective).

2.5.2. Dose and Formulation Selection Studies with H5N1 strain (AFLUNOV)

Based on Fluad-H5N3 results, 7.5µg and 15µg doses were investigated in the initial studies with the H5N1 vaccine strain.

Study V87P1: Participant flow

	Number (%) of Subjects		
Vaccine group	**7.5 µg**	**15 µg**	**Total**
	Adults (18-60 yrs)		
Enrolled	157	156	313
Completed protocol	148 (94%)	150 (96%)	298 (95%)
Premature withdrawals	9 (6%)	6 (4%)	15 (5%)
AE or death	1 (< 1%)	0	1 (< 1%)
Withdrawal of consent	4 (3%)	5 (3%)	9 (3%)
Lost to follow-up	4 (3%)	0	4 (1%)
Protocol deviation/violation	0	1 (< 1%)	1 (< 1%)
	Elderly (> 60 yrs)		
Enrolled	87	86	173
Completed protocol [a]	84 (97%)	75 (87%)	159 (92%)
Premature withdrawals	3 (3%)	11 (13%)	14 (8%)
AE or death	0	1 (1%)	1 (< 1%)
Withdrawal of consent	1 (1%)	8 (9%)	9 (5%)
Lost to follow-up	2 (2%)	2 (2%)	4 (2%)
Protocol deviation/violation	0	0	0

Source: Table 14.1.1.2; According to protocol, some subjects did not take part in the booster part of the study, but are considered completers.

A large phase II study, conducted in 2006/2007, evaluated the immune response to a pre-pandemic Fluad formulation with H5N1. A total of 486 healthy subjects were stratified into non-elderly adult (18-60 years) and elderly (>60 years) age groups as recommended in EMA guidelines. Two vaccinations of either 7.5 µg or 15 µg both adjuvanted were given 3 weeks apart. The sample size was chosen to test for non-inferiority of 2 x 7.5 µg vaccinations to 2 x 15 µg vaccinations with regard to the immune response. A booster vaccination was given at day 202 (i.e., 6 months after the first vaccination) in approximately half of the subjects enrolled in each of the 7.5 µg and 15 µg groups and subjects were followed up for a further 6 months.

The lower limit of the 95% CI for HI GMT ratio of 7.5µg over 15µg group for the total population (adults below and above 60 years) was >0.5; all point estimates for the percentages of seroprotected and seroconverted subjects, in addition to the GMRs, by both HI and SRH, were higher after two vaccinations of the 7.5µg compared with two vaccinations of the 15µg formulations in adults below 60 years. In adults above 60 years the immune responses showed greater similarity between both formulations.

Results for the smaller sample size study in adults below 60 years (**V87P2**, see table 3) confirmed that point estimates by all three serological assays were higher for the 7.5µg than for the 15µg AFLUNOV group. Additionally, consistently with the results of the Fluad-H5N3 studies, immunogenicity results of the non-adjuvanted 15µg H5N1 vaccine used in this study confirmed the need of including the MF59 adjuvant in the vaccine formulation.

In conclusion, an analysis across studies supported the decision to choose the lowest antigen dose (7.5µg HA) for further investigation and licensure of the candidate vaccine, due to the fact that all CHMP requirements were met in the three dose finding studies V7P3, V87P1 and V87P2 and no advantage was detectable for the 15 µg HA neither for the 30 µg HA formulation.

2.5.3. Main studies

Study V87P13

A large phase III study has being conducted on 3372 and 275 adults below and above 60 years, respectively. Subjects received (randomization 4:1) either two vaccinations 3 weeks apart i) of 7.5µg AFLUNOV 3 weeks after a seasonal unadjuvanted trivalent influenza vaccine (Agrippal) or ii) of seasonal vaccine Fluad 3 weeks after a placebo injection. This study is pivotal for the assessment of AFLUNOV safety. An immunogenicity subset was also included in the study. The sample size of the immunogenicity subset was considered sufficient to provide adequate estimates for the endpoints specified in the CHMP criteria, and was not based on any statistical hypothesis. The study comprises a 6-month safety follow-up that was still ongoing when the original dossier was submitted. Final results of the 6-month safety study were submitted by D121 as addendum to CSRV87P13.

Study V87P12

An additional phase III study investigated immunogenicity and safety of two vaccinations with 7.5µg Fluad-H5N1 of the same lot used in V87P13, administered 1, 2, 3 or 6 weeks apart to non-elderly adult subjects (ages 18 to 60 years). A total of 240 subjects were randomized at a 1:1:1:1 ratio.

Study V101P1

The phase II study V101P1 investigated concomitant administration of a conventional seasonal vaccine or a pre-formulated tetravalent (3 seasonal strains + H5N1 strain) adjuvanted vaccine together with the pre-pandemic vaccine to assess the potential impact on the immune responses to either H5N1 or influenza seasonal strains (A/H1N1, A/H3N2, and B). Study V101P1 is further described in the supportive studies section.

Methods

Laboratory methods

As previously established, immune responses can be measured 3 weeks after administration of H5 vaccines (CPMP/BWP/214/96; Nicholson et al., 2001; Stephenson et al., 2003; Bresson et al., 2006). Hemagglutination inhibition (HI) and single radial haemolysis (SRH) assays were used to measure immune responses based on the interpandemic experience with seasonal influenza vaccines (CPMP/BWP/214/96). Given the limitations of the HI assay to measure the immunogenicity of H5 strains, a modified HI using horse instead of bird erythrocytes (Stephenson et al., 2004a) was used. Moreover in all studies presented in this application three serological assays (HI, SRH, and MN) were used in parallel to assess antibody responses to H5N1 viral antigens. The SRH assay, based on the method of Schild et al.1975 as recommended by CPMP/BWP/214/96, appeared to work equally well for

seasonal and H5N1 pandemic influenza strains. MN was performed according to previously established methods described by Nicholson (Nicholson et al., 2001).

In the absence of specific correlates of protection for pandemic influenza vaccines, as suggested by the pre-pandemic guideline (CHMP/VWP/263499/2006) all three CHMP criteria as assessed by HI and/or SRH assays should be met to satisfy the licensure requirements for a pre-pandemic vaccine.

In the absence of corresponding correlate of protection for MN against influenza A/H5N1 viruses, and based on available reports (Bresson et al., 2006; Lin et al., 2006; Treanor et al., 2006), ≥4-fold increase from baseline in neutralizing antibody was considered appropriate measure of immune response to H5N1.

Interlaboratory Consistency

For all H5N1 studies, except V87P3, HI and MN assays were performed at the Novartis Clinical Serology Laboratory, Marburg, Germany, and SRH at the University of Siena, Italy. In study V87P3 the HI and MN assays were performed at the HPA Laboratories, Colindale, London, UK. Correlation and concordance analyses, based on subsets of sera samples from study V87P1 tested at HPA, showed agreement for both HI and MN assay results produced by Novartis and HPA laboratories. Additionally, the correlation between the SRH assays performed at the University of Siena and the HI and MN assays performed by the other two laboratories was also high. Overall, based on the high correlation between inter- and intra-laboratory HI, MN and SRH assays, all serology assays appeared to be measuring the same immune response with an acceptable degree of consistency. This degree of consistency was not observed in study V87P13, where the HI data did not match the SRH results, however this could be linked to the different study design.

Inclusion/exclusion criteria

The inclusion and exclusion criteria for all studies with AFLUNOV V87P1, V87P2, V87P3, V87P12, V87P13 and V101P1 were in general identical. Subjects were not to be enrolled into the study if:

1. They had any serious disease such as: cancer, autoimmune disease, advanced arteriosclerotic disease or complicated diabetes mellitus, chronic obstructive pulmonary disease, acute or progressive hepatic disease, acute or progressive renal disease, congestive heart failure.
2. They were hypersensitive to eggs, chicken protein, chicken feathers, influenza viral protein, neomycin or polymyxin, or any other component of the vaccine.
3. They had a history of neurological symptoms or signs, or anaphylactic shock following administration of any vaccine.
4. They had a known or suspected impairment/alteration of immune function resulting, for example, from: immunosuppressive therapy, immunostimulants, HIV infection or HIV-related disease, etc.
5. Women who were pregnant, or women able to bear children but not willing to practice acceptable contraception for the duration of the trial.
6. Within the past 4 weeks they had received: another vaccine, any investigational agent.
7. Within the past 3-7 days, they had experienced: any acute disease, infections requiring systemic antibiotic or antiviral therapy, fever.
8. They were taking part in another clinical study, had surgery planned during the study or had other conditions which might interfere with the study.

Study Participants

All study participants were aged 18 years and older (see Table 3). Within each study, demographic characteristics were stratified by age to include both adults below and above 60 years and were generally balanced among groups within each age. Overall a high number of study participants were included in all studies to evaluate the safety and immunogenicity of the prepandemic vaccine. With regard to demography and other baseline characteristics for both non-elderly (18-60 years) and elderly (>60 years) adults of the enrolled population, the study criteria were fulfilled completely. However the number of elderly >70 years of age is limited to only 69 individuals.

Treatments

Refer to the Quality section for the composition of AFLUNOV (previously named Fluad-H5N1) influenza vaccine containing 7.5 µg A/Vietnam/1194/2004-like (H5N1 Clade 1) HA antigen. The formulations prepared with 15ug or more of the same HA contained identical amounts of excipients and adjuvants.

Treatments are described in table 4. In Study V87P12 AFLUNOV was administered 1, 2, 3, or 6 weeks apart; in study V87P13 treatments were as follows:

Study Day	Adults		Elderly	
	NonAdjSeasonal/ AdjPanH5N1	Placebo/ AdjSeasonal	NonAdjSeasonal/ AdjPanH5N1	Placebo/ AdjSeasonal
day 1	NonAdjSeasonal	Placebo	NonAdjSeasonal	Placebo
day 22 (-2/+14)	AdjPanH5N1	AdjSeasonal	AdjPanH5N1	AdjSeasonal
day 43 (-2/+14)	AdjPanH5N1	AdjSeasonal	AdjPanH5N1	AdjSeasonal

AdjPanH5N1: Adjuvanted pandemic H5N1 (Fluad-H5N1); AdjSeasonal: Adjuvanted seasonal (Fluad) ; NonAdj Seasonal: Non Adjuvanted seasonal (Agrippal)

- Investigational Vaccine

AdjPanH5N1: AFLUNOV (see above for composition)

- Reference Vaccines

AdjSeasonal: A 0.5mL dose of AdjSeasonal, trivalent inactivated MF59-adjuvanted subunit influenza vaccine, contained the purified viral envelope-glycoproteins neuraminidase (NA) and hemagglutinin (HA), including 15 µg of HA of the A/H1N1, A/H3N2 and B antigens recommended for the influenza season 2008/2009 in the Northern Hemisphere.

NonAdjSeasonal: A 0.5mL dose of NonAdjSeasonal, non-adjuvanted trivalent inactivated subunit influenza vaccine, contained the purified viral envelope-glycoproteins NA and HA, including 15 µg of HA of the A/H1N1, A/H3N2 and B antigens recommended for the influenza season 2008/2009 in the Northern Hemisphere.

Objectives

All studies conducted with H5N1 influenza strain requested different objectives to describe the immunogenicity and cross-reactivity as well as CMI of the candidate vaccine as described in table 3. Specifically:

Study V87P12

- To evaluate the magnitude of antibody responses to two doses of MF59-adjuvanted egg-derived pandemic monovalent A/H5N1(MF59-PanH5N1) influenza vaccine, each containing 7.5µg of A/H5N1 antigen administered 1, 2, 3, or 6 weeks apart.

Study V87P13

- To evaluate the immunogenicity of two doses of adjuvanted pandemic H5N1 (AdjPanH5N1), each containing 7.5 µg of H5N1 antigen, versus the homologous A/Vietnam/1194/2004 strain.
- To evaluate the immunogenicity of two doses of adjuvanted pandemic H5N1 (AdjPanH5N1), each containing 7.5 µg of H5N1 antigen, versus the heterologous A/turkey/Turkey/1/2005 strain.

Outcomes and endpoints

The primary measures of immunogenicity were GMT (as determined by HI assay and MN assay), GMA (by SRH assay), GMR, Percentages of Subjects With Seroconversion or Significant Increase in HI Titer and SRH Area, Percentages of Subjects Achieving the thresholds of HI ≥ 40, SRH area ≥25 mm2, MN ≥20, MN ≥40, MN ≥80, four-fold rise in MN titers. HI, SRH and MN data provide a surrogate marker for efficacy in this application, although, as for all pandemic influenza vaccines, no direct correlation has been demonstrated between the immune response and clinical efficacy. For further information on the assays see section 3.5.3 Main Studies/Laboratory methods.

Study V87P12

Immunogenicity was evaluated antibody titres by HI, SRH and MN assays on blood samples taken from all subjects:

- before first vaccination (day 1)
- on the day before the second vaccination on day 7, 15, 22 or 43
- three weeks after the second vaccination.

Study V87P13

The measures of immunogenicity versus both homologous and heterologous A/H5N1strains, collected for all evaluable subjects in study V87P13 included:

- Geometric mean titers/area (GMTs/GMAs) on days 22, 43 and 64, as determined by HI, SRH, and MN.
- Day 43/ day 22 and day 64/day 22 geometric mean ratios (GMRs).
- Percentage of subjects achieving seroconversion or significant increase in antibody titer on days 43 and 64, as measured by HI and SRH.
- Percentage of subjects achieving an HI titer ≥40/ SRH area ≥25mm² on days 22, 43 and 64.
- Percentage of subjects with MN titers ≥20, ≥40, ≥80 on days 22, 43 and 64.
- Percentage of subjects achieving at least a four-fold rise in MN antibody titer on days 43 and 64 compared to day 22.

Sample size

Study V87P12

The sample size in study V87P12 was chosen in order to comply with the CHMP requirements outlined in document CPMP/BWP/214/96 (50 subjects for immunogenicity per group). 240 subjects were

enrolled in four groups. All enrolled subjects were included in the Full Analysis Set (FAS) for immunogenicity and in the Safety set.

Study V87P13

Sample size in study V87P13 was chosen in order to achieve an overall safety database that allowed for detecting adverse events with an underlying incidence of 0.1% in adults or 1% in elderly subjects with a probability of 95%. Subjects were enrolled into two age stratifications: 3372 adults (18 to 60 years) and 275 elderly (>60 Years). Total 3371 subjects were vaccinated according to the randomization list. For the immunogenicity analyses, 258 subjects were enrolled in adults age group (18 to 60 years) and 272 subjects were enrolled in the elderly age group (>60 years). Total 95% adults and 93-95% elderly subjects were included in Full Analysis Sets (FAS) at second, third and fourth visit.

Randomisation

When applicable, randomisation was stratified according age group (18-60, >60 years). Within each stratum, the randomisation ratio was 4:1 (Fluad-H5N1: control) in study V87P13 and 1:1:1:1 into each of the 4 vaccine schedule groups in study V87P12.

Blinding

Study V87P13 was performed as observer blind study, while study V87P12 was performed as an open label study (comparing different vaccine schedules).

Statistical methods

Only descriptive analyses were performed. Safety data were summarised by providing frequencies and percentages of subjects experiencing each adverse reaction. The CHMP considered this approach appropriate.

Results

Study V87P13

Participant Flow

	Number (%) of Subjects			
	Adults		Elderly	
Vaccine Group	NonAdjSeasonal+ AdjPanH5N1	Placebo+ AdjSeasonal	NonAdjSeasonal+ AdjPanH5N1	Placebo+ AdjSeasonal
Enrolled	2691	681	219	56
Completed study (day 64)	2592 (96%)	653 (96%)	213 (97%)	54 (96%)
Premature withdrawals	99 (4%)	28 (4%)	6 (3%)	2 (4%)
Death	0	0	0	0
Adverse Event	7 (<1%)	8 (1%)	2 (<1%)	1 (2%)
Withdrew consent	47 (2%)	11 (2%)	3 (1%)	1 (2%)
Lost to follow-up	27 (1%)	7 (1%)	0	0
Protocol Deviation/violation	16 (<1%)	2 (<1%)	1 (<1%)	0
Unable to Classify	2 (<1%)	0	0	0

Recruitment

This was a multicentre study (Germany, Finland) conducted in 2008/09.

Conduct of the study

Immunogenicity was evaluated by HI assay, and additionally by MN and SRH, before and after each AdjPanH5N1 or AdjSeasonal vaccines injection at day 22, day 43 and day 64.

Baseline data

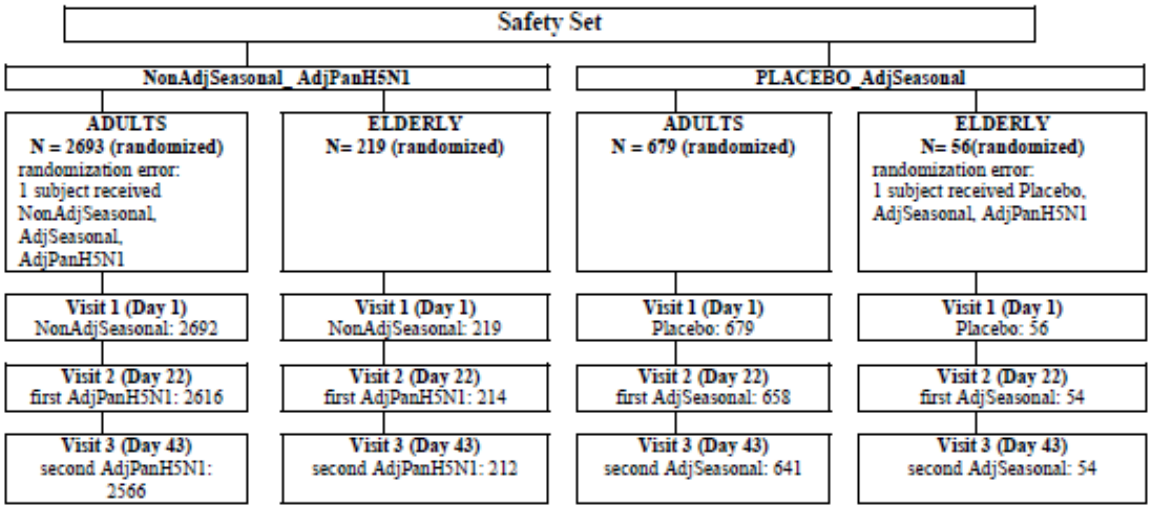
Demographic and baseline characters of the subjects enrolled into each of the vaccine groups were balanced in both the age groups of the safety set and immunogenicity set. Most of the subjects enrolled were of Caucasian origin in both the vaccine groups and age groups and almost all the subjects met the entry criteria. The CHMP considered that demography and baseline characteristics of the 504 subjects of the immunogenicity subset were balanced across different vaccination groups and within both the non-elderly adult and elderly age stratifications.

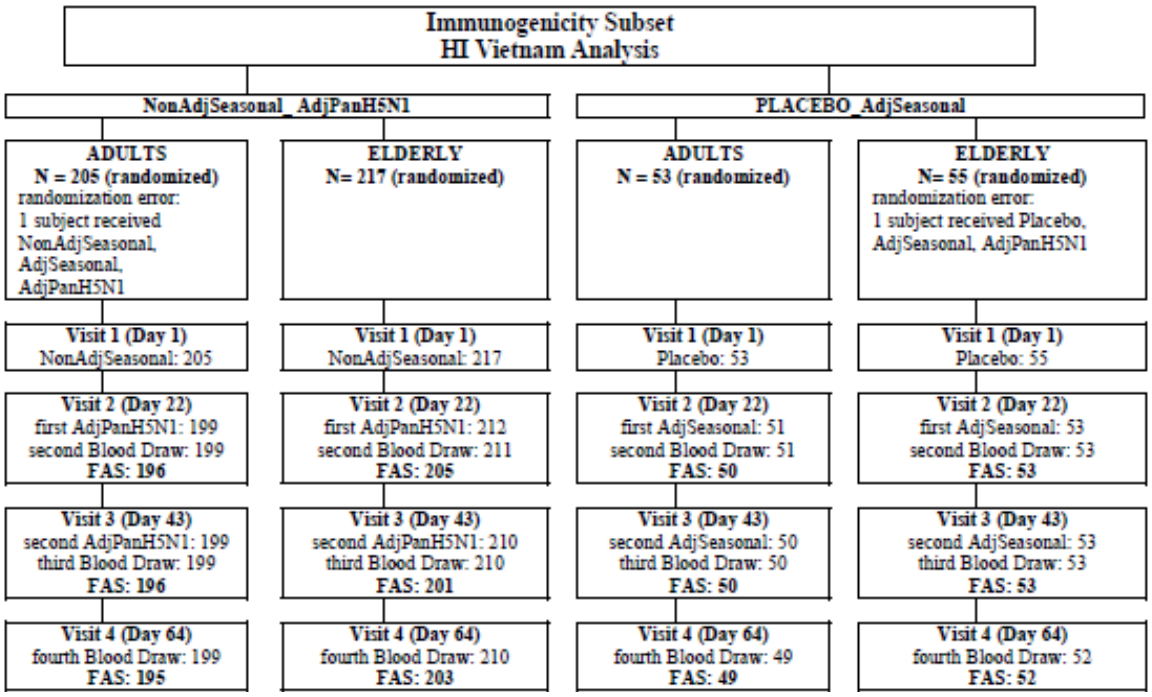
In studies conducted with H5N1 only a few subjects were seropositive by MN at baseline (MN titres>40 2% adults, 1% elderly in V87P13). In elderly subjects in study V87P1, a range of 11% to 24% (depending on dosage and assay) showed seroprotection to H5N1 at baseline, while for V87P13 this range was as described in the Outcomes and estimation paragraph. However it should be considered that baseline positive titres might reflect cross-reactivity due to previous exposure to the neuraminidase included in the H5N1 vaccine.

The Applicant submitted data taking as baseline serostatus values measured at day 22 only for the V87P13 study. Day 22 was indeed the visit at which subjects received the first H5N1 vaccine; hence by definition this was the H5N1 baseline. Day 1 value as baseline for the V87P13 trial is 3 weeks prior to the H5N1 vaccine. For all other trials the Applicant provided serostatus at Day 1 (before vaccination studied).

Numbers analysed

The following figure shows the numbers analysed within the study V87P13. Upon assessment drop-out rate and randomization error were deemed very low within study V87P13.





Outcomes and estimation

Immunogenicity against A/Vietnam/1194/2004 vaccine strain:

When assessed by MN assay, 65% of non-elderly adults and 55% of elderly adults exhibited at least a 4-fold increase from baseline in MN titres after two doses of AFLUNOV compared to 0% (non-elderly) and 10% (elderly) in the placebo group.

	Adults	Elderly
	N=197	N=208
4-Fold Increase Day 43 to Day 22	28 (14%) (10-20)	37 (18%) (13-24) N=207
4-Fold Increase Day 64 to Day 22	129 (65%) (58-72)	115 (55%) (48-62)

MN assay: Subjects with MN Titers ≥20/≥40/≥80 against A/Vietnam/1194/2004 Strain, by Age Group – FAS

	Adults	Elderly
	N=197	N=209
MN titer≥20		
Day 22	5 (3%) (1-6)	4 (2%) (1-5)
Day 43	53 (27%) (21-34)	51 (25%) (19-31) N=207
Day 64	156 (79%) (73-85)	149 (72%) (65-78) N=208
MN titer≥40		
Day 22	3 (2%) (0-4)	2 (1%) (0-3)
Day 43	32 (16%) (11-22)	39 (19%) (14-25) N=207
Day 64	132 (67%) (60-74)	118 (57%) (50-64) N=208
MN titer≥80		
Day 22	2 (1%) (0-4)	0 (0%) (0-2)
Day 43	18 (9%) (6-14)	26 (13%) (8-18) N=207
Day 64	98 (50%) (43-57)	68 (33%) (26-40) N=208

When assessed by HI and in contrast to a previous study V87P1, results by baseline serostatus showed that only 2/3 of the CHMP criteria were met after the second vaccination (Day 64) in both adult and elderly groups (see also the next paragraph on analysis across trials). When assessed by SRH, all criteria (3/3) were met in both age groups but a high proportion of seropositive subjects at baseline was observed. Therefore, since by SRH assay the level of baseline seropositivity was high, the Applicant was requested to provide the baseline serostatus data by SRH:

Percentages of subjects with SRH areas ≥25mm^2, by baseline serostatus ≥4mm^2 and <4mm^2, by age group - FAS

	18 ≤ AGE ≤ 60				AGE > 60 (AGE ≥ 61)			
	< 4		≥ 4		< 4		≥ 4	
	AGR_H5N1	PL_FLUAD	AGR_H5N1	PL_FLUAD	AGR_H5N1	PL_FLUAD	AGR_H5N1	PL_FLUAD
	N=69	N=19	N=128	N=31	N=66	N=18	N=144	N=35
Day 22	0 (0%) (0-5)	0 (0%) (0-18)	38 (30%) (22-38)	1 (3%) (0.082-17)	0 (0%) (0-5)	0 (0%) (0-19)	53 (37%) (29-45)	3 (9%) (2-23)
Day 43	24 (35%) (24-47)	4 (21%) (6-46)	71 (55%) (46-64)	15 (48%) (30-67)	17 (26%) (16-38)	3 (17%) (4-41)	78 (55%) (46-63) N=142	19 (54%) (37-71)
Day 64	60 (87%) (77-94)	9 (47%) (24-71)	120 (94%) (88-97)	17 (57%) (37-75) N=30	54 (82%) (70-90)	6 (35%) (14-62) N=17	117 (82%) (75-88) N=143	24 (69%) (51-83)

Source: Table 2.1

Percentages of subjects with seroconversion or significant increase in SRH areas, by baseline serostatus ≥4mm^2 and <4mm^2, by age group – FAS

	18 ≤ AGE ≤ 60				AGE > 60 (AGE ≥ 61)			
	< 4		≥ 4		< 4		≥ 4	
	AGR_H5N1 N=69	PL_FLUAD N=19	AGR_H5N1 N=128	PL_FLUAD N=31	AGR_H5N1 N=66	PL_FLUAD N=18	AGR_H5N1 N=143	PL_FLUAD N=35
Seroconversion Day 43 to Day 22	24 (35%) (24-47)	4 (21%) (6-46)			17 (26%) (16-38)	3 (17%) (4-41)		
Significant Increase Day 43 to Day 22			48 (38%) (29-46)	14 (45%) (27-64)			30 (21%) (15-29) N=142	16 (46%) (29-63)
Seroconversion or Significant Increase Day 43 to Day 22	24 (35%) (24-47)	4 (21%) (6-46)	48 (38%) (29-46)	14 (45%) (27-64)	17 (26%) (16-38)	3 (17%) (4-41)	30 (21%) (15-29) N=142	16 (46%) (29-63)
Seroconversion Day 64 to Day 22	60 (87%) (77-94)	9 (47%) (24-71)			54 (82%) (70-90)	6 (35%) (14-62) N=17		
Significant Increase Day 64 to Day 22			94 (73%) (65-81)	19 (63%) (44-80) N=30			77 (54%) (45-62)	19 (54%) (37-71)
Seroconversion or Significant Increase Day 64 to Day 22	60 (87%) (77-94)	9 (47%) (24-71)	94 (73%) (65-81)	19 (63%) (44-80) N=30	54 (82%) (70-90)	6 (35%) (14-62) N=17	77 (54%) (45-62)	19 (54%) (37-71)

These results from SRH assay showed that all 3 CHMP criteria were met in subjects seronegative at baseline as well. It was noted that in study V87P13 all subjects in the AFLUNOV arm received first a dose of seasonal non-adjuvanted vaccine and then at day 22 received the first AFLUNOV administration. In the protocol, a blood drawing at day 0 was not included; therefore it was not possible to ascertain whether the high proportion of seropositive subjects at baseline by SRH in this study was due to the previous seasonal vaccination or to pre-existing immunity. Similar data was obtained with MN assay by baseline serostatus, supporting the immunogenicity of Aflunov.

Immunogenicity against A/turkey/Turkey/1/2005 Heterologous strain:

In the analysis of cross-reactivity potential of H5 specific antibodies following two doses of AFLUNOV (A/H5N1/Vietnam/1194/04, NIBRG-14; clade 1), some levels of immune response against heterologous influenza virus variant A/H5N1/turkey/Turkey/05 (NIBRG23) clade 2.2 was detectable, with seroprotection rates ranging at day 64 from 23% to 59% in adults and from 25% to 48% in elderly by HI and SRH respectively. Only 1/3 CHMP criterion was matched after two vaccinations both in adults and elderly (seconversion rates measured by SRH). At 3 weeks after second vaccination (day 64) 36% of adults and 25% of elderly achieved 4-fold increase in MN titres.

Ancillary analyses

Concomitant administration of AFLUNOV with seasonal influenza vaccine (Study V101P1)

This study was conducted to evaluate the effect of three different immunization schedules on the immune response to the H5N1 A/Vietnam/1194/2004 strain (Clade 1) and specifically aimed to investigate the concomitant administration of AFLUNOV with seasonal influenza vaccine. This randomized, placebo-controlled, observer-blind, multi centre, phase II study was performed over a period of approximately 7 months for each volunteer at two sites in Germany in a population of subjects 18 years of age and above. A total of 601 subjects were enrolled and randomized: 199 received a pre-formulated tetravalent influenza vaccine (3 seasonal influenza strains + Fluad-H5N1 + MF59- adjuvant) and concomitantly a placebo on day 1, then 3 weeks later a dose of Fluad- H5N1; 203 which received a dose of Fluad-H5N1 and concomitantly a placebo on day 1, then 3 weeks later a dose of the tetravalent vaccine; and 199 which received one dose of Fluad-H5N1 and concomitantly one dose of a seasonal trivalent vaccine for the 2007/08 influenza season (Agrippal) on day 1, then 3 weeks later received a second dose of Fluad-H5N1.

The results from this study showed that immune response to the H5N1 strain was not affected when AFLUNOV and a conventional seasonal influenza vaccine were administered concomitantly (for the

H5N1 strain all 3 CHMP criteria were met after two injections of AFLUNOV by both HI and SRH assays). Similarly, immune responses to the seasonal strains, as assessed by HI assay, were not affected (all 3 CHMP criteria were met for all three seasonal strains 3 weeks after receiving one dose of seasonal vaccine). Taken together these results indicated that immune responses met CHMP requirements for both pre-pandemic and seasonal influenza vaccines when the two vaccines were administered concomitantly.

Analysis performed across trials (pooled analyses and meta-analysis)

Immunogenicity Results against homologous H5N1 strain

Immunogenicity results for the A/H5N1 Vietnam/1194/04 strain by all three assays for the five studies used to support AFLUNOV immunogenicity in this application are presented in Table 4.

Table 4: Immune Responses to the A/H5N1/Vietnam/1194/04 strain after Two Vaccinations of 7.5µg Fluad-H5N1, by HI, SRH and MN Assays

		Non-elderly Adults (18-60 years)					Elderly (>60 years)	
		V87P1	**V87P2**	**V87P3**	**V87P12**	**V87P13**	**V87P1**	**V87P13**
HI Assay	No. of subj	N=151	N=14	N=29	N=60	N=196	N=81	N=205
	% SP (95% CI)	**73 (65-80)**	**86 (57-98)**	52 (33-71)	**74 (61-85)**	61 (53-67)	**75 (64-84)**	57 (50-64)
	GMR (95% CI)	**16 (12-21)**	**21 (8.41-52)**	**6.62 (3.76-12)**	**22 (13-37)**	**7.1 (5.52-9.14)**	**9.52 (6.55-14)**	**5.15 (4.15-6.4)**
	% SC (95% CI)	**73 (65-80)**	**79 (49-95)**	**52 (33-71)**	**74 (61-85)**	**56 (49-63)**	**67 (55-77)**	**50 (43-57)**
SRH Assay	No. of subj	N=149	N=14	N=29	N=58	N=197	N=84	N=210
	% SP (95% CI)	**85 (79-91)**	**86 (57-98)**	**93 (77-99)**	**88 (77-95)**	**91 (87-95)**	**80 (70-88)**	**82 (76-87)**
	GMR (95% CI)	**7.74 (6.6-9.07)**	**8.12 (4.61-14)**	**9.69 (7.54-12)**	**3.82 (3-4.88)**	**4.03 (3.54-4.59)**	**4.96 (3.87-6.37)**	**2.9 (2.53-3.31)**
	% SC (95% CI)	**85 (78-90)**	**86 (57-98)**	**90 (73-98)**	**71 (57-82)**	**78 (72-84)**	**70 (59-80)**	**63 (56-69)**
MN Assay	No. of subj	N=151	N=14	N=29	N=60	N=197	N=84	N=209
	% MN ≥40 (95% CI)	85 (78-90)	93 (66-100)	55 (36-74)	73 (60-84)	67 (60-74)	79 (68-87)	57 (50-64)
	GMR (95% CI)	11 (8.87-13)	8.14 (3.78-18)	3.32 (2.15-5.14)	7.49 (5.62-9.98)	6.21 (5.29-7.29)	4.54 (3.44-6.01)	4.42 (3.79-5.15)
	% ≥4-fold increase (95% CI)	83 (77-89)	86 (57-98)	55 (36-74)	73 (60-84)	65 (58-72)	58 (47-69)	55 (48-62)

V87P3: unprimed arm; V87P12: 3 week apart schedule arm; V87P13 previously vaccinated with Agrippal. SP = seroprotection; SC = seroconversion or significant increase; GMR = geometric mean ratio day 43/day 1 in V87P1, V87P2, and V87P3, and day 64/day 22 in V87P13. Note: **Bold** values when the respective CHMP criterion for HI and SRH assays is met. Results for study V87P1 are for the PP population; for studies V87P2, V87P3, V87P12, and V87P13 are for the FAS (for PP and FAS definition)

After two 7.5µg vaccinations with AFLUNOV all three CHMP criteria were met by SRH in all studies and in both age groups. As measured by HI assay, CHMP criteria were met in all studies except for adults below 60 years in studies V87P3 and V87P13 and for adults over 60 years in study V87P13 (for whom the seroprotection criterion was not met). The percentage of subjects achieving MN titers ≥ 40 and 4-fold increase of titers from baseline were generally high. As observed for HI and SRH results, MN responses in the 7.5µg and 15µg formulations were overall similar. For elderly subjects in V87P1 and V87P2 and for both groups in V87P13 the results are lower.

Antibody Persistence and Immune Response to a Third (Booster) Vaccination

Data on antibody persistence 6 months after primary vaccination for AFLUNOV are provided by studies V87P1, V87P2, and V87P3, and for further 6 months after a 3rd dose in study V87P1 and V87P2. Across studies, for both adults below and above 60 years, at 6 months (day 202) after primary vaccination HI, SRH, and MN titers reduced from 1/2 to 1/5th of their post vaccination level, still remaining above baseline. Following booster vaccination (studies V87P1 and V87P2) by all three assays in both age groups titers increased to similar or higher values than after primary vaccination. All 3 CHMP criteria were met by HI and SRH. At 6 months after the third vaccination, by all assays and in both age groups, GMT decreased but remained at least at the same level as 6 months after the primary vaccination and 2- to 6- fold higher than baseline. As seen for the primary vaccination, antibody persistence and booster dose responses for 7.5µg and 15µg formulations of AFLUNOV were generally similar.

Taken altogether these results showed that although antibody titres decreased over 6 months, they quickly increased to high levels after a booster shot, suggesting there was immunological memory. Therefore two vaccinations with 7.5 µg AFLUNOV primed the population and induced immunological memory, so that the third vaccination with the same vaccine could be considered a homologous booster.

Cross-reactivity against Heterovariant H5N1 Strains

Immune responses to the heterologous strain A/H5N1/turkey/Turkey/1/05 (NIBRG23; clade 2.2) were tested in four studies as described in table 5.

Table 5: Immune Responses to the Heterologous A/H5N1/turkey/Turkey/05 Strain after Two Vaccinations of 7.5 µg AFLUNOV by HI, SRH and MN Assays

		Non-elderly Adults (18-60 years)				**Elderly (>60 years)**	
		V87P1[b]	**V87P3[c] (Unprimed)**	**V87P12**	**V87P13**	**V87P1[b]**	**V87P13**
HI	No. of subj	N=70	N=29	N=60	N=197	N=36	N=207
	GMT (day 43) (95% CI)	18 (13-24) *N=69*	7.93 (5.13-12)	11 (8.29-16)	12 (9.77-14)	14 (8.4-22)	12 (10-14)
	GMR (95% CI)	ND[a]	1.98 (1.22-3.21)	2.3 (1.67-3.16)	1.92 (1.64-2.25)	ND[a]	1.79 (1.56-2.06)
	% SP (95% CI)	36 (25-49) *N=69*	21 (8-40)	28 (17-41)	23 (18-30)	36 (21-54)	25 (19-32)
	% SC (95% CI)	ND[a]	21 (8-40)	28 (17-41)	19 (14-25)	ND[a]	19 (14-25)
SRH	No. of subj	N=70	N=29	N=60	N=197	N=37	N=209
	GMA(day 43) (95% CI)	23 (19-28)	34 (28-40)		23 (21-26)	15 (9.91-22)	20 (18-23)
	GMR (95% CI)	ND[a]	**7.67 (6.09-9.67)**	**4.51 (3.63-5.61)**	2.37 (2.1-2.67)	ND[a]	1.74 (1.57-1.94)
	% SP (95% CI)	**71 (58-80)**	**90 (73-98)**	65 (52-77)	59 (52-66)	57 (39-73)	48 (41-55)
	% SC (95% CI)	ND[a]	**86 (68-96)**	**65 (52-77)**	**49 (42-56)**	ND[a]	**32 (26-39)**
MN	No. of subj	N=70	N=29	N=60	N=197	N=37	N=208
	GMT (day 43) (95% CI)	19 (15-24)	16 (11-23)	29 (23-38)	30 (26-35)	12 (9.4-16)	23 (20-26)
	GMR (95% CI)	ND[a]	1.59 (1.12-2.26)	2.95 (2.3-3.77)	2.77 (2.4-3.2)	ND[a]	2.01 (1.78-2.26)
	% MN ≥40	27	10	31	39	11	30

	(95% CI)	(17-39)	(2-27)	(19-44)	(32-46)	(3-25)	(24-37)
	% ≥4-fold increase (95% CI)	ND[a]	10 (2-27)	31 (19-44)	36 (29-43)	ND[a]	25 (19-31)

[a]NIBRG23; Clade 2.2; [b]Baseline not tested; [c]Unprimed subjects. GMT = geometric mean titer; SP = seroprotection; SC = seroconversion or significant increase; GMR = geometric mean ratio day 43/day 1 in V87P1, V87P3, and V87P12, and day 64/day 22 in V87P13; vac = vaccination; ND = not determined; Note: **Bold** values when the respective CHMP criterion for HI and SRH assays is met. Results for study V87P1 are for the PP population; for studies V87P2, V87P3, V87P12, and V87P13 are for the FAS

After two vaccinations with AFLUNOV some levels of immune response to the heterologous strain was detectable: at least one CHMP criterion was met by SRH assay whereas no criterion was fulfilled by HI and MN assays.

In study V87P1 the immune response to a heterologous strain following administration of a booster vaccination with the same pre-pandemic vaccine, i.e. a third vaccination with AFLUNOV 6 month after the two primary vaccinations, was assessed in 70 adults and 36 elderly. After the third vaccination, the immune response (GMT and seroprotection) to the heterologous strain increased by all assays: seroprotection rates were 70% by HI, 83% by SRH and 73% of subjects had an MN titer ≥1:40.

In study V87P3, in addition to the naïve subjects (unprimed arm), subjects who were primed 6-8 years previously with two vaccinations either with MF59-adjuvanted or non-adjuvanted H5N3 vaccines received two vaccinations of 7.5µg AFLUNOV. Data at 3 weeks after the first AFLUNOV vaccination showed that subjects previously primed with two doses of a different pandemic vaccine strain (H5N3) developed a very effective anamnestic immune response to a variant H5 strain (A/Vietnam/1194/04) used for vaccination 6-8 years later.

Cell mediated immunity (CMI)

CMI was investigated in studies V87P2 and V87P3. One injection of AFLUNOV was sufficient to induce an increase in the frequency of H5-specific CD4 T-cells with a memory TH0/TH1 phenotype, with high survival potential in vivo and the ability to expand and differentiate into effector cells upon infection. Two vaccinations were needed to expand long lasting H5N1-specific memory B-cells that further expanded upon boosting either with a vaccine formulated with the same pandemic strain or with a novel pandemic antigen.

Subjects with underling conditions associated with influenza complications.

None of the studies in the Fluad-H5N1 group development program were designed to evaluate specific high-risk groups. The Applicant was requested to perform an analysis of immunogenicity and safety results across studies for subjects exposed to AFLUNOV who reported in their medical history at enrolment medically relevant co-morbidities. This analysis was conducted on the pooled database for studies V87P1, V87P2, V87P3, V87P13, in order to achieve a bigger sample size. Individual at risk for influenza complications were defined as those who reported, in the medical history, the following diseases:

- Mild to moderate Congestive Heart Failure. (Class I-III)
- Mild to moderate Chronic Obstructive Pulmonary Disease (Stage 1-2)
- Mild to moderate Asthma (Step 1-3)
- Hepatic disease (Grade A)
- Renal insufficiency (Stage 1-3)
- Neurological/neuromuscular, or metabolic disorders including diabetes mellitus

- Obesity

This analysis showed that, within each study and age group, CHMP criteria were less frequently met for the subset of subjects with co-morbidities than for the whole study population, which could be due to the small number of subjects with co-morbidities. Seroconversion rates and GMRs were mostly similar to the whole population, suggesting that the immune response to H5N1 in these subjects is similar to that elicited in healthy individuals.

Immunogenicity and safety data in the elderly.

In order to ascertain the availability of sufficient efficacy and safety data in the elderly target population as claimed by the Applicant, they were requested to perform a stratified analysis of immunogenicity and safety results from studies enrolling elderly. In the pivotal safety study V87P13, 205 and 53 elderly aged >60 years were enrolled in the AFLUNOV and in the comparator groups respectively. All subjects enrolled were aged 61-65 years and no subject in either of the two groups was older than 70 years. In study V87P1 the number of subjects aged >60 years is 159, 69 of which were aged > 70 years. Despite the relatively small sample sizes, after two vaccinations with 7.5µg AFLUNOV, all three CHMP criteria for the A/H5N1/Vietnam/1194/04 strain were met in elderly regardless of the age strata by both HI and SRH assays. With the exception of the seroprotection criterion for the >80 years which was not met by either of the assays, likely due to the really small number of subjects (N=5), all criteria were met also with the 15µg formulation of the vaccine.

Clinical studies in special populations

No studies were performed in special populations. The following study V87P6, conducted in children from 6 months to 17 years of age, was not part of the core dossier, as it does not fall under the scope of the requested indication (adults above 18 years of age).

V87P6

This was a randomized, controlled, observer-blind study designed to evaluate immunogenicity, safety and tolerability of two doses of AFLUNOV compared to two doses of trivalent, interpandemic MF59-seasonal vaccine, each administered 3 weeks apart in children from 6 months to 17 years of age. Between 94 and 145 individuals were exposed to H5N1 per cohort. The secondary immunogenicity objectives were to evaluate i) cross-protection, ii) persistence of specific antibodies 12 months after primary vaccination and iii) magnitude of antibody response to a 12 months booster dose of AFLUNOV. Less than 1% overall showed seroprotection at baseline by HI assay. Baseline titres bordered the limit of detection also by SRH and MN assays. AFLUNOV containing 7.5 µg of H5N1 antigen per injection was immunogenic and all CHMP criteria were met after two vaccinations for all age groups. Persistence of immune response as well as effect of a booster vaccination were equally efficient in all age categories investigated in the overall clinical study program.

Supportive study

Study V87P12: Vaccination schedule

The 3 weeks apart vaccination schedule selection for AFLUNOV was based on the earlier pre-pandemic vaccine studies with H5N3 vaccines and on previously published studies. In addition, this study was conducted to evaluate the immunes responses of two 7.5µg Fluad-H5N1 vaccinations administered at 1, 2, 3, or 6 weeks apart in adults below 60 years.

Table 6: Immunogenicity Results 21 Days After 2nd Vaccination, Study V87P12, FAS

		DAY1+8	DAY1+15	DAY1+22	DAY1+43
		N=60	N=60	N=60	N=60
HI	% SP (95%CI)	55% (42-68)	**76% (63-86) N=59**	**74% (61-85) N=58**	**79% (67-89) N=58**
	GMR (95%CI)	**7.73 (4.64-13)**	**21 (13-35) N=59**	**22 (13-37) N=58**	**36 (22-61) N=58**
	% SC (95%CI)	**53 (40-66)**	**76 (63-86) N=59**	**74 (61-85) N=58**	**79 (67-89) N=58**
SRH	% SP (95%CI)	**86 (75-94) N=59**	**98 (91-100) N=59**	**88 (77-95) N=58**	**97 (88-100) N=58**
	GMR (95%CI)	**2.92 (2.29-3.72) N=59**	**3.26 (2.56-4.15) N=59**	**3.82 (3-4.88) N=58**	**4.57 (3.58-5.84) N=58**
	% SC (95%CI)	**64 (51-76) N=59**	**75 (62-85) N=59**	**71 (57-82) N=58**	**90 (79-96) N=58**
MN	MN ≥ 40 (95%CI)	38 (26-52)	76 (63-86) N=59	73 (60-84)	90 (79-96) N=59
	GMR (95%CI)	2.22 (1.67-2.96)	6.78 (5.07-9.06) N=59	7.49 (5.62-9.98)	17 (13-23) N=59
	4-Fold Increase (95%CI)	35 (23-48)	75 (62-85) N=59	73 (60-84)	90 (79-96) N=59

All 3 CHMP criteria as assessed by HI and SRH at 3 weeks after the second vaccination were met when the second vaccination was given 2 weeks or more after the first one.

2.5.4. Discussion on clinical efficacy

AFLUNOV is a H5N1 vaccine intended for use in a pre-pandemic situation to protect from infection by zoonotic H5N1 strains as well as by pandemic H5N1 influenza virus, should such a human strain ever evolve. Overall, the data presented in this dossier provided evidence that two vaccinations with AFLUNOV 7.5ug HA are sufficiently immunogenic since all CHMP criteria were met by SRH and MN for both the adult (18 – 60) and the elderly (> 60) population. Although there are no CHMP criteria for MN, percentages of subjects achieving MN titers >40 or a 4-fold increase of titers were comparable or only slightly lower than the SRH rates for seroprotection and seroconversion, respectively. With regard to the HI assay, lack of consistency in results across pivotal clinical studies and suboptimal results from the largest study V87P13 were observed and discussed. The difference in the results across studies depending on the assay used was not unexpected as already seen with Focetria. However, a definitive explanation for the variability in immunogenicity by HI across pivotal studies V87P1 and V87P13 could not be found.

In toddlers, children and adolescents aged 6 month to 17 years all CHMP criteria defined in guideline CPMP/BWP/214/96 were fulfilled, although these surrogate markers for protection were established for adults and the ranges for children are not validated yet. Surprisingly HI results in these age categories correspond much better to SRH data compared to the adult and elderly population. In section 4.2 a statement was introduced regarding the limited experience with H5N1 in children between 6 months and 17 years of age. The available information in children was reflected in section 5.1 of the SmPC.

Overall the data on cross protection after two vaccinations with AFLUNOV provided some evidence of a priming effect of the vaccination and were supportive of the induction of immune response to

heterologous strains. However the results on cross-protection were sub-optimal; the number of subjects contributing in most studies was small; in most studies, the effect of the vaccination could not be completely interpreted due to lack of data on serostatus at baseline. Therefore a cautious approach was considered necessary on this issue and a statement on the limited cross-reactivity induced by AFLUNOV has been included in sections 4.4 and 5.1 of the SmPC. In study V87P1, following administration of the booster dose, GMTs and the percentages of subjects with a seroprotective titer to a heterologous H5N1 strain highly increased, as measured by all three serological assays. Therefore, cross-reactivity was enhanced by priming. The Applicant was asked to submit the follow up of study V87P1 (V87P1E1) as a follow-up measure (FUM 4) and these data were provided on the 15 of September. Study V87P3 showed that a heterologous booster with AFLUNOV could induce adequate serological and cell mediated immune responses in subjects primed against the H5N3 strain 6 to 8 years previously, suggesting that a strategy of pre-pandemic vaccination with an adjuvanted vaccine would allow boosting with a single dose of the actual pandemic strain, once this is known, even 6-8 years later. This booster ability of the MF59-adjuvanted vaccine shows its protective function as a pre-pandemic vaccine. Therefore, despite the low cross reactivity to the heterologus A/H5N1/turkey/Turkey/1/05 strain following two vaccinations with AFLUNOV, these two vaccinations could act like a prerequisite for induction of an effective immune response upon vaccination to a different pandemic strain at a later time point. These results were reflected in section 4.2 and 5.1 of the SmPC.

Cell-mediated immunity was investigated in studies V87P2 and V87P3 using vaccine formulations with and without MF59 adjuvant. Although results looked promising, since no CMI correlates for protection exist for influenza or any other vaccines, relevance of read-outs from CMI assays cannot reliably be interpreted. The evaluation of cell-mediated immunity was also among the primary objectives of study V101P1. CMI results from this study were lacking at the time of opinion, therefore the Applicant committed to submit these data in fulfilment of a FUM by January 2011.

Concomitant administration of AFLUNOV with a conventional subunit seasonal influenza vaccine did not negatively impact the immune response to either the pandemic H5N1 strain or to the seasonal strains (study V101P1).

Supportive study V87P12 overall demonstrated that the time point for giving a second dose of AFLUNOV is flexible at least within a window of 6 weeks following the first vaccination.

Due to the limited sample size, a clear conclusion on immunogenicity of AFLUNOV in the population most at risk for influenza complication could not be drawn. This was reflected in section 4.4 of the SmPC. Additional studies to evaluate efficacy, safety an tolerability of AFLUNOV in patients with co-morbidities and immunocompromised were requested to the Applicant as follow up measures (FUM 5 and 6).

With regards to the elderly population, overall immunogenicity and safety data were available for a total of 364 subjects >60 years of age. This fulfilled the criteria for the size of the safety database of pre-pandemic vaccines (EMEA/CHMP/VWP/263499/2006) but overall data on immunogenicity in elderly >70 years came from 69 subjects. Therefore, although extensive experience has been gathered in elderly with seasonal influenza vaccines, the limited availability of both immunogenicity and safety data in the elderly above 70 years was reflected in section 4.2 and 5.1 of the SmPC.

2.5.5. Conclusions on clinical efficacy

This application was based on a large number of clinical trials with a sufficient number of vaccinated subjects to demonstrate acceptable immunogenicity of AFLUNOV after two doses of 7.5 µg H5 HA-

antigen given 21 days apart. Evaluation of immune responses was performed according to standard test procedures as described in the relevant Note for Guidance on Harmonisation of Requirements for Influenza Vaccines applying HI, SRH and MN assays. All CHMP criteria for qualification of an acceptable influenza vaccine have been met using the SRH assay for both, the adult (18 – 60) and the elderly (> 60) study population. Immunogenicity of influenza vaccine that is its ability to induce a satisfactory immunologic response is considered a surrogate for clinical efficacy in providing protection from infection. Studies on actual clinical protection from H5N1 are not possible due to the limited spread of the infection essentially limited to animal exposure.

2.6. Clinical safety

The overall clinical safety data included:

1. safety analyses on the pooled AFLUNOV safety database, which included subjects from studies V87P1, V87P2, V87P3 (all subjects exposed to AFLUNOV in study V87P3 were included in the pooled safety analyses regardless of the priming status) and V87P13. Safety data for studies V87P12 and V101P1 were presented as stand alone mainly in support of different vaccination schedules and concomitant administration of a trivalent subunit seasonal influenza vaccine, respectively.
2. the analysis of the safety profile of the MF59-adjuvanted seasonal influenza vaccine, Fluad;
3. Fluad post-marketing surveillance data.

Patient exposure

The incidence of adverse reactions has been evaluated in six clinical trials involving approximately 4,000 adults and elderly receiving AFLUNOV (at least 7.5 µg HA, adjuvanted). There were 3678 subjects 18-60 years of age, 264 subjects 61-70 years of age, and 41 subjects greater than 70 years of age. The pivotal safety study V87P13 was specifically designed to make a direct comparison between the safety profile of AFLUNOV and that of the MF59-adjuvanted seasonal vaccine Fluad and contributed most of the data for the pooled analysis. This phase III study was performed at multiple study sites, in a population of healthy subjects aged 18 to 60 years and over 60 years of age. The study sample size was calculated so that the overall safety database from the clinical trials would be sufficiently large to detect rare (at ≤0.1% frequency) adverse events (AEs) in adults below 60 years, and uncommon (at ≤1% frequency) AEs in adults above 60 years. For more details on study V87P13 see the clinical efficacy section of this AR.

The safety analysis for Fluad was based on a pooled clinical safety database of 14,586 subjects ≥18 years of age (1383 aged 18-64 years, 12,913 aged >65 years, and 290 subjects with underlying disease) from 42 historical clinical trials (plus an additional 8 extension studies). Fluad post-marketing surveillance data was obtained from over 40 million doses distributed since the initial licensure in September 1997 until April 30, 2008.

None of the studies in the AFLUNOV development program were designed to evaluate specific high-risk groups. The only study performed in children at the time of opinion was V87P6, discussed later on.

Adverse events (AEs)

The assessment of safety was based on the recommendation of the New Vaccines guidelines (CPMP/EWP/463/97, 1999). Overall, in the clinical trials conducted with AFLUNOV in adults aged ≥18, safety measures included:

- Solicited AEs (i.e., local and systemic reactions) and selected indicators of reactogenicity (e.g., use of analgesic/antipyretic medication) on the day of vaccination and each of the following 6 days.
- Unsolicited AEs (and related concomitant medications), as defined by ICH E2A, collected as predefined in the respective study protocols.

The results of adverse events were composed in the following way:

1. Solicited local and systemic AEs
2. Unsolicited AEs
3. Adverse Events after the Booster and 6 months follow-up
4. AEs after different vaccine schedules.

1. <u>Solicited local and systemic AEs</u>

Table 7: Overview of subjects with solicited reactions, pivotal study V87P13

	Number (%) of Subjects With Solicited Reactions			
Age Group	**Adults**		**Elderly**	
After first vaccination (Days 1-7)	**NonAdj Seasonal N=2688**	**Placebo N=678**	**NonAdj Seasonal N=219**	**Placebo N=56**
Any	1858(69%)	339(50%)	118(54%)	25(45%)
Local	1456(54%)	202(30%)	84(38%)	15(27%)
Systemic	1207(45%)	229(34%)	66(30%)	18(32%)
Other	338(13%)	65(10%)	18(8%)	4(7%)
After second vaccination (Days 22-28)	**AdjPanH5N1 N=2607**	**AdjSeasonal N=657**	**AdjPanH5N1 N=214**	**AdjSeasonal N=54**
Any	1770(68%)	507(77%)	113(53%)	30(56%)
Local	1532(59%)	452(69%)	89(42%)	22(41%)
Systemic	1134(43%)	343(52%)	71(33%)	17(31%)
Other	246(9%)	106(16%)	16(7%)	4(7%)
Third vaccination (Day 43-49)	**AdjPanH5N1 N=2559**	**AdjSeasonal N=639**	**AdjPanH5N1 N=212**	**AdjSeasonal N=53**
Any	1413(55%)	385(60%)	94(44%)	29(55%)
Local	1200(47%)	342(54%)	66(31%)	21(40%)
Systemic	844(33%)	206(32%)	55(26%)	16(30%)
Other	185(7%)	45(7%)	16(8%)	3(6%)

Pain was the most frequently reported local reaction across all study vaccinations (7,5µg dose), followed by induration and erythema. A general tendency towards decreased reports of local reactions was observed with subsequent vaccinations. No consistent increase or decrease in local reactogenicity was observed when higher, 15µg antigen dose was administered. As expected, overall reactogenicity was higher for adjuvanted vaccine groups than for the Non-adjuvanted 15µg group.

Most frequently reported systemic AEs were myalgia, headache and fatigue. When comparing the two antigen dose groups, no consistent increase or decrease of systemic adverse events was observed when the higher (15µg) dose formulation was used. Moreover there was a general trend towards

decreased reports of systemic reactions after the second and booster vaccination compared with the first. Most solicited systemic reactions were mild to moderate in severity, with exception of the booster dose in the 7.5 µg group (studies V87P1, V87P2): in the group of non-elderly (pooled data) after the third dose 5% suffered from severe Myalgia and 3% severe Arthralgia. Also in study V87P3 no severe local reactions have been seen, except for a few cases after the booster. The percentage of elderly reporting solicited systemic reactions was lower than those among non-elderly.

In comparison with the seasonal Fluad data, all systemic reactions, except for myalgia (witch occurred only by 14% of the seasonal Fluad recipients), were reported by similar percentages across groups.

2. Unsolicited AEs

Overall in study V87P13 after both vaccinations, similar percentages of AFLUNOV and seasonal Fluad recipients reported unsolicited AEs, both "any" and possibly or probably related, as shown in table 8.

Table 8: Percentage of all and at least possibly related most common adverse events by preferred term reported by descending frequency collected within 21 days after any injection (study V87P13)

Preferred Term	Percentage of Subjects with Adverse Events			
	All		At Least Possibly Related	
	AdjPanH5N1	AdjSeasonal	AdjPanH5N1	AdjSeasonal
Adults	**N=2611**	**N=658**	**N=2611**	**N=658**
Nasopharyngitis	7	8	2	3
Upper Respiratory Tract Infection	6	5	1	1
Headache	5	3	1	<1
Rhinitis	3	4	1	1
Oropharyngeal Pain	3	4	2	1
Gastroenteritis	2	2	<1	0
Sinusitis	2	2	<1	<1
Injection Site Hemorrhage	1	2	1	2
Cough	2	1	1	<1
Diarrhea	2	2	1	1
Back Pain	2	2	<1	0
Fatigue	2	2	1	1
Elderly	**N=214**	**N=54**	**N=214**	**N=54**
Upper Respiratory Tract Infection	4	9	1	4
Headache	3	6	0	4
Rhinitis	2	6	<1	2
Cough	1	4	<1	4
Diarrhea	0	4	0	4
Dyspepsia	<1	4	<1	2
Nasopharyngitis	3	0	0	0
Oropharyngeal Pain	3	2	2	2
Injection Site Hemorrhage	2	2	2	2
Gastroenteritis	2	0	0	0

Source: Table 14.3.1.1.10 Table 14.3.1.1.13

In Study V87P1 and V87P2, regardless of vaccine relatedness, the frequency of other unsolicited AEs within 3 weeks post vaccination was very low. During the 6 months follow-up period (i.e. onset more than 21 days after vaccination) AEs were only reported by subjects in the AFLUNOV 7.5ug group (2

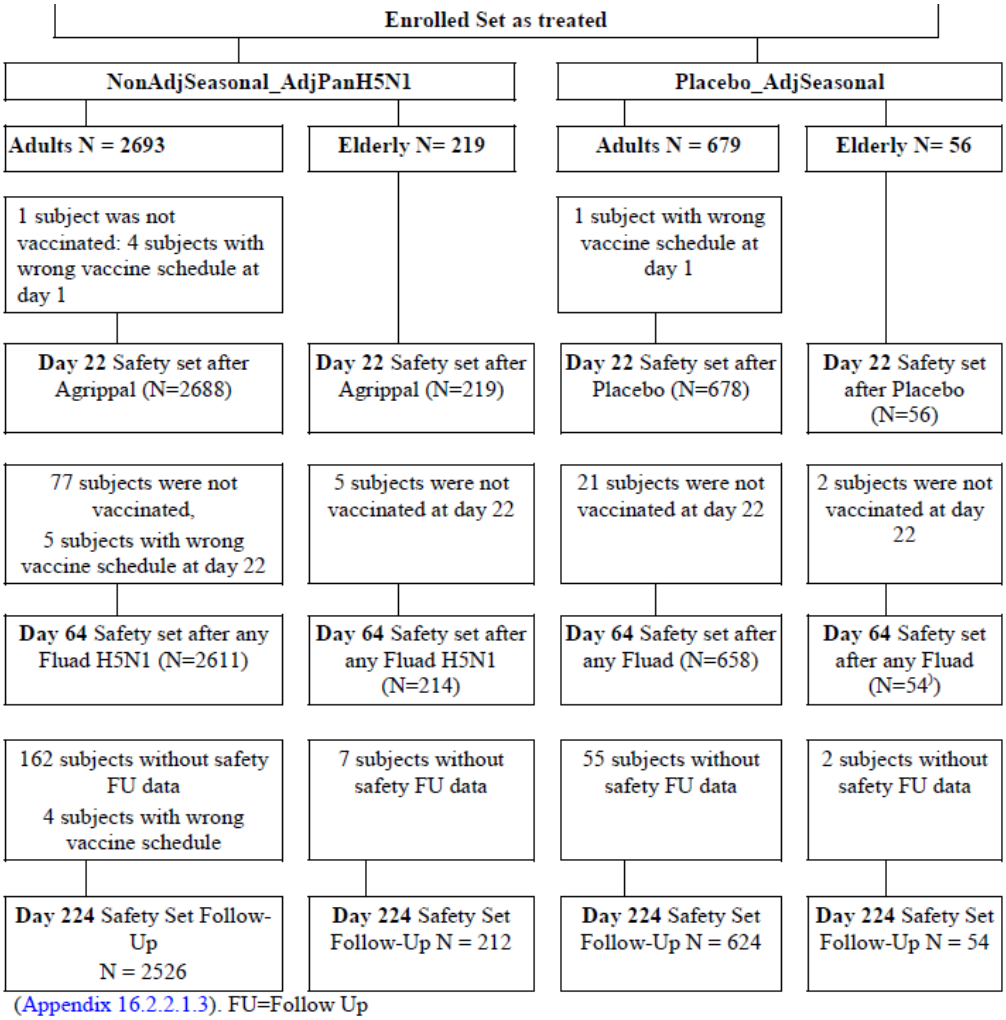
subjects after the second and 1 subject after the booster vaccination). No AE was judged by the investigator as probably or possibly related to vaccination. Reactogenicity after any vaccination was generally higher in the AFLUNOV groups than in the non-adjuvanted 15 group, as expected.

3. Adverse Events after the Booster and 6 months follow-up

AEs were collected 7 days after the booster vaccination, whilst unsolicited AEs were collected 3 weeks and SAEs up until 6 months after the booster dose. V7P37E1 study has assessed the effect of one dose. Most studies have tested only a two dose schedule: V87P12, V101P01 and V7P37. A third dose of AFLUNOV has been administered in studies V87P1, V87P2 and V87P5. The pooled analysis of data on safety of a booster dose of the current AFLUNOV formulation is limited to 87 adults and 38 elderly. Slightly higher numbers can be considered if formulations with higher antigen content (15 ug) are included. Based on available data no clear and consistent trend for an increased or decreased reactogenicity was observed in subjects receiving a booster dose.

On D121 of the procedure, the Applicant has submitted the 6-months safety follow-up data from study V87P13 collected until the end of the study (day 224) in the Addendum to CSRV87P13.

Subjects completion flowchart



(Appendix 16.2.2.1.3). FU=Follow Up

During the period from day 65 to day 224, only Serious Adverse Events (SAEs), Adverse Events (AEs) that lead to a physician's visit, AEs that lead to withdrawal from the study, and concomitant medications associated with these events were collected. The analysis was provided both for adults and elderly. However, the investigated population does not properly represent the elderly, due to the

very limited cases aged over 65. The safety profile of NonAdjSeasonal_AdjPanH5N1 group was generally similar to that of Placebo_AdjSeasonal group for both adults and elderly.

Adults

The percentages of AEs, SAEs, possibly or probably related AEs, AEs necessitating a physician's visit and AEs leading to hospitalization were similar between vaccinated and placebo groups. The most commonly reported preferred terms were: Nasopharyngitis, Sinusitis, and Upper Respiratory Tract Infection. The percentage of subjects reporting SAEs was 1% across groups. However in the NonAdjSeasonal_AdjPanH5N1 group gastrointestinal disorders were reported more frequently than in the placebo group and it was noted among SAE. Six cases of AEs were considered possibly related to the study vaccine in the NonAdjSeasonal_AdjPanH5N1 group and included also a case of vaccination failure, laboratory confirmed. The possibility of vaccination failure is reported in section 5.1 of the SmPC.

Elderly

A higher percentage of subjects in the NonAdjSeasonal_AdjPanH5N1 group (19%) reported at least one AE when compared with Placebo_AdjSeasonal group (15%). The reported terms after visit four included: musculoskeletal pain, urinary tract infections, conjunctivitis. Serious Adverse Events (SAEs) resulted similar in frequency in both groups. They were the expected events corresponding to the age group and were judged not related to the study vaccine. The most commonly reported preferred terms were Sinusitis, Nasopharyngitis, Bronchitis, Back Pain, and Urinary Tract Infection.

A higher percentage of eye disorders (1%) was found in the NonAdjSeasonal_AdjPanH5N1 compared with the Placebo_AdjSeasonal group (0%). Among AEs necessitating a physician visit, 6% of Infections and Infestations and 3% of musculoskeketal events were found in the NonAdjSeasonal_AdjPanH5N1 vs 2% in the control group.

4. AEs after different vaccine schedules (study V87P12)

Overall a higher percentage of subjects reported local or systemic reactions after the first vaccination than after the second vaccination in all vaccine schedule groups. Pharyngitis was the most commonly reported AE in the safety population, reported at highest frequency (by 10% subjects) in day1-22 group. None of the other spontaneously reported AEs were recorded at >5% frequency in a vaccine schedule group. There were two reports (of erythema and pain) in one subject in day1-43 group and one report (Pruritus) in day1-15 group that were assessed to be possibly related to the vaccination.

Serious adverse event/deaths/other significant events

Deaths

One death (a 77 year old man following an acute myocardial infarction) was reported in study V87P1 and it was considered unrelated to AFLUNOV. A total of four deaths, none assessed as vaccine-related, occurred in study V87P4. All deaths occurred during the 6-month follow-up period after second vaccination. No other deaths were reported in the 6 studies in adults ≥18 years.

Sever AEs

In the six studies with AFLUNOV all SAEs were judged by the investigator as unrelated to the study vaccine except 2 cases of adults below 60 years and 3 SAEs in 2 adults above 60 years of age in the study V87P13. These 5 SAEs were assessed possibly or probably related to the vaccine: anaphylactic reaction, bronchial hyperreactivity and pneumonia, muscular weakness, leukocytoclastic vasculitis (all recovered, except the last two, which were still ongoing at the time of assessment).

Laboratory findings

No laboratory evaluations were assessed for the evaluation of safety in studies V87P1, V87P2, V87P3, V87P12 and V87P13.

Only in study V101P1 laboratory data regarding safety were evaluated. Blood was collected in a subset of 150 subjects immediately prior to vaccination on day 1 and on day 43 for serum chemistry and haematology clinical laboratory testing. These included haemoglobin, red blood count (erythrocytes), MCV, MCHC, MCH, white blood count (leukocytes), platelet count, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), electrolytes and creatinine.

In the analysis of serum chemistry at baseline, a total of 10 subjects demonstrated lab values in the upper level of normal range. Elevated ALT was reported in 4 subjects; elevated AST was reported in 3 subjects; less that 4% of subjects demonstrated elevated levels in the rest of the parameters. A total of 23 subjects demonstrated lab values in the upper level of normal range in the analysis of the final blood draw. Elevated ALT was reported in 5 subjects; elevated sodium was reported by 9 subjects; less than 4% of subjects demonstrated elevated levels in the rest of the parameters.

In the analysis of haematology at baseline, a total of 7 subjects demonstrated lab values in the upper level of normal range. Less than 4% of subjects demonstrated such levels in any of the parameters assessed. A total of 5 subjects demonstrated lab values in the upper level of normal range at the final analysis. Less than 2% of subjects demonstrated such levels in any of the parameters assessed.

Safety in special populations

Study V87P6 (children 6 months- 17 years of age)

Safety analyses were stratified by age; no other analyses of intrinsic factors were undertaken.

Solicited Local and Systemic reactions after first and second vaccinations (day 1-7 and day 22-28)

For the 201 **toddler** subjects, overall reactogenicity was highest after the first vaccination and decreased after the second vaccination. Most solicited reactions were mild or moderate, with no more than 2% of each solicited local or systemic reaction classified as severe in either vaccine group. Few of these reactions continued past the day 7 observation window and none continued at the end of the study. Erythema and tenderness were the most common solicited local reactions and irritability was the most common solicited systemic reaction in toddlers in both vaccine groups.

Of the 136 **child** subjects, 72% of AFLUNOV and 80% of MF59-Seasonal Fluad recipients reported at least one solicited local or systemic reaction after the first injection, and 68% and 56% after the second injection. Most solicited reactions were mild or moderate, with no more than 5% of each solicited local or systemic reactions classified as severe in either vaccine group. Pain was the most common solicited local reaction; no more than 5% of children complained of severe pain after any injection and headache was the most common systemic reaction in both vaccine groups.

For the 134 **adolescent** subjects, at least one solicited local or systemic reaction was reported for 91% of AFLUNOV and 88% of MF59-Seasonal Fluad recipients after the first injection, with a decrease to 82% and 78% respectively after the second injection. Most of the solicited reactions were mild or moderate, with no more than 5% of each solicited local or systemic reaction classified as severe in either vaccine group. Pain was the most common local reaction. No more than 5% of adolescents complained of severe pain, and headache was the most common solicited systemic reaction in both vaccine groups.

Solicited Local and Systemic reactions after booster vaccination (day 382-388)

Overall, the percentage of subjects reporting local reactions within 7 days of booster vaccination was higher than after both first and second dose, ranging across age cohorts from 60% to 81%. The most commonly reported reactions were tenderness for toddlers, pain at the injection site for children and adolescents followed by erythema. Within each age cohort most reactions were mild or moderate in severity. No severe local reactions were reported for children. Few local reactions continued past the 7-days observation window and none continued at the end of the study.

Overall, the percentage of subjects reporting solicited systemic reactions within 7 days of booster vaccination ranged across age cohorts from 45% to 69%. The most commonly reported systemic reaction was irritability and unusual crying for toddlers. There was an increase of solicited local and systemic adverse reactions after the booster vaccination compared with the primary administration.

Unsolicited AEs 21 days following first and second vaccinations (day 1-43)

The percentages of subjects with any AEs (other than solicited local and systemic reactions), regardless of their assessment of relatedness, were similar between the vaccine groups (AFLUNOV versus Seasonal).

Unsolicited AEs (other than local and systemic reactions) 21 days following booster vaccination (days 382-403)

In total 15 subjects (8 toddlers, 3 children and 4 adolescents) were reported to have at least one possibly/ probably related AE each. All possibly/probably related AEs were known side effects of influenza vaccination. A trend towards an increased local and systemic reactogenicity after the administration of the booster dose was observed. In particular in the toddlers, severe local and systemic reactions after booster administration were much higher compared to the reactions observed after the first two administrations.

Pregnant individuals

Although in all studies pregnancy was an exclusion criterion, a total of 13 pregnancies were reported after administration of AFLUNOV in studies V87P1, V87P12, V87P13 and V101P1.

Study V87P1: one pregnancy was reported; the woman delivered a healthy live-born baby.

Study V87P12: two pregnancies were reported; one woman received the two AFLUNOV vaccinations 3 weeks apart and one 6 weeks apart; both women delivered healthy live-born babies.

Study V87P13: seven pregnancies have been reported in the original application; however the study was still ongoing. Overall there were 14 women (two in the Placebo/Fluad group and 12 in the Agrippal/AFLUNOV group) who became pregnant during the entire study duration. Ten women (two in the Placebo/Fluad group and eight in the Agrippal/AFLUNOV group) delivered a live-born baby. One of these babies had a congenital abnormality (Agrippal/AFLUNOV group), another baby who was born without abnormalities (Agrippal/AFLUNOV group) however was diagnosed with a congenital cystic adenomatoid malformation by routine ultrasound at 20 gestational weeks which then disappeared at 32 gestational weeks and the baby was born healthy. Three spontaneous abortions in the first trimester and one therapeutic abortion occurred, all in women from the AFLUNOV group.

Study V101P1: Three pregnancies were reported during the study. One live born delivery of a healthy baby (in a woman from AFLUNOV/Placebo + Tetravalent group), one spontaneous (in a woman from Tetravalent/Placebo + AFLUNOV group) and one therapeutic (in a woman from Tetravalent/Placebo + AFLUNOV group) abortion were reported.

Safety related to drug-drug interactions and other interactions

Concomitant Administration with Seasonal Influenza Vaccine (Study V101P1)

AFLUNOV was administered either before or after a tetravalent vaccine pre-formulated with H5N1 and the three seasonal influenza strains or concomitantly with a subunit seasonal influenza vaccine as first vaccination followed by a second AFLUNOV administration. Regardless of the timing of administration, reactogenicity of AFLUNOV was consistent with that observed for the other studies and for the pooled safety database. In particular, when AFLUNOV was administered concomitantly with Agrippal, overall reporting of solicited reactions did not increase. As expected, solicited local reactions were more frequently reported for AFLUNOV than for Agrippal.

Discontinuation due to adverse events

Overall in the AFLUNOV studies, premature withdrawals due to AEs were infrequent: within the 21 days following primary course vaccination, less than 1% of the subjects across the two age groups withdrew prematurely due to an AE. No subject withdrew during the 21-day period following the booster dose. During the 6-month follow-up period after second/third vaccination, only one elderly subject (2%) of the total 66 withdrew due to an AE after the booster dose.

Post marketing experience

No post marketing experience is available for AFLUNOV.

Seasonal Fluad (surface antigen, inactivated, adjuvanted with MF59) has been marketed since authorisation in 1997 and more than 27 million doses have been distributed. In clinical trials in total 14,028 subjects were administered one vaccination, 596 subjects received two vaccinations and 150 subjects three vaccinations. Analysis of post-marketing data collected demonstrated a good safety profile.

2.6.1. Discussion on clinical safety

According to the Guideline on Influenza vaccines prepared from a virus with the potential to cause a pandemic and intended for use outside of the core dossier context (CHMP/VWP/263499/2006), a database of approximately 3000 adult subjects should be sufficient to evaluate the safety profile. All together in the submitted studies actually 3678 adult and 305 elderly subjects were exposed to 7.5 µg AFLUNOV. The mean age of non-elderly AFLUNOV recipients included in studies V87P1, V87P2, V87P3 and V87P13 was 40.9 years. Additionally 255 subjects received a higher dose of 15µg Fluad-H5N1. Such a sample size is sufficient to detect rare adverse events in adult (at ≤ 0.1 % frequency) and elderly (at ≤1%) and is in line with the above mentioned guideline.

However, most of the enrolled elderly population of the pivotal safety study V87P13 was in the age stratum 60-65 years, and only few subjects aged >70 years were included in the study. The exclusion of subjects with co-morbidities and the relative young age of the elderly population make the results of the study not directly applicable to the clinical setting of people over 70; moreover elderly with co-morbidities will likely be among those to whom the vaccine will be prescribed. The Applicant submitted a pooled analysis of safety and efficacy data from studies V87P1, V87P2, V87P3 and V87P13 focused on subjects whose medical history at enrolment reported diseases associated with increased risk of complications from seasonal influenza. Results from this analysis showed that AFLUNOV profile of solicited and unsolicited AE in the subset of patients with co-morbidities was overall similar to that

observed in the pooled safety database of the AFLUNOV dossier. These data are hampered by the fact that i) these patients are not representative of chronically ill patients with potentially severe co-morbidities (all patients were judged healthy by the investigators at the time of enrolment) ii) the number of subjects included in the analysis is small: a total of 280 adults and 109 elderly (>60 years) exposed to one of the two 7.5 µg or 15 µg AFLUNOV formulations. Considering the lack of clinical data in patients with co-morbidities, the Applicant was requested to perform i) a study to evaluate the efficacy, safety and tolerability of AFLUNOV in patients with co-morbidities; ii) a study to evaluate efficacy and safety of AFLUNOV in immunocompromised patients (FUMs 4 and 5).

The results of the different studies have shown that two doses of AFLUNOV, each dose containing 7.5µg of A/Vietnam/1194/2004 antigen, were safe and well tolerated, both in adult and elderly. No consistent increase or decrease in local reactogenicity was observed when higher, 15µg antigen dose was administered. No possibly/probably related death was reported. Injection site pain was the most frequently reported local reaction, while myalgia and headache were the most frequently reported systemic reactions. Reactogenicity was generally higher for the adults than the elderly. The rate of AEs, SAE or Withdrawals has been comparable between the different groups. A general tendency towards decreased reports of local and systemic reactions was observed with the second vaccinations. An increase of solicited local and systemic adverse reactions after the booster vaccination compared with the primary administration has been found in the paediatric study (V87P6).

Results from the six-month follow up study V87P13 have shown that the safety profile of the NonAdjSeasonal_AFLUNOV group was similar to that of Placebo_AdjSeasonal group for both Adults and Elderly, although a higher percentage of subjects in the AFLUNOV group (19%) reported AEs compared to the control group (15%).

The pooled analysis of data on safety of a booster dose of the current AFLUNOV formulation is limited to 87 adults and 38 elderly. Slightly higher numbers can be considered if formulations with higher antigen content (15 ug) are included. Based on available data no clear and consistent trend for an increased or decreased reactogenicity, compared to that observed after the first dose, was observed in subjects receiving a booster dose .

Data on pregnancy from clinical trials are very limited and difficult to interpret without appropriate comparison with unvaccinated pregnant women. However it should be considered that more than 90,000 pregnant women have been immunised with Focetria vaccine which contains the same amount of MF59 as AFLUNOV. Further data with Focetria are still awaited, but no major cause of concern has been identified so far. However, as AFLUNOV is intended for use in a non-emergency situation, a cautious approach should be preferred and a wording suggesting deferring administration after pregnancy was included in section 4.6 of the SmPC.

So far, there was no consistent difference in safety profiles of the four different vaccine schedule groups receiving two vaccinations of 7.5µg of H5N1 antigen (study V87P12).

With regard to the concomitant use of a seasonal influenza vaccine together with AFLUNOV, no clinically relevant increase of reactogenicity has been found in study V101P1 with approximately 600 enrolled subjects.

2.6.2. Conclusions on clinical safety

No specific safety concern has emerged from the analysis of the safety data set submitted for AFLUNOV. However, considering that the vaccine is intended for use in pre-pandemic settings, a

cautious approach should be used for pregnant women and elderly due to the limited number who have been vaccinated with AFLUNOV in the clinical studies.

2.7. Pharmacovigilance

The Applicant provided a detailed description of the updated system of pharmacovigilance. From the assessment of all documents a clear picture has emerged of the organisation of the whole system as well as of the PhV activities to be conducted by the Applicant during the pre-pandemic and the pandemic phases. The system described appears to meet the needs for a pre-pandemic vaccine. A statement signed by the applicant and by the qualified person for pharmacovigilance has been provided, indicating that the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means to notify any adverse reaction occurring either in the Union or in a third country. The CHMP considered that the PhV system as described by the Applicant fulfils the requirements and provides adequate evidence that the Applicant has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk management plan

The MAA submitted a risk management plan, which included a risk minimisation plan and an Efficacy Follow-up Plan.

Pharmacovigilance activities during the pandemic period

During the pandemic period, an enhanced pharmacovigilance activity will be implemented, in line with the CHMP recommendations for pandemic influenza vaccines. Key elements of the enhanced pharmacovigilance activity for spontaneously reported events will be as follows:

- Due to a probable disruption of the routine pharmacovigilance system, resources will be concentrated on timely and effective monitoring of the safety profile of AFLUNOV.
- Simplified PSURs will be submitted on a monthly basis with discussion of AESI in a dedicated section.
- Weekly signalling meeting using Proportional Reporting Ratio and denominator-based sensitivity analyses as appropriate.
- Disproportionality analyses of spontaneous reports using Proportional Reporting Ratio (PRR).
- Denominator-based sensitivity analyses using expected versus observed reporting rates.
- PASS study
- Pregnancy registry
- In the case of H5N1 pandemic, the Applicant should perform a pregnancy study to evaluate safety and effectiveness of AFLUNOV in pregnant women (FUM).

Table Summary of the risk management plan

Safety issue	**Proposed pharmacovigilance activities**	**Proposed risk minimisation activities**
Important identified risks:		
None	Not applicable	None
Important potential risks:		
Vaccination failure Convulsion Vasculitis Anaphylaxis Neuritis Encephalitis Guillain Barré Syndrome Demyelinisation Immune thrombocytopenia Bell's palsy	• routine pharmacovigilance (pre-pandemic) • enhanced pharmacovigilance (pandemic)	• SmPC labeling is considered sufficient in pre-pandemic phase
Missing information		
Vaccine Effectiveness	• The Company remains committed to provide a study protocol as soon as concrete proposals can be made. This information will also be updated in each PSUR.	N/A
pregnant and lactating women	• routine pharmacovigilance (pre-pandemic) • enhanced pharmacovigilance (pandemic)	SmPC, Section 4.6 *Limited data was obtained from women who became pregnant during the course of clinical trials with AFLUNOV (H5N1) and H1N1v vaccine adjuvanted with MF59C.1. Since AFLUNOV is expected not to be used in an emergency situation, its administration during pregnancy might be deferred as a precautionary approach.* *There are no data regarding the use of AFLUNOV during breast-feeding.*
Use in children	• routine Pharmacovigilance for off label use • Planned clinical trials (PIP) • PIP modification requested to change inclusion/exclusion criteria	SmPC, section 4.2 *<u>Paediatric population:</u>* *There is limited experience in children between 6 months and 17 years of age (see section 5.1).*

Immunocompromised patients	• Requested new study to evaluate efficacy, safety and tolerability. Clinical Study Report to be provided by December 2014	SmPC, section 4.1 *This indication is based on immunogenicity data from healthy subjects (…).* SmPC, section 4.4 *Very limited data in subjects with co-morbidities, including immunocompromised subjects are available for this H5N1 vaccine.*

The CHMP, having considered the data submitted in the MA application is of the opinion that the following risk minimisation activities are necessary for the safe and effective use of the medicinal product:

- the SmPC will be updated whenever new relevant safety data are reviewed and analysed.
- In the pre-pandemic phase, vaccinated individuals that come in contact with an avian influenza virus (e.g. poultry workers, cullers, veterinarians) could be followed-up (symptoms and seroconversion titres)
- The protocols of effectiveness studies, when developed and agreed with public health authorities, should be included in the RMP updates. If national authorities have established plans to monitor vaccine effectiveness for specific pandemic vaccines, those plans, if publicly available, should also be incorporated in the RMP.
- During the pandemic period, the same conditions as those described in the document on CHMP recommendations for pandemic influenza vaccines will apply.
- The plan for the pandemic period should include a dedicated surveillance study on immunocompromised subjects; all planned studies should be ready to start at the time of (or before) pandemic declaration; protocols (as well as the organization structures and ethical approvals) should be provided before the WHO's Phase 6 declaration.
- Stickers reporting at least brandname and batch number should be used to assure the traceability of the vaccination during both prepandemic and pandemic phase. Moreover a specific educational plan dedicated to HCPs and patients should be put in place by the Applicant.
- The Applicant should continue to assess the cross-reaction of sera of subjects immunised with AFLUNOV (produce by Vietnam strain) against emerging drifted strains of H5N1 as they are identified, in order to monitor the usefulness of pre-pandemic vaccine (FUM).

User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.8. Benefit-risk balance

Benefits

- Beneficial effects

The H5N1 strain is considered as a likely candidate from which pandemic influenza may evolve. In contrast to the recent influenza pandemic caused by the A/H1N1 (A/California/7/2009) strain, which seemed to be easily transmitted between humans but had a lower mortality rate, the avian origin H5N1 strains caused so far influenza outbreaks with rare human transmission but high mortality rate (63%). In case of a H5N1 pandemic, the use of a pre-pandemic vaccine, even if not perfectly matched to the pandemic virus and perhaps giving very low protection, may nevertheless prevent some infections and deaths whilst waiting for the specific pandemic vaccine.

Overall the production of a vaccine against a potential influenza viral strain during the interpandemic period may: i) permit early vaccination at the beginning of a pandemic when the "first track pandemic" vaccine is not yet available; ii) prime during pre-pandemic stages to reduce mortality against a closely matched pandemic strain in those countries were infections are occurring; iii) reduce the chance of an emergence of a reassortant pandemic strain by vaccinating those (e.g. veterinarians, poultry workers…) at high risk of both avian and human virus infection.

The production process of AFLUNOV is based on long experience with the other Applicant's influenza vaccine, the interpandemic Fluad. In addition, objections raised in the context of the Focetria MAA have been taken onto consideration in the present application. Therefore, the process is well established and raises no substantial quality concerns.

The benefit of a prepandemic vaccine is assessed primarily based on the CHMP immunogenicity criteria and the use of animal challenge data. As already established for several other vaccines, the addition of an adjuvant (regardless of the HA dose) increases the vaccine's reactogenicity. However, important advantages of including an appropriate adjuvant are immune potentiation and antigen sparing, which are especially important in pandemic situations. The vaccine was proven to induce satisfactory antibody responses in healthy adults below and above 60 years of age, with low HA antigen quantities. However, although no major concerns exist on the adequacy of a two dose schedule, it may occasionally be insufficient to complete full maturation of a developing anti H5 immune response, in particular as regards potential immunity against H5N1 variants different from the vaccine strain. Additionally, results from the only submitted paediatric study to date suggested a sufficient immune response also in the group aged 6 months-17 years (study V87P6).

In subjects primed against the H5N3 strain, a heterologous booster with AFLUNOV administered after 6 to 8 years from the priming induced high and rapid serological and cell mediated immune responses against both strains and a variety of others. Thus the strategy of pre-pandemic vaccination with an adjuvanted vaccine would allow boosting with a single dose once the actual pandemic strain is known even 6-8 years later. This suggested that immunological memory had been induced by the H5N3 vaccination series which persisted several years and that B cells can rapidly be expanded upon an adjuvanted booster vaccination containing a different pandemic strain.

Concomitant administration of AFLUNOV with a conventional subunit seasonal influenza vaccine did not negatively impact the immune response to either the pandemic H5N1 strain or to the seasonal strains.

- Uncertainty in the knowledge about the beneficial effects.

At present the benefit of AFLUNOV can only be assumed from the data on the immunological responses elicited following a primary series and a booster against the vaccine strain and against antigenically drifted strains of influenza A/H5N1 virus. SRH and MN assays were considered the most appropriate serological tests for assessing immunogenicity of AFLUNOV, as it has already been the case for Focetria H5N1. However it is not fully understood why immunogenicity results obtained by the Applicant with HI assay are not always satisfactory. In particular, the lack of consistency across pivotal studies for some results measured by HI and the suboptimal results obtained with the HI assay in the largest study V87P13 are still unexplained.

The expected benefit of AFLUNOV is to prime immunological response in fully susceptible subjects against H5N1 virus and therefore to allow shorter time to induce appropriate protection against clinically apparent infection and/or severe disease in case of an influenza pandemic due to H5N1. This is based on an assumption that vaccination with AFLUNOV containing antigens derived from A/Vietnam /1194/2004 will provide a clinically useful degree of cross-protection against a H5N1 strain causing the pandemic. Results reported for AFLUNOV/Prepandemic Influenza Vaccine H5N1 from heterologous challenge showed limited cross-reaction. For these reasons the applicant is requested to continue to evaluate cross-reactivity and cross-protection in the post-authorisation period against emerging strains considered to have some potential to cause a pandemic.

Risks

- Unfavourable effects

AFLUNOV is commonly or very commonly associated with a range of local and systemic adverse reactions but these are not often of severe intensity and the safety profile would not preclude the use of the vaccine in healthy adults aged 18-70 years. The experience of AFLUNOV in the elderly and in a population with co-morbidities is limited. No data are available in immunocompromised patients.

Although limited safety data are available for AFLUNOV in the elderly population, post-marketing experience with Fluad (containing adjuvant MF59) in elderly subjects is extensive. Overall, considering the cumulative exposure, including all age groups, the reporting frequency of all adverse events is 1.4 cases per 100,000 sold doses. The observed differences in AEs between adults and elderly subjects are consistent with what observed in clinical trials and do not seem to represent a safety risk.

Data on pregnancy outcomes are limited.

- Uncertainty in the knowledge about the unfavourable effects

The current safety database of AFLUNOV was considered to be sufficient to describe adverse reactions that occur uncommonly and to give an indication of any rare events. However, there are some adverse reactions known to be very rarely associated with influenza vaccines and it was not possible to predict if higher rates might be observed with AFLUNOV compared with, for example, seasonal influenza vaccines. Safety in elderly subjects above 70 years of age was not assessed.

As the vaccine is intended for use in a non-emergency situation, careful consideration should be given to its administration to pregnant women, for whom vaccination could be deferred till the end of pregnancy. Sporadic cases of pregnancy were reported in some studies, but the number of cases was very small and no firm conclusion could be drawn. However, safety of AFLUNOV in specifically vulnerable populations or in risk populations might be extrapolated from Focetria H1N1 which has been widely used during the current pandemic. Final data on pregnancy outcomes with Focetria H1N1 are awaited, thus final conclusions on the safety profile of AFLUNOV during pregnancy cannot be drawn at present.

Benefit-risk balance

- Importance of favourable and unfavourable effects

Data on the immunological responses elicited following a primary series and a booster vaccinations against the vaccine strain and against antigenically drifted strains of influenza A/H5N1 virus, supported the claim that AFLUNOV, in the interpandemic period may

i) permit early vaccination at the beginning of a pandemic when the "first track pandemic" vaccine is not yet available;

ii) prime during pre-pandemic stages to reduce mortality against a closely matched pandemic strain in those countries were infections are occurring;

iii) reduce the chance of an emergence of a reassortant pandemic strain by vaccinating those (e.g. veterinarians, poultry workers…) at high risk of both avian and human virus infection.

These benefits together with the acceptable safety profile of AFLUNOV are considered of significant clinical relevance and overcome the uncertainties due mainly to the limited experience in elderly and in subjects with co-morbidities or immunocompromised. Of note, the limited experience with AFLUNOV during pregnancy suggests as a precautionary approach to defer the administration of the vaccine.

2.8.1. Conclusions on benefit-risk balance

In conclusion, as with any rare, catastrophic event, it is impossible to determine the likelihood of an H5N1 pandemic. Therefore the benefit of a pre-pandemic vaccine is not easy to predict. Vaccination with pre-pandemic influenza virus vaccine (if specific) could be an effective way to reduce the threat of a possible influenza pandemic while an acceptable safety profile can be concluded from the currently available safety data base. Thus, following thorough evaluation of immunogenicity and safety data provided in the MAA the CHMP is of the opinion that a two doses regimen of AFLUNOV has a favourable benefit/risk ratio in the prophylaxis of infection by H5N1 subtype of Influenza A virus.

The overall benefit-risk balance of AFLUNOV is positive.

2.8.2. Risk management plan

A risk management plan including an Efficacy Follow-up Plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- in case of pandemic, pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns
- additional risk minimisation activities were required (as listed in paragraph 3.7).

2.9. Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered that the risk-benefit balance of AFLUNOV in the prophylaxis of infection by H5N1 subtype of Influenza A virus was favourable and therefore recommended the granting of the marketing authorisation.

Furthermore, the CHMP takes note that the agreed Paediatric Investigation Plan is not fully completed yet as only some of the measures are completed. The CHMP reviewed the already available paediatric data of studies subject to this plan and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.