European Medicines Agency
Evaluation of Medicines for Human Use

Doc.Ref.EMEA/540171/2008

WITHDRAWAL ASSESSMENT REPORT
FOR

Aflunov

Common name:
Prepandemic influenza vaccine (H5N1) (surface antigen, inactivated, adjuvanted) (A/VietNam/1194/2004)

Procedure No. EMEA/H/C/804

This Withdrawal Public Assessment Report is based on the Day 180 assessment report, which is the latest assessment report adopted by the CHMP prior to the Applicant's withdrawal of the marketing authorisation application.

This Withdrawal Public Assessment Report does not include all available information on the product as the CHMP assessment of the applicant's responses to Outstanding Issues raised by CHMP was still ongoing. It should therefore be read in conjunction with the Questions and Answers Document on the withdrawal of the marketing application for this product, which provides an overview on all available information on the product at the time of the Applicant's withdrawal.

7 Westferry Circus, Canary Wharf, London E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 16
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ADR	Adverse reactions
AE	Adverse event
BA	Bioavailability
BWP	Biologics Working Party
CDC	Centers for Disease Control
CHMP	Committee for Human Medicinal Products
CI	Confidence interval
CSR	Clinical Study Report
CTAB	Cetyl trimethylammonium bromide
FAS	Full analysis set
GCP	Good Clinical Practise
GLP	Good Laboratory Practise
GMP	Good Manufacturing Practise
GMR	Geometric mean ratio
GMT	Geometric mean titer
HA	Haemagglutinin
HI	Hemagglutination inhibition
HPA	Health Protection Agency
HPAI	Highly Pathogenic Avian Influenza
ITT	Intention to terat
IM	Intramuscular
IU	International units
LoQ	List of Questions
NA	Neuraminidase
MAA	Marketing Authorisation Application
MN	Microneutralization
MPH	Monovalent Pool Harvest
MRP	Mutual Recognition Procedure
NIBSC	National Institute for Biological Standards and Controls
NIH	National Institutes of Health
PIP	Paediatric Investigation Plan
PP	Per-protocol
RG	Reverse Genetics
SAE	Serious adverse event
SPC	Summary of Product Characteristics
SRH	Single radial hemolysis
SRID	Single radial immunodiffusion
VEG	Vaccine Expert Group
VWP	Vaccine Working Party
WHO	World Health Organization

I. RECOMMENDATION

Based on the review of the data and the Applicant's response to the CHMP LoQ on quality, safety and efficacy, the CHMP consider that the application for Aflunov in the Prophylaxis of H5N1 avian influenza in adults and elderly (18 years of age and over), is not approvable since a major objection still remains, which precludes a recommendation for a marketing authorisation at the present time.

The major objection precluding a recommendation of a marketing authorisation pertains to the principal following issue:

Taking into account the results of the CHMP requested inspection of the pivotal study V87P4 and in addition the outcome of an earlier Lithuanian GCP inspection, and the events at two further sites in Poland, the GCP Inspectors have provided the following assessment of the GCP compliance of study V87P4:

- The trial cannot be considered to have been conducted in accordance with GCP as required by Annex I of Directive 2001/83/EC.
- The statement provided in the clinical overview and in the Clinical Study Report concerning GCP compliance can no longer be considered valid.
- It is recommended that the study V87P4 is not used for evaluation in connection with the examination of Aflunov MAA.

Therefore, the CHMP considers that the data from this trial should not be taken into account for the evaluation of Aflunov MAA.

II. EXECUTIVE SUMMARY

Disease Background

The H5N1 avian influenza strain is therefore the most likely candidate from which a pandemic strain may evolve. The current WHO phase of pandemic alert is the following: the new influenza virus subtype H5N1 is causing disease in humans, but is not yet spreading efficiently and sustainably among humans.

The "Guideline on Dossier structure and content of marketing authorisation applications for influenza vaccines derived from strains with a pandemic potential for use outside the core dossier context" (EMEA/CHMP/VWP/42326/2007) identifies three potential scenarios of use of vaccines against a potential influenza pandemic viral strain:

- in WHO Phase 3 before there is any evidence of transmission of a potential pandemic strain between humans; in such case, the degree of protection that might be conferred to immunised individuals in an actual pandemic situation cannot be predicted since the cross-antigenicity between strains in the avian influenza vaccines and the pandemic strain will be unknown;
- in WHO Phase 3 and/or later phases (4-5) for protection of those at high risk of exposure to avian influenza (e.g. poultry workers, veterinarians, laboratory workers);
- in WHO Phases 4 and 5 for pandemic preparedness (e.g. if the vaccine strain is considered a close enough match to a virus that had been shown to have significant transmission between humans).

Novel influenza viruses may emerge following the reassortment of two co-circulating viral strains or from a series of genetic mutations in one strain. Pandemic influenza outbreaks occur when the resulting virus strain transmits 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. The daily incidence of cases for a new pandemic may peak in as little as 2 months after the first reported case and attack rates may rise above 40% (*Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. Nature 2006 Apr 26*).

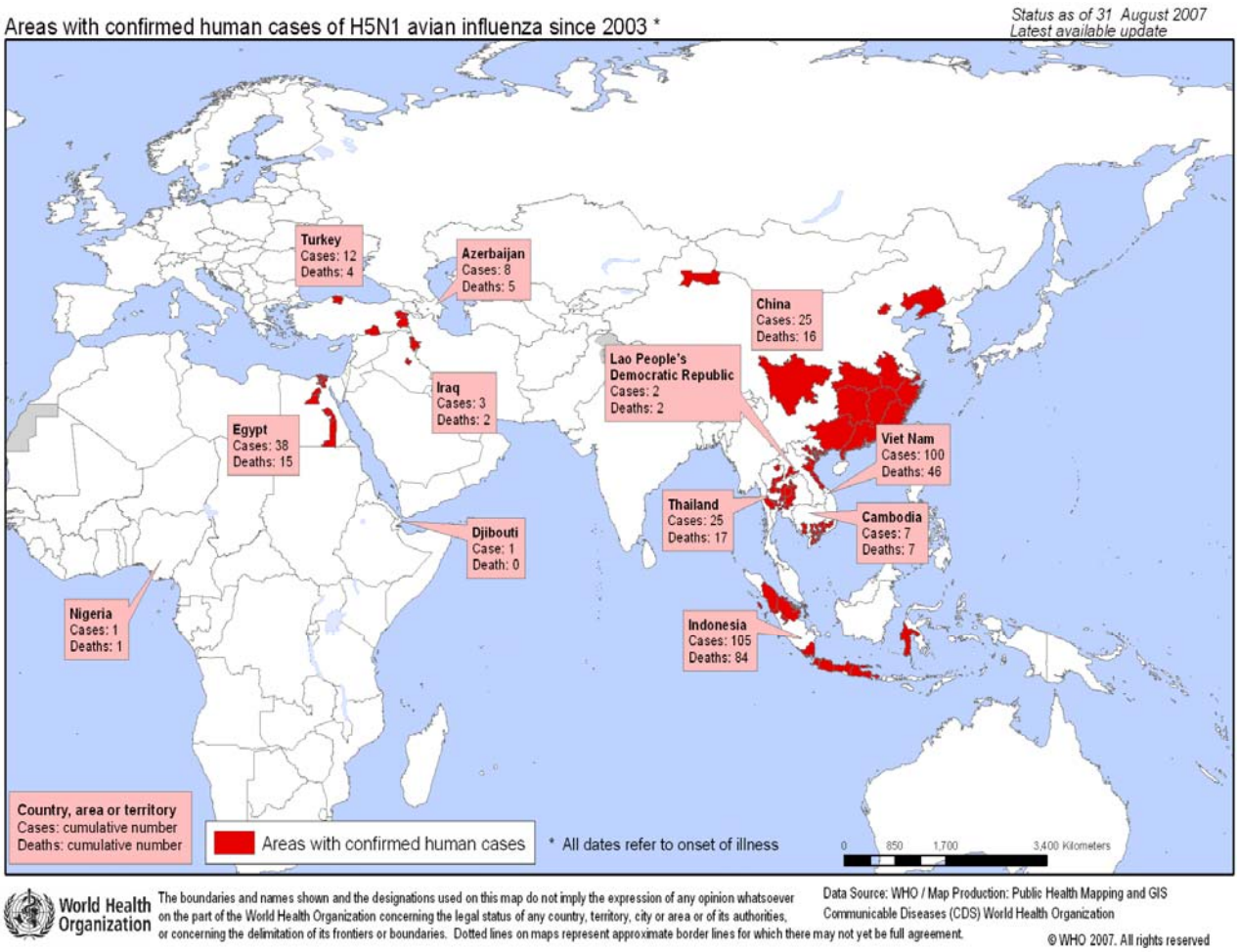
The first wave of a pandemic outbreak may be largely over within 4 months. A specific monovalent vaccine against the emerging strain will therefore have to be developed, registered and produced within a very short time. Although EMEA has consequently introduced guidelines for a fast-track licensing procedure for pandemic vaccines it is unlikely that manufacturers would be able to license and produce sufficient quantities within such a short time after identification and confirmation of a pandemic influenza viral strain.

Since supply of the "fast track pandemic" vaccine will be limited, the production of a vaccine against a potential influenza pandemic viral strain during the interpandemic period may:

- 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.
- Prime during pre-pandemic stages (WHO phases 3 to 5) to reduce mortality against a closely matched pandemic strain.
- Reduce the chance of an 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 both avian and human virus infection.

Even a vaccine of low efficacy may mitigate a pandemic.

From mid-2003 until August 2007, a cumulative number of 327 confirmed human cases of avian influenza A/H5N1 (199 deaths) have been reported to WHO, originating from 10 countries worldwide. About 50% of these cases have been reported in 2006 and 2007, and it is likely that an increasing number of countries will be affected in the coming years.



II.1 About the product

Product Development Rationale

This application is intended to register a **pre-pandemic vaccine** (Aflunov, indicated in the dossier as Fluad-H5N1) for prophylaxis of avian A/H5N1 influenza, outside of the context of a mock-up core dossier, i.e. allowing for use before a pandemic is declared.

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

The specifications for the active ingredient (except antigen content) and composition of adjuvant, in addition to the other excipients, are the same. The quality and stability profiles of both vaccines are also identical.

Each dose of Aflunov contains:

- A/Vietnam/1194/2004(H5N1)-like strain 7.5(*) per 0.5 ml dose *expressed in µg haemagglutinin
- Adjuvant: MF59C.1 composed of 9.75 mg of squalene, polysorbate 80, sorbitan trioleate, sodium citrate, citric acid.

- Excipients: sodium chloride, potassium chloride, potassium dihydrogen phosphate, disodium phosphate dihydrate, magnesium chloride hexahydrate, calcium chloride dihydrate, and water for injections

The H5N1 virus seed used in the production of the vaccine was produced from Reverse Genetics (RG) technology. The Drug Substance in Fluad H5N1 are purified surface antigens prepared from the strain of influenza virus with a pandemic potential supplied by the NIBSC or other recognised WHO influenza reference centre. Monovalent Pooled Harvests comply with the relevant Ph.Eur. monograph of influenza vaccines, surface antigen, inactivated. The MF59C.1 adjuvant is an oil-in-water emulsion, composed mainly of squalene that is an intermediate metabolite in the synthesis of cholesterol, already contained in the licensed influenza vaccine Fluad.

Novartis V&D S.r.l. submitted through a Centralised Procedure a new Marketing Authorization Application (MAA) for the vaccine Aflunov (referenced as Fluad H5N1). This vaccine is intended for the prophylaxis of influenza before the pandemic situation is declared.

This application takes 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).

Fluad H5N1 is manufactured using the same process and contains the same adjuvant used for Fluad, a Novartis seasonal, trivalent influenza vaccine licensed thorough a MRP (procedure No. IT/H/0104/001) and marketed in several European countries as well as outside Europe. This submission consists of a dossier for Fluad H5N1 derived from a vast collection of data generated on selected vaccine licensed formulations equivalent to Fluad H5N1 (Novartis' Agrippal and Fluad).

On 18 October 2005 the company Chiron (now Novartis) requested Scientific Advice for the pandemic mock-up application (Centralised Procedure No.EMEA/H/C/000710/0000). CHMP advice was finalised during the plenary meeting on 12-15 December 2005 (EMEA/CHMP/SAWP/421363/2005).

A pandemic mock-up application, in accordance to the CPMP/VEG/4717/03 "Guideline on Dossier structure and Content for Pandemic Influenza Vaccine Marketing Authorization Application" was submitted in January 2006, and obtained positive opinion by the CHMP and was granted a marketing authorisation under exceptional circumstances in the EU in May 2007 (EU/1/07/385/001-4). This mock-up vaccine is based on studies using H5N1, H9N2 and H5N3 strains manufactured and controlled according to an identical process as the one presented in this application.

The licensed vaccine Fluad contains purified haemagglutinin (HA) and neuraminidase (NA) antigens from the surface of the three influenza virus strains, type A and type B, recommended annually for immunization by the WHO (World Health Organization) and the EMEA. The influenza virus strains are individually grown in embryonated chicken eggs and inactivated by formaldehyde treatment before purification of the surface antigens and final formulation. The antigenic content and composition of Fluad is the same as that for Novartis' influenza subunit vaccine, Agrippal, except for the addition of the adjuvant. Agrippal has been registered in Italy since October 1986 and has been the subject of a Mutual Recognition Procedure in all the other EU Member States (IT/H/102/01), which was successfully completed on December 22, 1998 (Day 90).

In compliance with the various applicable guidelines the nonclinical support for Fluad H5N1 is based on pharmacology and toxicology studies performed with the 'parent' vaccine, Fluad, a mouse and ferret challenge studies with A/NIBRG-14 (H5N1) antigens adjuvanted with MF59 (Fluad H5N1), and a reproductive toxicity study with Fluad H5N1 in rabbits.

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. The relationships between Fluad H5N1, Fluad, Agrippal, MF59W.1 or MF59-0 (water formulations), and MF59C.1 (citrate formulation) is illustrated below:

Adjuvant	Composition
MF59-0	MF59W.1
MF59W.1	Squalene, polysorbate 80, sorbitane trioleate, in water
MF59C.1	Squalene, polysorbate 80, sorbitane trioleate, citric acid monohydrate, sodium citrate dehydrate, in water

Vaccine	Drug substance	Strain/dosage	Adjuvant	Comments
Agrippal	Surface antigen, Inactivated	Trivalent (15 µg HA/ strain)	Not present	Seasonal vaccine. Licensed in EU (for adults and children)
Fluad	Surface antigen, Inactivated	Trivalent (15 µg HA/ strain)	MF59C.1 (0.25 ml)	Seasonal vaccine. Licensed in EU (for elderly ≥65 years)
Focetria	Surface antigen, Inactivated	Monovalent H5N1 (7.5 µg HA/ strain)	MF59C.1 (0.25 ml)	Pandemic Mockup vaccine. Licensed in EU
Fluad H5N1 (Aflunov)	Surface antigen, Inactivated	Monovalent H5N1 (7.5 µg HA/ strain)	MF59C.1 (0.25 ml)	Avian flu vaccine. Same process than Fluad. Submiss. in Oct. 2006 (for adults and elderly)

Antigen choice

Although there are various strains of avian influenza A virus, only H5N1, H7N3, H7N7, and H9N2 are known to cause human infections. Of these, H5N1 is responsible for the recent human cases above reported.

Dosage of hemagglutinin (HA) and schedule

Based on the EMEA-recommended HA content for interpandemic vaccines (CPMP/BWP/214/96) and the expected initial naivety of the population against a newly emerged pandemic strain, Novartis studies on pre-pandemic Fluad formulated with H5N3 (Fluad-H5N3) investigated the effect of two injections of vaccines containing different amount of antigen: 7.5, 15, and 30 µg HA/injection (V7P37 and V7P37E1). The results show that with all doses most recipients develop serum antibodies after two injections. Although a subsequent trial run by the USA National Institutes of Health (NIH), (study DMID 04-019) on a Fluad-like vaccine including antigens from H9N2 (Fluad-H9N2) showed that even two injections of 3.75 µg HA each also induced a satisfactory humoral immune response, subsequent formulations did not use such low antigen content.

For the present evaluation the relevance of the immunogenicity induced by the vaccine containing 3.75 µg HA/dose from the H9N2 formulation is unclear since it is known that H9 viral antigens induce greater immune responses than antigens from H5 viruses. Overall, immunogenicity data from pre-pandemic Fluad-like vaccine formulated with H5N3 or H9N2 support the conservative decision of a two injection schedule of 7.5 µg HA H5N1. A schedule of 2 x 7.5 µg HA allows for, in theory, a 3-fold increase of production capacity, compared with the seasonal trivalent influenza vaccines.

Adjuvant

Studies on inactivated vaccines using potentially pandemic strains have shown that more antigen per injection and more than one injection are needed to elicit immune responses comparable to those following a single injection of a seasonal trivalent inactivated influenza vaccine. Use of an adjuvant appears to reduce the amount of antigen required to elicit a sufficient immune response.

As clinical trial and post-marketing data on seasonal Fluad demonstrate that MF59 is equally safe and potent when combined with different influenza strains, it was selected as the adjuvant for the Novartis H5N1 avian pre-pandemic vaccine (Fluad-H5N1). As the composition and quantity of MF59 in both seasonal Fluad and Fluad-H5N1 are identical, this adjuvant is expected to have the same safety and potency to protect against pandemic influenza strains.

This protection may extend to drifted strains. Hence, even a poorly matched MF59-adjuvanted vaccine can prime a previously naive population and may confer a certain degree of protection against the pandemic influenza virus. Taken together, the evidence suggests that MF59 permits lower dosing while simultaneously mitigating pandemics caused by other H5 strains.

The Parent Vaccine, Seasonal Fluad

Seasonal Fluad (surface-antigen, inactivated adjuvanted with MF59) has been licensed since 1997 and currently is the only adjuvanted influenza vaccine on the global market. Fluad was registered for the prophylaxis of influenza in Europe in 1997 and is currently authorized in 23 countries worldwide. Consequently there is an extensive clinical database on its safety and immunogenicity, which, as previously suggested, does not change after modification of the strain composition.

Since the proposed antigen content of the pre-pandemic vaccine (2 x 7.5µg H5N1) is 3-fold less than that of seasonal Fluad (15µg H1N1, 15µg H3N2, 15µg B) while the adjuvant content remains the same, the Fluad safety database would be expected to overestimate the incidence rate for adverse reactions to Fluad-H5N1. The "head to head" phase III trial (V87P4) has shown that safety profiles following two injections with 7.5µg Fluad-H5N1 are at least as good as those following two injections of seasonal Fluad.

The clinical safety database on seasonal Fluad, which does not yet include the Fluad control (over 1000

subjects) in the phase III study V87P4, has been evaluated in clinical trials in adolescents (N=116), 18-64 years (N=812) and the ≥65 years (N=12,818), beginning in the 1992/93 influenza season. Approximately 14,000 subjects were administered one vaccination, 596 subjects received two vaccinations and 150 subjects three vaccinations. During this period, more than 27 million doses have been distributed.

Analysis of post-marketing data collected from September 1997 to April 2006 demonstrates a good safety profile (Table 2.5.5-3).

Table 2.5.5-3 Case Distribution (N=385) by Special Search Categories

Search Category	Number of cases [a]	Percentage of cases reported [a]	Percentage of ADR-Case Profile	Reporting rate (100,000 doses)
Systemic reactions	113	29	27	0.5
Injection site reactions	132	34	31	0.5
Allergic reactions	39	10	9	0.1
Neurological events	58	15	14	0.2
Vascular disorders	9	2	2	0.03
Others	74	19	17	0.3
Total	425[a]	109 [a]	100	

Target Population

While authorized seasonal Fluad is indicated for those aged 65 years and over, it is possible that in the next pandemic about half the associated deaths would initially occur also among those younger than 65 years of age. The proposed indication for Fluad-H5N1 therefore includes all adults (i.e., ≥18 years of age). and it is anticipated that a variation for the inclusion of the paediatric indication will be filed as soon as the appropriate clinical data will be available.

The CHMP Day 120 LoQ solicited the compliance of a paediatric investigation plan.

As a preliminary outline of the paediatric studies planned, the company informs that a Phase II clinical study to assess the safety and immune response of two doses, administered three weeks apart of Aflunov in paediatric subjects is planned to start within 2007 in Finland.

A protocol for this study has been submitted [*Study V87P6: A Phase II, Randomized, Controlled, Observer-blind, Single-center Study to Evaluate the Immunogenicity, Safety and Tolerability of Two Doses of FLUAD-H5N1 Influenza Vaccine in Subjects aged 6 months to 17 years*].

II.2 General comments on compliance with GMP, GLP, GCP

No GMP issues have been raised with the MAA for Aflunov.

GLP aspects

The non-clinical testing for Fluad supports the efficacy and safety of Fluad H5N1 and in general conforms with the available regulatory guidance for the non-clinical testing of influenza vaccines and adjuvant. For the pharmacological aspects, the immunogenicity and non-clinical efficacy (challenge studies) were the objects of the non-GLP studies in mice and ferrets. The toxicology program reported in the specific part of the dossier, was designed on appropriate global regulatory requirements for the non-clinical testing of vaccine and adjuvant and was performed, except for single-dose toxicity studies, in GLP studies that assessed local tolerability, single-and repeat-dose toxicity, and recovery or delayed responses.

Finally the nonclinical studies that have been performed specifically with Fluad H5N1, (immunogenicity/challenge study in ferret model (Study CBI-PCS-008) and the "Reproductive toxicity study in rabbits" (Study No.UBA00021) are in compliance with GLP.

Relevant documentation is present with the various study reports on GLP aspects.

GCP aspects

CHMP has adopted a GCP inspection request of some sites in Poland of the pivotal clinical trial V87P4. The results of the GCP inspection are considered an integral part to the further evaluation of the dossier.

A GCP inspection of the pivotal study V87P4, requested by CHMP, was conducted at 5 sites in Poland.
Taking into account the results of the CHMP requested inspection, as summarised in the Integrated Inspection Report (IIR) issued on 7 April 2008, and in addition the outcome of an earlier Lithuanian GCP inspection and the events at two other sites in Poland, the GCP inspectors provided the following assessment of the GCP compliance of study V87P4 in order to evaluate the full impact of the inspection findings as well as the critical GCP issues at sites not inspected in the context of the CHMP inspection request:
a) The data from 4 out of 23 sites has been/has to be excluded;
b) The data from other 4 inspected sites have been found on inspection to be suitable for assessment purpose;
c) The remaining 15 sites have not been inspected.

Considering that 4 out of 23 sites and 1042 out of 4560 subjects (corresponding to the 23% of subjects) have been excluded due to serious violation of GCP, the quality of the sponsor control must be regarded as questionable. The majority of the findings resulting in the exclusion of sites from the study analysis have not been identified by the sponsor or sponsor's representatives' action, but due to inspection or other external intervention (the sponsor did report the use of an unapproved patient recruitment advertisement to the Lithuanian ethics committee leading to the halting of the study there and the ensuing investigation, and the exclusion of that site (031) was identified in the submitted clinical study report). Of the remaining sites, the majority (15) have not been inspected and their quality cannot be assured. Other findings made during the inspection such as inclusion of some vulnerable people into the study, inadequate medical records, changes to the inclusion criteria without appropriate competent authority/ethics committee approval and lack of feedback of results to the sites also support the conclusion of a lack of adequate quality oversight of the study by the sponsor and the sponsor's representative.

Therefore based on all above facts:

- The trial cannot be considered to have been conducted in accordance with GCP as required by Annex I of Directive 2001/83/EC.

- The statement provided in the clinical overview and in the Clinical Study Report concerning GCP compliance can no longer be considered valid.
- It is recommended that the study V87P4 is not used for evaluation in connection with the examination of Aflunov MAA.

II.3 Type of application and other comments on the submitted dossier

This dossier is based on the Focetria dossier that received positive CHMP opinion on February 2007 and a Marketing Authorisation under exceptional circumstances in the EU in May 2007 (EU/1/07/385/001-4).

The Aflunov MAA was the first application for a pre-pandemic vaccine. All the EMEA guideline were released after the application; also the assessment of the submitted risk management plan was premature as at that time the ad hoc expert group had not yet written the Core RMP for pre pandemic vaccine.

III. SCIENTIFIC OVERVIEW AND DISCUSSION

III.1 Quality aspects

A complete and independent application was filed by Novartis Vaccines & Diagnostics S.r.l. for Aflunov**,** an Influenza Vaccine for Human Use (Monovalent, H5N1, Surface Antigen, Inactivated, Adjuvanted with MF59C.1), for the prophylaxis of H5N1 avian influenza in adults and elderly (18 years of age and over).

This application has been prepared taking into account the concepts expressed in the CHMP/VWP/263499/2006 guideline and in accordance with the recommendations of the Scientific Advice.

As Pandemic viral strains may be highly pathogenic, they could need attenuation by Reverse Genetics (RG) technology in order to allow their use in production of egg-derived vaccines. The avian virus seed, strain H5N1, prepared from RG technology has been used for the production of Aflunov.

Aflunov is manufactured with the same process and has the same adjuvant as Fluad, a Novartis seasonal, trivalent influenza vaccine that has been licensed since 1997 in Italy and since 2000 in 13 EU countries (Mutual Recognition Procedure No. IT/H/0104/001). It is currently on the market and from 1997 to 2006, more than 25 million doses of Fluad have been distributed worldwide. The company's application for the pandemic vaccine "Focetria", based on the same H5N1 RG strain, and produced by the same manufacturing process, was approved by CHMP and granted a marketing authorisation under exceptional circumstances in the EU in May 2007 (EU/1/07/385/001-4).

The MF59C.1 adjuvant contained in Fluad, Focetria, and Aflunov is an oil-in-water emulsion, composed mainly of squalene that is an intermediate metabolite in the synthesis of cholesterol.

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. 45µg HA/dose).

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

Most of the quality related issues pertaining to Aflunov have already been raised during evaluation of the Focetria dossier and have been addressed and taken into account by the company for the present application. As a consequence, most of the Day 120 quality-related issues can be rated as solved for the present Aflunov application.

No major objections for both the drug substance and the drug product were included in the Day 120 CHMP List of outstanding issues.

Regarding the "other concerns", all questions have been satisfactory addressed.

Active substance

The Drug Substance of the product is the Monovalent Pooled Harvest (MPH) of H5N1 avian influenza vaccine, surface antigen, inactivated. It is a buffered suspension containing predominantly the purified outer membrane proteins, Haemagglutinin (HA) and Neuraminidase (NA), of the H5N1 avian influenza virus strain.

Production of MPH involves the cultivation of the avian influenza virus strain in embryonated chicken eggs, harvesting of allantoic fluid, concentration 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 cetyl trimethylammonium bromide (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.

In general the manufacturing process is well documented, and the in-process controls through the production and the purification process are extensive and assure compliance with the Ph.Eur., where required.

A few issues, referring to the drug substance, were raised in the D120 CHMP list of questions.

As requested, the Applicant has completed the description of a few steps of the manufacturing process, and specified the critical parameters specifically used for the manufacturing of the H5N1 NIBRG-14 strain batches presented in the application.

With regard to the stability of the drug substance, the data provided are consistent with a shelf -life of 18 months when stored at 2-8°C. The applicant will continue to collect further stability data for the drug substance to evaluate the possibility of an extension of the shelf-life according to the stability protocol submitted; furthermore the potential impact of a long holding period on the quality of the finished product and its shelf life should be addressed during stability studies. In addition, currently retesting period for biological substances is not acceptable.
Concerning the primary seed quality-related issues that have come up in the course of the assessment in relation to the use of a reverse genetic (RG) derived influenza strain, the Applicant has committed to adhere to the requirement to update the dossier according to any future novel regulatory guidance, and to submit additional data for confirming the genetic stability of the RG derived virus strain included in the vaccine.

Finished Product

The Drug Product is a combination of MPH, MF59C.1 adjuvant bulk and buffer solutions.

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. MF59C.1 Adjuvant Bulk is manufactured in German Novartis manufacturing site (Marburg) and then transported to Novartis Rosia (Italy).

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

In general, the manufacturing process is well documented, and the in-process controls assure compliance with the Ph.Eur., where required. A few issues, referring to the drug product, were raised in the D120 CHMP list of questions.

All the questions concerning the manufacturing and control of the drug product has been addressed satisfactorily and only a couple of issues remains to be solved by follow up measures.

With regard to the stability of the drug product, the data provided are consistent with a shelf life of 18 months when stored at 2-8 ° C. Updated stability data for the pre-filled syringes of H5N1 Aflunov are provided justifying the proposed shelf life of 18 months when stored at 2-8°C. The Company commits to report any out-of-specifications results or unexpected trend coming from any future stability study for Aflunov.

If the marketing authorisation holder wants to further extend the shelf life, non-clinical and/or clinical investigation might be considered necessary and the testing program should be discussed with the competent authorities, as stated in the “Guideline on influenza vaccine prepared from viruses with potential to cause a pandemic and intended for use outside of the core dossier context“ (EMEA/CHMP/VWP/263499/2006).

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

Conclusion on Quality aspects

In conclusion, all quality issues are resolved and, with regards to the quality of Aflunov, the application could be approved.

III.2 Non clinical aspects

Novartis V&D S.r.l. submitted through a Centralised Procedure a new Marketing Authorization Application (MAA) for the vaccine Aflunov (referenced as Fluad H5N1). This vaccine is intended for the prophylaxis of influenza before the pandemic situation is declared.

This application takes 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).

Fluad H5N1 is manufactured using the same process and contains the same adjuvant used for Fluad, a Novartis seasonal, trivalent influenza vaccine licensed through a MRP (procedure No. IT/H/0104/001) and marketed in several European countries as well as outside Europe.

This submission consists of a dossier for Fluad H5N1 derived from a vast collection of data generated on selected vaccine licensed formulations equivalent to Fluad H5N1 (Novartis' Agrippal and Fluad).

A pandemic mock-up vaccine, in accordance to the CPMP/VEG/4717/03 "Guideline on Dossier structure and Content for Pandemic Influenza Vaccine Marketing Authorization Application" has received a marketing authorisation under exceptional circumstances in the EU in May 2007 (EU/1/07/385/001-4). (Focetria) . This mock-up vaccine is based on studies using H5N1, H9N2 and H5N3 strains manufactured and controlled according to an identical process as the one presented in this application.

In the first application, in line with the available regulatory guidance for the non-clinical testing of influenza vaccines and adjuvant, the Company provided non-clinical programs for Fluad as support of the efficacy and safety of Aflunov. This was the pandemic-like vaccine based upon virus surface antigens, propagated in eggs, of strain A/HongKong/1073/99 (H9N2)-like strain (A/Chichen/Hong Kong/G9/97), adjuvanted with MF59C.1.

Although no major objections regarding the non-clinical section have been raised, some aspects have been evidenced. The analysis of these data indicated that Aflunov is immunogenic, and that the antibodies induced by the vaccine cross-react with at least one heterologous strain of H5N1 virus both in mice and ferret. The vaccine was effective in preventing clinical signs of illness/death and viral replication in brain, lung and spleen, in mice challenged with homologous or heterologous virus.
The protective efficacy data from the the studies on ferret are more persuasive, and therefore, at the time of the responses to LoQ the submission of the final report including statistical analysis of this study was warranted.

Pharmacology

The only question from the efficacy point of view regards the lack of the studies on cross-reactivity and cross-protection of the candidate vaccine. So EMEA asked the applicant to investigate this aspect in appropriate animal model.

In response to this question, Novartis has provided new data on cross-protection and cross-reactivity of the candidate vaccine by performing studies on mice and ferrets.

Mouse studies. A series of cross-protection/cross-reactivity experiments was conducted to assess the immune response and protection from homologous and heterologous viral challenge following vaccination with Aflunov and non-adjuvanted H5N1 antigens. As these data were generated and are owned by the NIH (National Institute of Health, US), an NIH report or a publication are not yet available. The Applicant updated the specific sections of the dossier (Sections 2.4, 2.6.2 and 2.6.3) and the results were described in detail in sub-section 2.6.2.2.

The details of responses were reported in the section of Assessment of the responses to the CHMP List of Questions.

Ferret studies.

To date, two ferret homologous challenge studies have been performed. The summaries of the results of these studies have been added in the relative sections of Nonclinical overview and summaries.

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*.

This study has already been presented in the dossier in section 4.2.1.1 [Study CBI-PCS-008]. Now new data on serological cross-reactivity have been obtained and the Sections 2.4, 2.6.2, and 2.6.3 of the dossier have been updated (a detailed summary of this study is presented in sub-section 2.6.2.2.1).

Concerning this study, EMEA asked the Applicant to clarify which parameters monitored in the ferret showed a significant effect of the vaccine compared to control. The response and the relative assessment were reported in the section of Assessment of the responses to the CHMP List of Questions. However in the last analysis, Novartis underlined that the results of the ongoing ferret study (see below) are very straightforward.

Study No. 673-N106850: *Evaluation of the protective efficacy of Fluad H5N1 vaccine in ferrets challenged with a highly pathogenic avian influenza (HPAI) virus.*

In this ongoing study, Novartis plans additional studies to demonstrating nonclinical cross-protection and cross-reactivity. In this study, ferrets were vaccinated with Fluad H5N1 prior to challenge with a highly pathogenic avian influenza (HPAI) virus, A/Vietnam/1203/04. The study design is summarized below:

NonClin Table n. 1 - Experimental design -Study No. 673-N106850

Group	N	Treatment	VaccinationDay	HA Dose (ug); Volume (mL)	Challenge day *
1	8	MF59 Control	0, 21, 42	0; 0.5	65
2	8	Fluad H5N1	0, 21, 42	7.5; 0.5	65
3	8	Fluad H5N1	21, 42	7.5; 0.5	65
4	8	H5N1+CpG	42	7.5; 0.53[a]	65
Study evaluations (from beginning on study Day57 until the end of the study on Day84) *-Body weights* *-Body temperatures* *-Clinical observations (twice daily)* **(Days 42, 65 and 67)** *-Clinical chemistry* *-Haematology* *-Antibody analysis (HAI and MN assays)* *-Viral titer in nasal washes*					

[a] the nominal volume contributed by the 250 micrograms of CpG (10 mg/mL as supplied) is 0.025 mL

*Ferrets were challenged on day 63 with approximately 0.6 mL of PBS containing a target 1×106 TCID50 Influenza A/Vietnam/1203/04 was instilled intranasally (0.3mL into each nostril).

Major findings: Four of eight control animals died. There was no mortality in animals that received Fluad H5N1 vaccine. Interim body weight, body temperature, nasal viral titers, and homologous immunogenicity data show that one or two doses of Fluad H5N1 were immunogenic, prevented body weight loss and fever, and decreased nasal viral titers.

These studies indicate that Aflunov is immunogenic, and that the antibodies induced by the vaccine cross-react with at least one heterologous strain of H5N1 virus both in mice and ferret. The vaccine was effective in preventing clinical signs of illness/death in mice and ferrets and viral replication in brain, lung and spleen, in mice challenged with homologous or heterologous virus and in ferrets challenged with homologous virus.

Concerning the studies on ferret, the protective efficacy data from the second study 673-N106850 are more persuasive, and therefore, submission of the final report including statistical analysis of this study is warranted.

Toxicology

In the first application, the Company has provided the reproductive toxicity study design of Fluad H5N1 in rabbits (Study N° UBA00021). This study is planned according to the EMEA guideline on pre-pandemic influenza vaccines (Doc. Ref. EMEA/CHMP/VWP/263499/2006) that states that animal reproductive studies should be performed. Therefore EMEA asked to the Company to complete this important study prior to authorization.

As requested, Novartis has concluded this GLP reproductive and developmental toxicity study in rabbits. The sections 2.4, 2.6.6, and 2.6.7 have been updated and are included in this application.

The study is briefly summarized below and is presented in detail in sub-sections 2.6.6.6, 2.6.7.13, and 2.6.7.14.

Study NoUBA00021: Intramuscular reproductive and developmental toxicity of Fluad H5N1 in rabbits, including a postnatal evaluation.

The object of this study was to assess the potential effects of Fluad H5N1 on reproductive and developmental toxicity in female rabbits and their fetuses or pups when administered by intramuscular injection at the 2 x clinical dose of 7.5 μg, before mating and during gestation. The study design is summarized below:

NonClin Table n. 2 - Study No. UBA00021 – Study Design

Group	Number of rabbits	Dosage (μg)/Injection volume (mL)[a]		Evaluations
		Day of study 1,15 and 29	Day of gestation 7 and 20	
Rabbits assigned to Caesarean-sectioning (day 29 of gestation)				Maternal and embryofetal toxicity and teratogenic potential
1	20	0/0.5	0/0.5	
2	20	15/0.5	15/0.5	
Rabbits assigned to natural delivery (day 29 of lactation)				
3	20	0/0.5	0/0.5	
4	20	15/0.5	15/0.5	

a Group 1 and 3 animals were administered saline as a control article. Group 2 and 4 animals were administered Aflunov (total volume 0.5 ml containing 15 μg antigen and 0.25 ml MF59 adjuvant). After 35 days on study, rabbits were mated (gestation day 0). Group 1 and 2 animals were euthanized at the end of the gestational period, on or about day 29.

The design of this study is well organized to assess both reproductive and developmental toxicity. Under the conditions of this study, Fluad H5N1 is well-tolerated, does not cause maternal or embryofetal toxicity, is not teratogenic, and has no effects on post-natal development. Additionally, the vaccine is immunogenic in maternal rabbits, in fetuses until to the first 4 weeks of life in F1 pups.

The results of this study are reported in detail in the Assessment of the responses to the CHMP List of Questions.

Conclusions of Non Clinical aspects

The issues on cross-reactivity and cross-protection can be considered solved, but the original studies reports are lacking. The Applicant should provide them in order to complete the present Application.

Considering that the study on "Reproductive and developmental toxicity of Fluad H5N1 in rabbits, including a postnatal evaluation", tested relatively high multiples of human doses for a vaccine product (five exposures to levels approximately 30-fold the expected clinical exposure to 7.5 μg on a body weight basis) it can be supposed that Fluad H5N1 is safe also in the human use from this point of view.

In Study N° 673/N106850 evaluation of the protective efficacy of Fluad H5N1 vaccine in ferrets challenged with HPAI (highly pathogenic avian influenza) was performed: it is noted that only 4/8 control animals died following challenge with the HPAI virus A/Vietnam/1203/04, whereas 100% of animals would be expected. The applicant should comment.

Data on viral replication in different tissues (e.g. brain and lung) on ferrets Study N° 673/N106850 with HPAI should be provided.

III.3 Clinical aspects

Clinical efficacy

Overview of Clinical Pharmacology

This section presents data and discusses the results for the early dose-findings studies on Fluad-H5N3 and Fluad-H9N2 using non-adjuvanted comparators. The results of these studies were the basis for the designs of the immunogenicity clinical trials on Fluad-H5N1.

Serology according to CHMP criteria and MN assay results for Fluad-H5N3 and Fluad-H9N2

In studies V7P37 and V7P37E1 on 7.5-30µg formulations of Fluad-H5N3, GMRs and seroconversion results based on HI assays were substantially lower than SRH assay after the second and third vaccinations and did not consistently meet CHMP criteria. HI seroprotection results did not meet CHMP criteria after any of the three vaccinations for any formulation. For individual subjects SRH GMT increases were 3 to 9 times larger than those assessed by HI assay, after the second vaccination, with an even larger difference after the third vaccination. It seems likely that these results are consistent with the earlier reports of lowered sensitivity of the HI assay to H5 viruses.

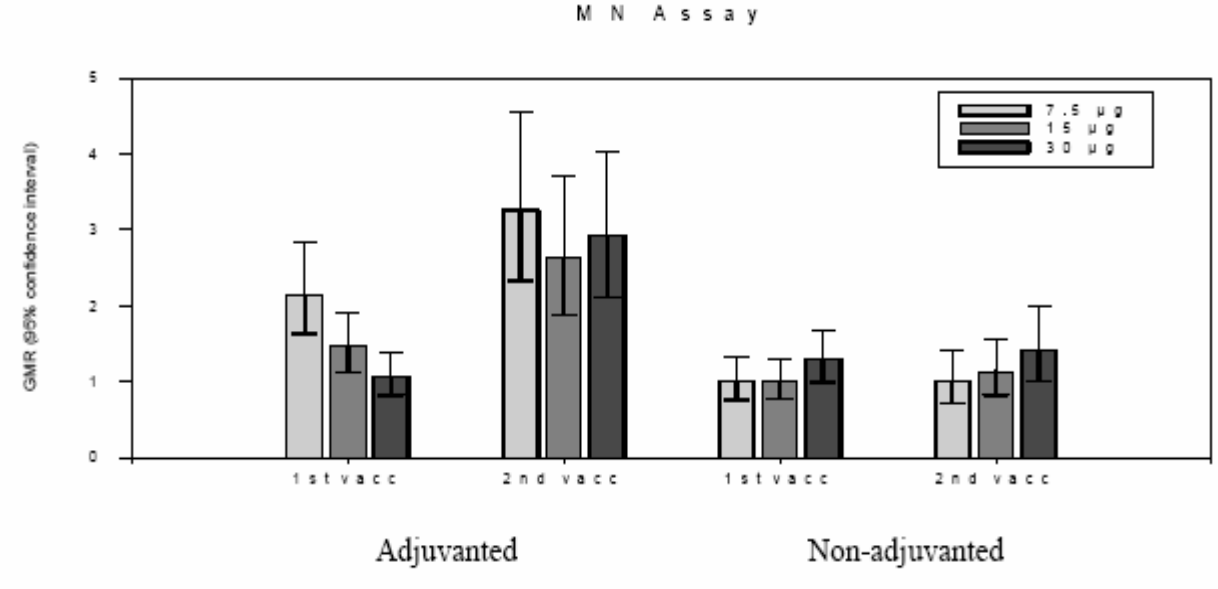
It is unlikely that the HI assay insensitivity to the H5 virus strain was due to a low potency since both SRH and MN assay results demonstrated strong immune responses. Subject samples analyzed using the MN assay in studies **V7P37** and **V7P37E1**, demonstrated 2.64 to 3.25-fold mean titer increases above baseline after the second vaccination and 18 to 33-fold increases above baseline after the third vaccination.

Although MN GMRs were not as high as those assessed by SRH assay (SRH = 18 to 23- fold above baseline) after the second vaccination, the boosted immune responses determined by both assays 21 days after the third vaccination was comparable (SRH, 34 to 35-fold above baseline). Additionally, in study V7P37, an apparent inverse dose-response was noted for Fluad-H5N3 (e.g. SRH: 7.5µg GMR met CHMP criterion, but not 15µg or 30µg GMRs after the first vaccination). Although similar responses have been reported with other adjuvanted vaccines, sample sizes were too small to speculate on the immunogenicity-enhancing effects of MF59 in relation to the relative quantities of antigen. In contrast, for Fluad-H9N2 after two injections of 3.75µg, 7.5µg, 15µg, and 30µg HA all three CHMP criteria were met by HI assay. Results with MN assay for percentages of subjects with four-fold increase were similar to seroconversion (i.e, 4-fold-rise/sign. increase) as assessed by HI. However, these immunogenicity data have little relevance to Fluad-H5N1 since H9 viruses induce greater immune responses than H5 viruses.

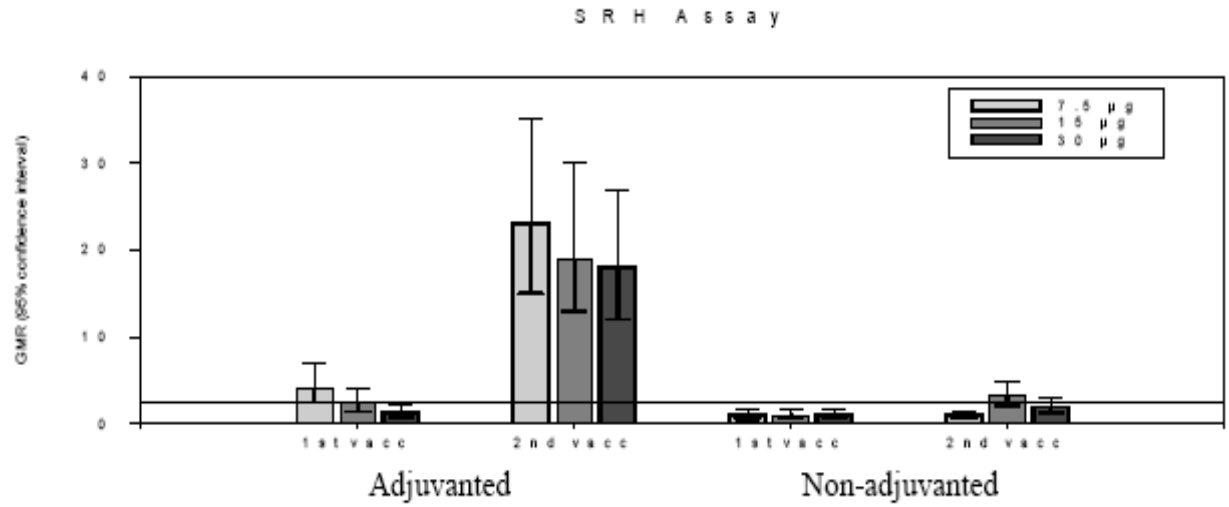
Selection of dose, dose schedule, and formulation

The first studies (**V7P37** and **V7P37E1**) used Fluad-H5N3 and non-adjuvanted comparator because at that time an H5N1 strain that could be propagated in eggs was not available. However, H5N3 and H5N1 are antigenically similar. For non-adjuvanted formulations, the 7.5µg, 15µg, and 30µg HA H5N3 did not induce a clear and consistent immune response after either one or two vaccinations. In contrast a single injection of 7.5-30µg of Fluad-H5N3 (Figure 2.5.3-1 and Figure 2.5.3-2) and 3.75 to 30µg of Fluad- H9N2 already induced increases in GMTs after the first vaccination. These were further increased following the second vaccination. As the sample sizes were small and the 95% CIs broad, there was no evidence that 15 and 30µg HA induced greater responses than 7.5µg HA Fluad-H5N3. Interestingly, and consistent with published reports, there was a tendency across assays for the highest titers to be observed in recipients of the adjuvanted formulation with the lowest antigen content (7.5µg) of Fluad-H5N3.

Cl_Figure n. 1 - Geometric Mean Ratios of Neutralizing Antibodies, Determined by MN, After One and Two Injections of Three Formulations of Fluad-H5N3 and Non-adjuvanted H5N3 Vaccine (V7P37)



Cl_Figure n. 2 - Geometric Mean Ratios of Heamagglutination Antibodies Determined by SRH After One and Two Injections of Three Formulations of Fluad-H5N3 and Non-adjuvanted H5N3 Vaccine (V7P37)



Overall, the results of this trial (V7P37) as assessed by MN and SRH assay suggest that the 2x7.5µg Fluad-H5N3 is sufficient to induce an immune response in 18-40 year olds with no benefit of higher formulations (i.e, 15µg or 30µg).

Persistence and Third Injection (V7P37E1)

In study titers against the H5N3 strain had returned to undetectable levels in adjuvanted and non-adjuvanted groups after 17 months as measured by HI and MN assays and SRH assay bordered on undetectable for recipients of 7.5 and 15µg HA but were higher for recipients of 30µg HA H5N3. A noteworthy 34- to 35-fold increase of titers over pre-booster levels were observed in recipients of Fluad-H5N3 when assessed by SRH and 18- to 33-fold increases were observed when titers were assessed by MN assay, suggesting a strong induction of immunological memory after the primary vaccination.

Protection against Heterovariants

The ability for MF59 to induce broad cross-reactive immune responses could be crucially important in the early response to an emerging pandemic, when global demand for vaccine would exceed production capability. The immunopotentiating ability of adjuvants could not only optimize the use of limited antigen, but also enhance broader cross-reactivity against drifted strains. Even assuming that the next pandemic strain is a mutation of the current H5N1 strain responsible for human infection, the antigenic characteristics of that mutation are not predictable.

***Comment**: Follow-up data on antibody persistence and immune response to a heterologous booster dose should be assessed, even post approval.*
In the meantime, the lack of data as regards this concern should be mentioned in section 5.1 of the SPC.

As shown in two H5N3 studies (V7P37 and V7P37E1), antibody titers were induced by Fluad-H5N3 against multiple clinical isolates of H5N1. The same cross-reactivity was not demonstrated for non-adjuvanted formulations, it can be hypothesized that MF59 could cross-protects and primes against antigenically similar avian strains. This is consistent with what has been repeatedly observed for inter-pandemic seasonal Fluad.

Inter-laboratory Consistency

Given the inter-laboratory variability reported for serologic assays, demonstration of inter-laboratory correlation is an important element to support the validity of the immunogenicity results. As a matter of fact, the assessment of correlation and concordance of immunogenicity results from the large phase 2 study, V87P1, showed a good agreement between the Novartis and the UK HPA for both the HI assay and the MN assay. These correlations were comparable to those noted between Baylor College of Medicine and CDC laboratories in study DMID 04-019. Intra-laboratory correlations were also high for the HI and MN assays.

The Applicant concluded that correlation and concordance studies, based on subsets of V87P1 samples, showed excellent agreement in both HI and MN assay results produced by Novartis and HPA laboratories and within the HPA laboratory. Thus, both laboratories appear to be measuring the same immune responses with a high degree of consistency. The correlations between the University of Siena laboratory SRH and HPA laboratory HI and MN assay results, between the University of Siena laboratory SRH and Novartis laboratory HI and MN assay results, and within the Novartis laboratory are lower than the correlations between HPA and Novartis, but are still quite high. In these instances, the laboratories also appear to be measuring the same immune response with an acceptably high degree of consistency.

Conclusions of Clinical Pharmacology

- Two injections of 7.5µg Fluad-H5N3, administered 21 days apart, produced sufficient levels of antibody to meet all three CHMP criteria when assessed by SRH but not all criteria by HI.
- MF59 adjuvant significantly enhanced the immune responses, persistence, and probably trend of cross-protection could be observed.
- The general agreement between GMRs and the percentages of subjects achieving at least 4-fold increases in titers by MN and SRH indicate that pre-pandemic Fluad-H5 formulations induced a clear immune response.

Overview of Efficacy

Clinical Studies

Two clinical studies have been conducted with H5N3 strains to investigate regimen, adjuvant, and antigen amount. Based on the results of those studies, three studies have been conducted using Fluad formulated with H5N1 (Table 1).

Cl_Table n. 1 - Overview of Studies

Study (Year)	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects Exposed: Test / Comparator	Duration	Healthy Subjects or Diagnosis of Patients	Study Design
V87P1 [a] (2006-2007)	Fluad-H5N1 Two injections of 7.5 or 15µg, IM: 6 months booster	485 Fluad-H5N1	13 months[b]	Healthy 18–60 and >60 year olds	Phase 2 Observer-Blind, Randomized
V87P2 [a] (2007) [a]	Fluad-H5N1 Two injections of 7.5- or 15-µg, IM; 6 months booster	27 Fluad-H5N1 13 Nonadjuvanted formulation [c]	13	Healthy 18–60 years olds	Phase 2 Observer-Blind, Randomized
V87P4 (2007)	Fluad-H5N1 Two injections of 7.5 IM	3168 Fluad-H5N1 1058 seasonal Fluad	7	Healthy 18–60 and >60 year olds	Phase 3 Observer-Blind, Randomized
V7P37 (1999)	Fluad-H5N3 Two injections of 7.5-, 15- or 30-µg IM	32 Fluad-H5N3 33 Nonadjuvanted formulation [c]	6 weeks	Healthy non-elderly adults 18-40 years	Phase 1 Observer-Blind, Randomized
V7P37E1 (2000)	Fluad-H5N3 One injection of 7.5-, 15- or 30-µg IM	17 Fluad-H5N1 11 Nonadjuvanted formulation[c]	3 weeks	Healthy non-elderly adults 18-40 years	Phase 1, Observer-blind Extension
DMID 04-019 (2005)	Fluad-H9N2 Two injections of 3.75-, 7.5-, 15- or 30-µg IM	48 Pre-pandemic Fluad: 48 Nonadjuvanted formulation[c]	7 months	Healthy non-elderly adults 18-34 years	Phase 1, Double-Blind, Randomized

[a] 6 week follow-up of primary vaccination completed [b] 6 month data complete: [c]without MF59: IM = intramuscular

Two H5N3 studies (V7P37 and V7P37E1), one H9N2 study, (DMID 04-019) and a small H5N1 (**V87P2**) study enrolled only 18-60 year olds. Two large Fluad-H5N1 phase 2 and 3 studies (V87P1 and V87P4) enrolled adults both above and below 60 years of age. In general the study populations were representative of the target population for avian influenza vaccination.

The first H5N3 study (**V7P37**) was conducted in healthy 18 to 40 year olds administered two injections of adjuvanted and non-adjuvanted vaccine formulated with 7.5, 15, and 30µg H5N3 viral strain. Seventeen months later, the same subjects were enrolled into an extension study (**V7P37E1**) and were given a third injection of the same formulation previously administered. An H9N2 study (DMID 04-019) was conducted by the NIH in the United States in 2005 on healthy adult subjects (18 to 34 years). This phase 1 study evaluated the immunogenicity and safety of 3.75, 7.5, 15, and 30µg HA Fluad-H9N2 and non-adjuvanted comparator. Although H9 viral strains are more immunogenic than H5 viral strains this study provided useful information on the immunogenicity and reactogenicity of pre-pandemic Fluad when formulated with other potential pandemic strains. This is a small sample size study, but the results of these studies suggested that two 7.5µg HA injections of H5N3 could be sufficient to induce protective antibodies if MF59 adjuvant was used.

Assessors' comment: The potential need of the third dose of vaccine to be administered to achieve a good immunogenicity should be evaluated in the pre-pandemic scenario, because the real benefit of a pre-pandemic vaccine use should be well defined.

Therefore, in 2006, after the availability of the H5N1 seed virus, a larger phase 2 confirmatory study (**V87P1**) was performed to evaluate whether 7.5 and 15µg Fluad- H5N1 had a similar immunogencity and safety profile. Healthy subjects were stratified into adults below and above 60 years of age. The sample size of the per protocol population, which comprised 464 of the 486 randomized subjects, was sufficient to test for non-inferiority (with 80% power) of the immune response induced by 2x7.5µg vaccinations compared with that induced by 2x15µg vaccinations, given 3 weeks apart. The 6 month safety follow-up is completed and all immunogenicity of the primary vaccination and in a subset, a booster, has been analyzed. Persistence, and safety for the 6 month follow-up of the primary vaccination are also evaluated. Six month follow-up safety data after the booster will be reported in an addendum to the CSR.

Assessors' comment: The CHMP guideline requests the complete evaluation of 6 month follow-up safety data after 2nd dose.

Study **V87P1** was powered to assess the non-inferiority of the 7.5µg formulation vs. the 15µg formulation. The lower limit of the 95% CI for HI GMT ratio of 7.5 over 15µg group for the total population was >0.5 (0.68), thus demonstrating that the immune response to 2x7.5µg was not inferior to that of the 2x15µg formulation. In fact all point estimates for the percentages of seroprotected and seroconverted subjects, in addition to the GMRs, were higher in the adults below 60 years after 2x7.5µg compared with 2x15µg injections. In adults above 60 years the immune responses showed greater similarity between both formulations. Based on results obtained by all assays, the decision was made to choose the 7.5µg formulation for registration.

A small phase 2 H5N1 study (**V87P2**) on adults below 60 years investigated the cellular immune responses induced by two injections of 7.5µg or 15µg Fluad-H5N1 or 15µg non-adjuvanted comparator. Humoral immunogenicity, as advocated by CHMP, and safety parameters were also assessed. All data has been analyzed and presented up to 3 weeks after the first and second injection (i.e., first 6 weeks). The 6 month safety follow-up of the primary vaccination is ongoing and this, together with immunogenicity and safety of a booster vaccination administered 6 months later will be reported in V87P2 CSR Part 2.

A large phase 3 H5N1 study (**V87P4**) was conducted on 2927 adults below 60 years and 241 elderly subjects aged above 60 years. All subjects have received 2 x 7.5µg Fluad-H5N1 or 2 injections (total 45 µg HA/injection) of seasonal Fluad (randomization 3:1). The sample size is sufficient to rule out AEs occurring at a rate of 0.1% in adults below 60 years and 1% in adults above 60 years. Safety has been

assessed and analyzed up to 3 weeks after the first and second injection (i.e., primary vaccination). The 6 month follow-up of Fluad- H5N1 and seasonal Fluad will be reported in an addendum/Part 2 CSR.

Assessors' comment: The CHMP guideline requests the complete evaluation of 6 month follow-up safety data after 2nd dose.

Study populations

The total population exposed to Fluad-H5N1 is shown in Table 4.

Cl_Table n. 4 - Study Populations Exposed to Fluad-H5N1

	18-60 Yr [b]		>60 Yr	
Dose	**7.5µg**	**15µg**	**7.5µg**	**15µg**
Enrolled	3098	169	328	86
First injection	3097	169	328	86
Second injection	3035	166	326	80
Booster injection	74	83	38	38

Within each study, demographic characteristics were well balanced among groups and were stratified by age to include both adults below and above 60 years. As expected a higher percentage of adults above 60 years had been vaccinated against influenza in the previous years. The population included in the immunogenicity analysis generally represents the overall population that would be expected to receive the vaccine if it was marketed.

Given the limitations of the HI assay to measure the immunogenicity of pandemic strains, three serological assays (HI, SRH, and MN) were used simultaneously to assess antibody responses to H5N1 viral antigens in all studies presented in this section.

The CHMP criteria for assessment of seasonal influenza vaccines were used to evaluate antibody responses to H5 obtained by HI and SRH. There is no corresponding correlate of protection for MN and therefore GMRs and 4-fold increases in neutralizing antibody titers above baseline may be the most appropriate measures of the immune response since they account for baseline and interlaboratory variation. In addition, several published reports of H5 vaccines have used the 4-fold increase in MN titers as a correlate of protection.

Immunogenicity Results

In the absence of a main assay to determine antibody titers and given the lack of consensus regarding a correlate of protection for the H5N1 avian influenza vaccine the efficacy results are assessed by:

- "Inhibiting antibodies" assayed by HI and SRH using the correlates of protection established by CHMP for interpandemic strains.
- Titers ≥40, GMRs, and four-fold increases above baseline in neutralizing antibodies assayed by MN.

Cl_Table n. 2 - HI and SRH Antibody Responses after Two Injections of Fluad-H5N1

Percentage of Subjects/GMR (95 % CI)									
		18-60 years					> 60 years		
		V87P1		V87P2		V87P4	V87P1		V87P4
		7.5µg	15µg	7.5µg	15µg	7.5µg	7.5µg	15µg	7.5µg
HI Assay		N=150	N=150	N=14	N=13	N=144	N=84	N=79	N=149
	% SP	63% (55-71)	61% (52-69)	79% (49-95)	31% (9-61)	53% (45-62)	65% (54-76)	68% (57-78)	56% (47-64)
	GMR	7.85 (6.02-10)	7.69 (5.89-10)	8.83 (4.52-17)	2.75 (1.37-5.52)	4.7 (3.86-5.73)	4.82 (3.44-6.76)	6.87 (4.84-9.76)	5.48 (4.47-6.71)
	% SC	63% (55-71)	60% (52-68)	79% (49-95)	31% (9-61)	53% (45-62)	57% (46-68)	63% (52-74)	52% (44-61)
SRH Assay		N=148	N=148	N=14	N=13	N=143	N=84	N=79	N=149
	% SP	86% (79-91)	85% (78-90)	86% (57-98)	38% (14-68)	81% (74-87)	80% (70-88)	81% (71-89)	70% (62-78)
	GMR	7.83 (6.68-9.18)	6.81 (5.81-7.99)	8.12 (4.61-14)	2.27 (1.26-4.09)	6.32 (5.33-7.49)	4.92 (3.84-6.32)	3.99 (3.08-5.17)	5.01 (4.16-6.03)
	% SC	85% (78-90)	80% (72-86)	86% (57-98)	38% (14-68)	79% (71-85)	70% (59-80)	68% (57-78)	66% (58-74)

The results for MN titers ≥40 and 4-fold increases were generally similar to those obtained for SRH seroprotection and seroconversion when compared for adults below 60 years in V87P1, V87P2, and V87P4. Generally for adults above 60 years the results were slightly lower when assessed by MN. As observed for SRH and HI, there was a tendency for higher responses in the 7.5 than 15µg formulation in adults below 60 years (Table 3). The closer agreement of GMR, seroprotection, and seroconversion rates between SRH and MN than between HI and SRH or MN, support reports of others that HI assay is not sensitive enough to measure antibodies to H5 pandemic strains.

Cl_Table n. 3 - MN Response after Two Injections of the Fluad-H5N1: Non-elderly and Elderly Adults

Percentage of subjects (95 % CI)								
	18-60 years					>60 years		
	V87P1		V87P2		V87P4	V87P1		V87P4
	7.5µg	15µg	7.5µg	15µg	7.5µg	7.5µg	15µg	7.5µg
	N=150	N=150	N=14	N=13	N=144	N=84	N=79	N=149
MN ≥20	91% (86-95%)	88% (82-93%)	ND	ND	78% (70-84%)	89% (81-95%)	82% (72-90%)	74% (67-81%)
MN ≥40	85% (78-90%)	81% (73-87%)	93% (66-100%)	54% (25-81%)	64% (55-72%)	79% (68-87%)	76% (65-85%)	56% (47-64%)
MN ≥80	66% (58-74%)	64% (56-72%)	ND	ND	31% (23-39%)	54% (42-65%)	58% (47-69%)	37% (29-45%)
MN 4-fold	83% (76-89%)	77% (70-84%)	86% (57-98%)	54% (25-81%)	63% (55-71%)	58% (47-69%)	61% (49-72%)	54% (45-62%)

As shown in Table 5, for Fluad-H5N1 7.5µg, all CHMP criteria were met by SRH in all studies and in both age groups. As far as HI is concerned, all three CHMP HI criteria were only met by adults below 60 years vaccinated with 7.5µg Fluad-H5N1 in study V87P2 and by adults over 60 years vaccinated with both the 7.5µg and 15µg formulations in study V87P1. Across all studies the seroprotection criteria was commonly not met with HI although GMTs increased in accordance with CHMP requirements, hence suggesting that naive subjects are less likely to attain high HI titers.

In addition there is a considerably lower immunogenicity that has been found with batches employed for the safety study V87P4 as compared to those of study V87P1. This might raise the question whether a third dose will be required for the complete protection at least in a pre-pandemic scenario.

Results achieved in the V87P2 should be interpreted carefully, because of the small number of subjects enrolled.

Cl_Table n. 5 - Assessment of CHMP Criteria after Two Injections of Fluad-H5N1

		18-60 years					>60 years		
		V87P1		V87P2		V87P4	V87P1		V87P4
		7.5µg	15µg	7.5µg	15µg	7.5µg	7.5µg	15µg	7.5µg
HI Assay		N=150	N=150	N=14	N=13	N=144	N=84	N=79	N=149
	% SP	-	-	+	-	-	+	+	-
	GMR	+	+	+	+	+	+	+	+
	% SC	+	+	+	-	+	+	+	+
SRH Assay		N=148	N=148	N=14	N=13	N=143	N=84	N=79	N=149
	% SP	+	+	+	-	+	+	+	+
	GMR	+	+	+	-	+	+	+	+
	% SC	+	+	+	-	+	+	+	+

Conclusions of Clinical Efficacy

- The detection of antibody by HI assay, is not adequately sensitive for the detection of antibody to avian hemagglutinin and cannot be used to assess H5 vaccines. Therefore, in order to have a more precise assessment of the immunogenicity of the pre-pandemic vaccine, two other assays, Single Radial Hemolysis and Micro Neutralization, have been used.

- All three assays show a significant increase in antibody titers after two injections administered three weeks apart and all CHMP immunogenicity criteria are met when using the SRH assay.

- The immune responses induced by 2x7.5µg Fluad-H5N1 was at least as good, if not better, than that induced by the 15µg formulation and induced immunological memory as shown by the booster response 6 months later. Based on these results the 7.5µg formulation, which in addition to optimal immunogenicity offers also an option for dose-sparing, has been selected for registration.

- The MF59-adjuvanted H5N1 (clade 1) vaccine also induced antibodies that cross-reacted against clade 2 H5N1 strain and there is no evidence that MF59 induced cell-mediated responses with a TH2 functional phenotype (generally associated with allergic reactions) while driving the production of memory cells.

III.3.1 Clinical safety

Since the antigen content of 7.5µg Fluad-H5N1 is 6-fold less than that for licensed seasonal Fluad, the safety database for the latter is expected to give a worst-case estimate of incidence rates of possible adverse reactions. The good safety profile of the licensed Fluad is based on extensive clinical experience in over 40 clinical trials on approximately 14,000 subjects in addition to post-marketing surveillance from more than 27 million doses sold.

In three H5N1 studies, safety profiles were evaluated in 3253 adults below 60 years following 6611 injections and two of these studies also evaluated safety in 414 adults above 60 years following 886

injections: totalling 7497 injections in 3667 subjects. Over 99% of subjects enrolled were vaccinated and included in at least one safety analysis.

Solicited AEs (local and systemic reactions) are selected local and systemic AEs routinely monitored in vaccine clinical studies as **indicators of vaccine reactogenicity**. It is recognized that each of these events, and particularly those of a systemic nature, may under some circumstances, in any individual subject, have a cause that is unrelated to the study vaccine. However, as a matter of convenience and in accordance with common clinical practice, all such events occurring within 7 days after immunization are herein termed solicited AEs.

Most solicited reactions to Fluad-H5N1 were mild or moderate in severity, onset near the time of injection, and lasted no more than a few days. The reactogenicity profiles of the 7.5µg and 15µg Fluad-H5N1 formulations were similar. Generally there was a higher incidence rate of reactions after the first when compared with the second and, where tested, the booster vaccination. Possibly or probably related AEs were infrequently experienced by the study population, were temporally related to the time of injection, were mild or moderate in severity and were caused by solicited adverse events ongoing past the 7-day observation window or by other known common side effects of influenza vaccination.

No possibly/probably related SAEs or clinically significant AEs were experienced by subjects of any study on prepandemic vaccines. The number of adolescents and adults below and above 60 years vaccinated with pandemic and seasonal Fluad vaccines are summarized in Table 6.

Cl_Table n. 6 - Safety Populations: Numbers of Subjects

Vaccine		Adolescents	18-60 years	>60years
Fluad-H5N1	Solicited and non-solicited AEs	0	3253	414
Fluad-H5N3	Solicited and non-solicited AEs	0	27	0
Fluad-H9N2	Solicited and non-solicited AEs	0	48	0
Licensed seasonal Fluad	Solicited AEs	116	806	3610
	Non-solicited AEs	116	812	12809

The safety profiles of three vaccines are reported in six studies:

- Fluad-H5N3 in studies V7P37 and V7P37E1
- Fluad-H9N2 in study DMID 04-019
- Fluad-H5N1 in studies V87P1, V87P2, and V87P4

The Fluad-H5N3 and Fluad-H9N2 studies were designed to investigate dose (7.5 - 30 µg and 3.75 - 30 µg HA, respectively) and adjuvanted versus non-adjuvanted formulations. These relatively small studies served as the basis of dose and formulation selection for the subsequent H5N1 studies.

Fluad-H5N3

The first study (**V7P37**) was conducted in 1999. This dose ranging (i.e., 7.5, 15, 30 µg HA/injection) Phase I pilot trial (Section 5.3.5.1) evaluated the safety of adjuvanted (Fluad-H5N3) and nonadjuvanted formulations of a monovalent H5N3 vaccine. The total planned enrollment was for 60 subjects 18-40 years of age. The first healthy subjects were enrolled and administered 7.5µg Fluad-H5N3 or nonadjuvanted comparator. These subjects were observed for at least a week after the first vaccination by telephone calls on days 3 and 7. Since no serious vaccine related event was reported in the first week after vaccination, additional subjects were randomized into the 15µg Fluad-H5N3 or nonadjuvanted vaccine groups then, after a further week, into 30µg Fluad-H5N3 or nonadjuvanted vaccine groups. Three weeks after the first injection, subjects received the same dose of a second injection. This ethically preferred

approach allowed for the sequential evaluation of the tolerability of one and two injections of 7.5, 15, and 30µg adjuvanted and nonadjuvanted H5N3 formulations.

Up to 65 subjects who were previously enrolled in **V7P37**, described above, could participate in the extension study **V7P37E1**. A total of 28 subjects were enrolled and revaccinated with the same formulation (7.5µg, 15µg, or 30 µg HA of Fluad-H5N3 or nonadjuvanted comparator) approximately 17 months after the primary vaccination. All 28 (100%) subjects completed the study as planned and were included in the analysis of safety.

Fluad-H9N2

A third study, **DMID 04-019**, was conducted on 96 healthy non-elderly adults (18-34 years) by the National Institute of Health (NIH), Maryland, in 2005. This was a dose ranging study (3.75, 7.5, 15, 30 µg H9N2 HA) using Fluad-H9N2 and nonadjuvanted comparator. The two injections were given four weeks apart. Because all injections contained lower amounts of HA than registered seasonal Fluad, subjects were enrolled into all groups concurrently. Blood was drawn before and 28 days after each vaccination.

Fluad-H5N1

The phase 2 H5N1 study (V87P1) investigated non-inferiority of 2 x 7.5µg to 2 x 15µg Fluad-H5N1. A total of 486 non-elderly (18-60 years) adult and elderly (>60 years) subjects were enrolled out of a planned 460. Two vaccinations were administered 3 weeks apart. Six months later, 50% of the subjects also received a booster. Analysis of safety data are presented for:

- Solicited AEs 7 days after each of the 3 injections after each 1st, 2nd, and 3rd vaccination, respectively)
- All spontaneously reported non-solicited AEs for 3 weeks after the first and second injection
- Non-solicited AEs leading to premature withdrawal, or necessitating physician visit or assessed as an SAE up until the booster vaccination (6 months after the primary course)
- All other SAEs are reported up until the 20 JUN 2007.

Long-term (6-month) follow up after booster vaccination is ongoing. This will be reported in an addendum to the CSR.

The phase 2 H5N1 study V87P2 investigated safety and immunogenicity. A total of 40 non-elderly (18-60 years old) subjects were enrolled out of a planned 45 and given two injections of 7.5 or 15µg Fluad-H5N1 or 15µg H5N1 nonadjuvanted vaccine, three weeks apart. Although all 3-week safety follow-up data is completed (safety population = 40), long-term (6-month) follow up is ongoing. Six months after primary vaccination, the subjects will be given a booster and followed up for safety for a further 6 months. This will be reported in part 2 of the CSR.

The only Phase 3 study presented in the dossier is study **V87P4**, an immunogenicity, safety and tolerability study. In order to comply with EMEA guidance, a total of 4400 subjects (planned 4000 non-elderly and 400 elderly subjects) were planned to be enrolled at a 3:1 ratio and to receive two injections of 7.5µg Fluad-H5N1 or seasonal Fluad vaccine three weeks apart.
The safety population consisted of 3155 H5N1 recipients (2927 aged 18-60 and 241 aged >60 years). All 3-week safety follow-up data is completed, long-term (6-month) follow up is ongoing and is reported in Part 2 CSR.

Assessors' comment: The lack of 6 months safety data corresponds to a major concern. As the acceptable risk for a pre-pandemic vaccine should be the lower, the applicant is requested to submit the completed data set on safety profile.

Age	Number of Subjects	
Adults: 18 to 60 years	Aflunov n=2927	FLUAD n=977
Total	N=3904	
Elderly: 61 years and above	Aflunov n=241	FLUAD n=810
Total	N=322	
Total Study V87P4	**3155**	**4226**
Planned	*Aflunov n=3300*	*FLUAD n=1100*
Total	*N=4400*	

The probability to detect at least one adverse event in the FLUAD-H5N1 vaccine group (overall and by age group) is reported in Table below by underlying rates of adverse events descending from 1% to 0.5‰. The probability of detection of at least one adverse event in the FLUAD-H5N1 vaccine group is consistent with the EMEA Guideline for influenza vaccines with avian strains (EMEA/CHMP/VWP/171037/2006).

Cl_Table n. 7 - Percentage of Subjects with Possibly or Probably Related AEs, by Age group, Vaccination and Worst Severity

		Fluad[d, e]			Seasonal Fluad[e]		
[c]Vacc. No.	Age group	Mild	Moderate	Severe	Mild	Moderate	Severe
1	<18 years	-[b]	-[b]	-[b] -	8% N=116	3% N=116	0 N=116
	18-60 years	2% N=3253	1% N=3253	<1% N=3253	1% N=812	1% N=812	<1% N=812
	>60 years	2% N=414	1% N=414	<1% N=414	1% N=12809	<1% N=12809	<1% N=12809
2	18-60 years	1% N=3201	1% N=3201	<1% N=3201	2% N=104	5% N=104	0 N=104
	>60 years	2% N=406	<1% N=406	<1% N=406	4% N=492	<1% N=492	0 N=492
3	18-60 years	-[a]	-[a]	-[a]	0 N=150	0 N=150	0 N=150

[a] to be assessed in ongoing V87P1: [b] Not done: [c]vacc - vaccination [d]Pooled across 7.5 and 15µg formulations: [e]Incidence rates were collected over 6 weeks for Fluad-H5N1 and throughout the seasonal Fluad studies

Phase 2 and 3 clinical trials have evaluated safety and immunogenicity of Fluad-H5N1 in non-elderly adults (V87P1, V87P2 and V87P4) and elderly (V87P1 and V87P4). The Phase 1 Fluad-H5N3 and Fluad-H9N2 dose-finding studies used a limited number of subjects (32 Fluad-H5N3 recipients and 48 Fluad-H9N2 recipients) and are considered as supportive for the safety profile of the Fluad-H5N1 vaccine.

Cl_Table n. 8 - Summary Table of Safety Studies

Study ID	Study Objectives	Study Design: Randomized Control Type Blinding	Test Products: Dosage Regimen; Route of Administration	Number of Subjects Enrolled	Type of Subjects (age)	Study Status;
V87P1	Immuno., persistence and safety of 2 or 3 injections of 7.5/15µg HA Fluad-H5N1. Non-inferiority between 7.5 and 15µg HA.	Phase 2 Randomized (1:1) Controlled Observer-blind	Fluad-H5N1: 7.5µg Fluad-H5N1: 15µg IM	486 subjects: 313 aged 18-60 years; (156-157/group) 173 aged >60 years (86-86/group)	Healthy 18-60 and >60 year olds	Ongoing (Part2 CSR: 6 month safety post-2nd and solicited AEs post-booster completed)
V87P2	Immuno., CMI and safety of 2 injections and booster of 7.5/15µg HA H5N1compared with Nonadj. vaccine.	Phase 2, Randomized (1:1:1) Controlled, Observer-blind	Fluad-H5N1: 7.5µg Fluad-H5N1: 15µg Nonadj.: 15µg IM	40 subjects: 27 in Fluad-H5N1 (13-14/vaccine group) 13 in Nonadj.	Healthy 18-60 year olds	Ongoing (Part 1 CSR: data up to day 43 completed)
V87P4	Safety and immuno. of 2 x 7.5µg HA Fluad-H5N1 compared to 2 injections of seasonal Fluad. (2 x 15µg HA A/H1N1, A/H3N2 and B)	Phase 3, Randomized (3:1), Controlled, Observer-Blind	Fluad-H5N1: 7.5µg Seasonal Fluad: 15µg IM	4226 Subjects: 3904 aged 18-60 years (2927 in Fluad-H5N1) 322 aged >60 years (241 in Fluad-H5N1)	Healthy 18-60 and >60 year olds	Ongoing (Part 1 CSR: data up to day 43 completed)
V7P37	Immuno. and safety of 2 x 7.5/15/30 µg of Fluad-H5N3 and Nonadj. vaccines.	Phase 1 Randomized (1:1) Controlled Observer-blind	Fluad-H5N3 (7.5/15/30 µg) Nonadj. (7.5/15/30 µg) IM	65 subjects: 32 in Fluad-H5N3 (10 - 11/group); 33 in Nonadj. (11/group)	Healthy 18-40 year olds	Complete (Full CSR with 6 weeks data)
V7P37E1	Immuno. and safety of a booster of Fluad-H5N3 and Nonadj. vaccines.	Phase 1 Randomized (1:1) Controlled Observer-blind	Fluad-H5N3 (7.5/15/30 µg) Nonadj. (7.5/15/30 µg) IM	28 subjects: 17 in Fluad-H5N3 4-7/group); 11 in Nonadj. (2-6/group)	Healthy 18-40 years	Complete (Full CSR with 3 weeks data)
DMID 04-019	Immuno. and safety of 2 x 3.75/7.5/15/30 µg of Fluad-H9N2 and Nonadj. vaccines.	Phase I Randomized (1:1) Controlled Double-blind	Fluad-H9N2 (3.75/7.5/15/30 µg) Nonadj. (3.75/7.5/15/30 µg) IM	96 subjects: 48 in Fluad-H9N2 (12/group); 48 in Nonadj. (12/group)	Healthy 18-34 years	Complete (Full CSR)

The number of subjects having received different antigen formulations of any pre-pandemic Fluad vaccine or a non-adjuvanted comparator vaccine and the number of doses administered are detailed in Table 9.

Cl_Table n. 9 - Fluad-H5N1: Numbers of Subjects and Injections

	Study	Prepandemic Fluad vaccine		comparator vaccines	
		Subjects who received vaccine	Doses of vaccine administered	Subjects who received vaccine	Doses of vaccine administered
18-60 years	V87P1	312	774	ND	ND
	V87P2	27	54	13	26
	V87P4	2914	5783	971	1924
	Total	3253	6611	984	1950
>60 years	V87P1	173	405	ND	ND
	V87P4	241	481	81	162
	Total	414	886	81	162
>18 years	**Total**	3667	7497	1065	2112

ND: not done

A total of 14486 subjects were enrolled into 44 seasonal Fluad clinical trials and vaccinated. Of these, 117 were adolescents (9–17 years), 916 adults (18-60) and 13454 elderly (>60 years). A total of 13737

subjects were used for the safety analysis of non-solicited AEs. (It should be noted that the design of a large safety trial, V7P35 [N=9198], did not include monitoring of solicited reactions).

Although not formulated with influenza strains with a pandemic potential, the safety profiles of seasonal Fluad vaccine trials are also reported. **Fluad seasonal vaccine** contains 15µg HA of each H3N2, H1N1, and B viral strains. The total safety database consists of 13,737 subjects from 44 studies. These studies were carried out in non-elderly (18-60 years) and/or elderly (>60 years) adults.
In one study (**V7P17**) a two vaccination schedule was administered, and in six studies, subjects were invited to participate in an extension study one year later. Subjects were exposed to a third vaccination in two extension studies. For all other studies subjects received one injection. The substantial clinical safety database for seasonal Fluad presents the safety profiles of subjects administered 6-fold the antigen contained in the otherwise identical vaccine, Fluad-H5N1 (7.5 µg HA formulation).

Conclusions of Clinical Safety

- Overall reactogenicity was similar between the FLUAD-H5N1 and FLUAD groups both for the adults and for the elderly.
- Reactogenicity was generally higher for the adults than the elderly after first injection (68% vs. 50%, respectively). Moreover, the severe reactogenicity for the FLUAD-H5N1 was lower than for the FLUAD group (3% vs. 7%, respectively with p-value<0.001) after the first dose, whereas similar severe reactogenicity was observed between the two groups in the elderly subjects and after the second injection for both age groups (ranged 1% - 2% for both groups with p-value ranging from 0.6 – 1.0).
- However, in both age groups and vaccine groups, the incidence of reactions tends to decrease after the second injection.
- For both the adults and the elderly, local reactions were reported more frequently than systemic reactions in both FLUAD-H5N1 and FLUAD groups.
- No fatal events were observed during pre-authorisation clinical studies.

Pharmacovigilance system

The applicant presented an incomplete Pharmacovigilance Plan, mainly based on the activities agreed for the core Pharmacovigilance Plan for pandemic flu vaccines (mock-up dossier).
Considering the importance of the RMP for a pre-pandemic vaccines, EMEA had organised an Ad-hoc expert meeting on the Risk Management plan for Avian influenza vaccines. According to the results of this meeting the EU- RMP for Fluad H5N1 has been revised.

Risk Management Plan

The objective of Pharmacovigilance Planning is to summarize important identified and/or potential risks prior to product launch, to identify important missing information for focus in the post-marketing period, and to detect and act appropriately on any relevant signal (including definition of potential at-risk populations). Industry and regulators have identified the need for better and earlier planning of pharmacovigilance activities before a product is approved or a license is granted.
The applicant submitted an up-dated RMP, in which is described the known Aflunov risk profile and the pharmacovigilance activity,

Cl_Table n. 10 - PhV action plan

Issue	*Concern*	*Actions*
Severe allergic reactions, convulsion, syncope, neuritis, encephalitis, Guillain-Barré syndrome, Bell's palsy, tthrombocytopenia and vasculitis	Potential safety concern based on pharmacological class (influenza vaccines in general)	- Close monitoring in the context of enhanced passive surveillance; se RMP
No data in children Limited data in elderly No data in pregnancy	Potential target population for this vaccine	Planned clinical trials - Study V87P6 study in children - Study V87P1 study in elderly - The applicant should plan an adequate register.
Effectiveness	Cannot be known before the outbreak of a pandemic	- Collaboration with National authorities will be pursued when an interest in the use of the vaccine will be expressed by any additional European countries.

The product development rationale has been described in detail elsewhere; as a preliminary outline of future paediatric studies planned, the company informs that a Phase II clinical study to assess the safety and immune response of two doses, administered three weeks apart of Fluad H5N1 in paediatric subjects is planned.

Introduction

From non clinical studies there are no emerging risk signal for Fluad and Aflunov. On the other hand, no dedicated secondary or safety pharmacology addressing potential effects on cardiovascular, respiratory and CNS parameters were performed with Fluad H5N1. However, based on the cardiovascular and neurological evaluations performed in dogs that received repeated intramuscular injections of MF59C.1 adjuvant, and the known safety of influenza antigens and Fluad in animals and humans, the risk of unanticipated secondary or safety pharmacological effects in vaccines receiving Fluad H5N1 should be considered extremely unlikely. In any cases, the applicant submitted a detailed list of potential risk for which closed monitoring must be performed during the post-authorization phase.

Regarding use of Aflunov during pregnancy and lactation the applicant declares that there is insufficient data available in preclinical and in humans studies to adequately assess the risks of immunisation with Fluad H5N1 during these situations. Vaccination should be assessed on an individual basis and in accordance with medical advice.

Assessors' comment: In case of use during pregnancy and lactation all the clinical and safety information should be collected, related also to the outcome of the pregnancy. The applicant should propose a useful register.

Also the humans safety database is limited, due to the nature of the medicinal product; all the conducted clinical studies show a comparable safety profile, similar with the experience with other authorized vaccines adjuvanted with MF59C.1.

The summary safety findings for the Fluad H5N1 study (V87P1) in adult (18-60 years) and elderly (≥ 61 years) subjects describes that the reactogenicity profile of both vaccines (with 7.5 µg HA/dose or with 15 µg HA/dose) did not raise concerns regarding vaccine safety. The safety profile of both vaccines was at least as good as that of seasonal influenza vaccines. As expected, for both the adults and the elderly, the most frequently reported reactions were injection site pain, myalgia and headache.

Potential indicators of **oculo-respiratory syndrome** (ORS) (i.e. coughing, wheezing, chest tightness, difficult breathing, sore throat facial edema, red eye and fever), irrespectively of the dose received, were reported by 21 (7%) of non-elderly adult and 6 (3.5%) of elderly subjects. This is in the same range as other reports on ORS in clinical studies with influenza vaccines. Adverse events judged as possibly or

probably related to vaccine administration were reported by 2 % - 5 % of the adult and elderly population and were mostly mild or moderate local or systemic reactions ongoing past the seven day observation window.

Cl_Table n. 11 - Overview V87P1 Safety: Subjects with at least a reactogenicity sign after any vaccination – Adults and Elderly

Type of Reaction	Number (%) of subjects					
	Adults			Elderly		
	Fluad 7.5 N=156	Fluad 15 N=156	Total N=312	Fluad 7.5 N=87	Fluad 15 N=86	Total N=173
Any reaction	114 (73%)	121 (78%)	235 (75%)	37 (43%)	39 (45%)	76 (44%)
Local reaction	95 (61%)	109 (70%)	204 (65%)	23 (26%)	28 (33%)	51 (29%)
Systemic reaction	87 (56%)	75 (48%)	162 (52%)	24 (28%)	28 (33%)	52 (30%)
Other reaction	20 (13%)	22 (14%)	42 (13%)	6 (7%)	7 (8%)	13 (8%)

The summary safety findings for the Fluad H5N1 study (**V87P4**) in adult (18-60 years) and elderly (> 60 years) subjects (n=3155 for Fluad H5N1) confirms the data available from study V87P1; in total 4207 of the 4226 enrolled subjects were included in the safety population which comprised: 3155 subjects for Fluad H5N1 of which 2914 were in the adult group and 241 were in the elderly group and 1052 subjects for Fluad of which 971 were in the adult group and 81 were in the elderly group.
The percentages of subjects reporting local reactions in the Fluad H5N1 group (40%-57% in adults and 26%-33% in elderly across injections) were similar to the Fluad group (42%-66% in adults and 16%-32% in elderly across injections).

- Adjuvant MF59C.1:

The adjuvant MF59C.1 is also used in the registered inter pandemic Fluad, hence post marketing data might be used as possible indicators for the safety of Fluad H5N1. These data, again presented elsewhere in detail, confirm the data in the limited clinical safety database. With the exception of a higher frequency of mild local reactions, more noticeable in the early launch phase, Fluad presents a post marketing safety profile very similar to that of other established influenza vaccines.

Safety specification

- Potential risks that require further evaluation:

Type B reactions are mainly attributable to the susceptibility of the subject or patient, such as allergic reactions. After the use of registered influenza vaccines there are a few side-effects reported that belong to these so-called type B reactions, like transient thrombocytopenia, anaphylactic reaction, vasculitis, acute demyelinisating encephalomyelitis (ADEM) or Guillain-Barré-Syndrome.
They appear due to a certain individual susceptibility resulting in an unpredictable different reaction in the immunosystem.
None of these kind of adverse reactions was observed during the clinical studies; really clinical trials of Fluad H5N1 have detected common adverse events, but cannot be sufficiently powered to identify low probability safety issues (e.g. incidence of Guillain-Barré Syndrome) or sub-group effects.
No new potential risks have been identified. However, as is known for seasonal influenza vaccines, there are a number of adverse events of special interest, which have been reported rarely in association with influenza vaccination:

– Neuritis

- Convulsions (non febrile)
- Allergic Reactions (serious)
- Syncopes
- Encephalitis (incl. myeloencephalitis)
- Thrombocytopenia
- Vasculitis
- Guillain-Barré Syndrome
- Bell's Palsy
- Autoimmune disease (e.g. multiple sclerosis, optic neuritis, diabetes mellitus)

- Populations not Studied in the Pre-Authorization Phase

Populations not studied in the pre-approval phase include:

- children and youth: A planned phase II study in children will provide additional data

The use of vaccine in children cannot be excluded completely, depending on the development of the influenza. Any cases of paediatric use will be documented and followed up, as needed.

Assessors' comment: The applicant should describe how the off-label use in special populations will be monitored.

According to what is known from registered influenza vaccines, there is no significant risk that requires further action at this point of time. From its posology and compounds the vaccine does not bear important risks for children.

- pregnant or lactating women,
- patients with relevant co-morbidities,
- immunocompromised patients.

Pharmacovigilance plan

The introduction of this vaccine to the market will be unusual, given its rationale, and the pharmacovigilance (PV) plan must consider several uncertainties: Clinical trials of Fluad H5N1 have detected common adverse events, but cannot be sufficiently powered to identify low probability safety issues (e.g. incidence of Guillain-Barré-Syndrome) or sub-group effects.
Safety experience with Fluad has relevance due to the production process. It may be argued that Fluad H5N1 is not that different from a seasonal strain change – however, even though the risk may be similar, this has to be weighed against the benefit, which is different for the pre pandemic strategy as compared to the seasonal situation.
Most programs for vaccine recommendation, distribution and administration are country specific. A positive outcome of the application for a marketing authorization would not necessarily lead to use of the vaccine in all countries.

Safety specification

- Potential risks that require further evaluation:

Regulators and industry agreed to especially focus on priority adverse events of special interest for the pandemic vaccine, for which common case definitions will be developed and used.
Type B reactions are mainly attributable to the susceptibility of the subject or patient, such as allergic reactions. After the use of registered influenza vaccines there are a few side-effects reported that belong to these so-called type B reactions, like transient thrombocytopenia, anaphylactic reaction, vasculitis, acute demyelinisating encephalomyelitis (ADEM) or Guillain-Barré-Syndrome.

They appear due to a certain individual susceptibility resulting in an unpredictable different reaction in the immunosystem.
None of these kind of adverse reactions was observed during the clinical studies; rarely clinical trials of Fluad H5N1 have detected common adverse events, but cannot be sufficiently powered to identify low probability safety issues (e.g. incidence of Guillain-Barré Syndrome) or sub-group effects.
No new potential risks have been identified. However, as is known for seasonal influenza vaccines, there are adverse events of special interest, which have been reported rarely in association with influenza vaccination:
The following events have been considered since they are already known with annual influenza vaccines: neuritis and other serious neurological events (including Bell´s palsy and Guillain-Barré-Syndrome), convulsions, anaphylaxis (fatal and life threatening), thrombocytopenia. The list may be updated as the discussion continues. It is suggested that these events are especially monitored also for Fluad H5N1 after launch. They will be analysed in the Periodic Safety Update Reports.

- Neuritis
- Convulsions (non febrile)
- Allergic Reactions (serious)
- Syncopes
- Encephalitis (incl. myeloencephalitis)
- Thrombocytopenia
- Vasculitis
- Guillain-Barré Syndrome
- Bell's Palsy
- Autoimmune disease (e.g. multiple sclerosis, optic neuritis, diabetes mellitus)

The differences between national influenza vaccination plans, especially as to possible pre-pandemic vaccination strategies, make it difficult to define harmonized methods for additional pharmacovigilance activities with respect to the safety of Fluad H5N1. In many countries insufficient or non-existing linkage between vaccination registries and epidemiological data create obstacles to the gathering of safety data, such as hospitalisations.
Some of the work initiated during several Workshop on Pandemic Influenza Vaccines Risk Management Plan may also provide insight and ideas for the pre-pandemic situation. Groups not studied in clinical trials need to be specifically evaluated. Children and youth, though not studied, are not in the currently proposed indication for Fluad H5N1, hence do not need special consideration at this time (see also section 1.2.7 on pediatric off-label use). Other groups include:
- pregnant or lactating women - patients with relevant co-morbidities - immuno-compromised patients.

The table below summarised the studies focused on the pharmacovigilance activities:

Study	Year	Nr. of Subjects	Healthy Subjects or Diagnosis of Patients	Objectives of the Study	Study Design	Test Product(s); Dosage Regimen; Route of Administration
V7P37	1999	32 test 33 control	Healthy adults 19-39 years	Safety Tolerability Immuno-genicity	Phase I Observer-Blind, Randomized	**H5N3 + MF59C.1** Two doses of 7.5, 15 or 30 µg I.M.
V7P37E1	2000	16 test 11 control	Healthy adults 19-39 years	Safety Tolerability Immuno-genicity	Phase I Observer-Blind, Randomized	**H5N3 + MF59C.1** Two doses of 7.5, 15 or 30 µg I.M.
DMID 04-019	2005	48 test 48 control	Healthy adults 18-34 years	Safety Tolerability Immuno-genicity	Phase I, Double-Blind, Randomized	**H9N2 + MF59C.1** Two doses of 3.75, 7.5, 15 or 30µg I.M.
V87P1	2006	313 (18-60y) 173 (≥61y) test	Healthy adults ≥ 18 years	Safety Tolerability Immuno-genicity	Phase II, Observer-Blind, Randomized	**H5N1 + MF59C.1** Two doses of 7.5 or 15 µg I.M.

IV. ORPHAN MEDICINAL PRODUCTS

N/A

V. BENEFIT RISK ASSESSMENT

Influenza pandemics have occurred at irregular intervals throughout human history. During the last century, three pandemics caused huge morbidity and mortality, social disruption and economic loss. The interval between the emergence of the pandemic strains in the Far East and the first cases in Europe was 3-4 months. International air travel has increased considerably since the 1970's making it therefore highly probable that the next influenza pandemic will have its first cases in Europe within weeks of its emergence. The first wave of a pandemic outbreak may be largely over within 4 months.

It is unlikely that manufacturers would be able to produce sufficient quantities of vaccine to confront an emerging pandemic. Thus the production of a vaccine against a potential influenza pandemic viral strain is vital at three stages:

• during the inter-pandemic period in order to reduce the chance of an 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 both avian and human virus infection,

• prime during pre-pandemic stages (WHO phases 3 to 5) to reduce mortality against a closely matched pandemic strain,

• permit early vaccination at the start of a pandemic (WHO phase 6) when the pandemic vaccine is not yet available.

Based on the global outbreaks of highly pathogenic H5N1 avian influenza, the H5N1 strain is the most likely candidate from which a pandemic strain would evolve. From the start of the outbreaks in mid-2003 until 29 June 2007, 317 people have been infected with laboratory-confirmed avian H5N1 influenza, 191 of whom died. The antigenic diversity of circulating H5N1 viruses means that vaccines prepared in the inter-pandemic period may not be appropriately matched to an emerging pandemic virus.

Therefore, cross-reactive immune responses to a range of antigenic variants are important for a pre-pandemic vaccine. Even a vaccine of relatively low efficacy may mitigate a pandemic. If there is an H5N1-based pandemic, a pre-pandemic vaccine, even if incompletely matched to the pandemic virus

(perhaps giving partial protection), will prevent more infections and deaths than waiting for the specific pandemic vaccine. In addition, vaccination is expected to be the most effective and efficient strategy to mitigate a pandemic situation.
MF59 has been shown to significantly enhance the immune response to a wide variety of vaccine constructs. Fluad-H5N1 at a dose of 7.5µg of antigen is able to reach protective levels of antibodies in healthy adults and the elderly, and induces T-cell memory already after the first dose. Antibody responses were boosted by subsequent vaccination with an adjuvanted vaccine, thus confirming that primary vaccination with Fluad formulated with an H5 viral strain primes naive populations. In addition, Fluad-H5N3 vaccine was able to induce broadly cross-reactive immune responses to a range of H5N1 antigenic variants and a clade 1 Fluad-H5N1 vaccine induced cross-reactive immune responses against an H5N1 clade 2 strain; thus suggesting that a priming strategy would be useful against a future H5N1 pandemic.
The overall reactogenicity profile of Fluad-H5N1 is similar to that of authorized seasonal Fluad, which is accepted to be safe and comparable to conventional, non-adjuvanted inter-pandemic influenza vaccines based on over 27 million doses administered. Most of the reactions were mild in nature, of short duration and qualitatively similar to those induced by conventional influenza vaccines. It is widely accepted that the adjuvant effect leading to increased immunogenicity is associated with a slightly higher frequency of local reactions (mostly mild pain) compared with conventional, non-adjuvanted influenza vaccines. There were fewer reactions after the second and third vaccination compared with the first. Overall, pre-pandemic Fluad-H5N1 has a good safety profile consistent with those observed for other H5N1 adjuvanted, subvirion, or whole virion adjuvanted vaccines.
As with any rare, catastrophic occurrence, it is impossible to determine the likelihood of an H5N1 pandemic. Therefore the benefit of a pre-pandemic vaccine is not easily to define. On the other hand, the cost of not having such a vaccine in the case of a pandemic would be grave: up to 50,000,000 died in the 1918 influenza pandemic. Based on over 3000 subjects included into clinical trials, there is every reason to expect that this vaccine (which will be administered at 1/6 of HA content/injection of seasonal Fluad) will be just as safe as any of the other influenza vaccines which are given to a large fraction of the European population on an annual basis and are well accepted due to their excellent safety profiles. The pre-pandemic vaccine will only have to be administered twice for priming, not on an annual basis.
Taken together, this represents a clear benefit for a Fluad-H5N1 pre-pandemic vaccine in the current pre-pandemic situation of the world.
In conclusion, Fluad-H5N1 containing 7.5µg HA has a good immunogenicity profile together with a favourable safety profile which is further supported by the safety experience gained with the seasonal Fluad, which is accepted as safe, based on over 27 million doses sold and more than 10 years post-marketing experience. Additional safety data on six months follow-up after the second dose and on the effect of a booster dose are to be submitted for complete assessment with the additional immunogenicity data on 400 subjects.

V.1 Benefits

⌦ Demonstrated benefits

- Two injections of 7.5µg Fluad-H5N3, administered 21 days apart, produced sufficient levels of antibody to meet all three CHMP criteria when assessed by SRH.
- The general agreement between GMRs and the percentages of subjects achieving at least 4-fold increases in titers by MN and SRH indicate that pre-pandemic Fluad-H5 formulations induced a clear immune response.
- Consistent with the finding of others HI was more sensitive to H9N2 and seasonal strains than to H5 viral strains.
- All three assays show a significant increase in antibody titers after two injections administered three weeks apart. and all CHMP immunogenicity criteria are met when using the SRH assay.
- MF59 adjuvant significantly enhanced the immune responses, persistence, and cross-protection.

- The immune responses induced by 2x7.5µg Fluad-H5N1 was at least as good, if not better, than that induced by the 15µg formulation and induced immunological memory as shown by the booster response 6 months later. Based on these results the 7.5µg formulation, which in addition to optimal immunogenicity offers also an option for dose-sparing, has been selected for registration.
- In both age groups and vaccine groups, the incidence of reactions tends to decrease after the second injection.
- For both the adults and the elderly, local reactions were reported more frequently than systemic reactions in both FLUAD-H5N1 and FLUAD groups.
- No fatal events were observed during pre-authorisation clinical studies.

⌦ **Uncertain benefits**

- Limited data in elderly
- Effectiveness to be evaluated

V.2 Risks

⌦ **Demonstrated risk**

- Neuritis; Convulsions (non febrile); Allergic Reactions (serious); Syncopes; Encephalitis (incl. myeloencephalitis); Thrombocytopenia; Vasculitis; Guillain-Barré Syndrome; Bell's Palsy; Autoimmune disease (e.g. multiple sclerosis, optic neuritis, diabetes mellitus).

⌦ **Uncertain risk**

- no data in children
- pregnant or lactating women,
- patients with relevant co-morbidities and/or immuno-compromised.
- 6 months safety data

V.3 Conclusions

The pre-pandemic vaccine Aflunov is intended for use during the pre-pandemic phase or the first wave of avian pandemia, depending on each National recommendation and according to WHO guidelines that identified three basic principles that should guide decisions concerning access to therapeutic and prophylactic measures: (1) efficiency (maximizing health benefits, preferably in terms of saving most lives); (2) equity (avoiding discrimination); and (3) accountability (including measures to increase public awareness, facilitate consultation and improve transparency).
The principles guiding prioritization may differ for the administration of vaccines. While vaccinating high-risk groups first might be appropriate in some circumstances, it would be unethical to have a system that would exclude persons who are at a lower, but still real, risk of infection. Under such circumstances, members of "low risk" groups could be at a greater disadvantage than those deemed to be at high risk. Finally, efficiency considerations might support vaccinating people at the highest risk of spreading the virus (e.g. those at higher risk of infection) even if those people do not have the greatest risk of dying from their infection. But, as noted above, it may not be possible to predict which groups will be at higher risk of infection or death in the early stages of a pandemic.
According to the possible use, the efficacy and the safety profile of Aflunov was evaluated in the first phase in a subgroup of patient; with the responses to the LoQ120 the analysis on the complete dataset on the immunogenicity has confirmed the preliminary results: the demonstrated benefits claim the positive efficacy, as sufficient levels of antibody were meet with all three CHMP criteria when assessed by SRH and immunogenicity was also demonstrated by MN. Analysis of HI assay results demonstrated the non-inferiority of 7.5µg dosage over 15µg dosage groups for the A/H5N1 influenza antigen. By HI after the second injection two out of three CHMP (CPMP/BWP/214/96) criteria were met in the adult population (the proportion of subjects achieving seroprotection was not met) whereas all three CHMP criteria were met by the elderly subjects above 60 years.

The safety profile was evaluated in a complete dataset, as requested by CHMP guideline; Novartis started the study V87P4 in January 2007.

This trial [Study V87P4] was carried out in Czech Republic, Lithuania and Poland, a total of 3168 subjects were enrolled and received Fluad-H5N1 (2927 adults and 241 elderly). This, together with trials V87P1 and V87P2, could meet the minimum requirements of the EMEA guideline CHMP/VWP/42326/2007.
This evaluation included the primary safety evaluation in all subjects and immunogenicity data in a subset, with data collected up to 3 weeks after the second dose (day 43).
The follow-up data for the evaluation of safety at 6 months after the last dose of vaccine will be submitted as soon as possible.

Considering the possible off-label use of Aflunov during the pandemic phase, the CHMP have agreed with the need of a PIP. In addition, recent data analysis of human H5N1 cases (WHO 2006 – human H5N1 data) show that the median age of all confirmed cases was 20 years. 50% of cases occurred in individuals younger than 20 years of age and among cases under 10 years of age, 60% being aged 5-9 years. The highest case fatality rate was reported in individuals aged 10-19 years (73%).
Consequently the applicant has planned a phase II study on the potential vaccine for pandemic and pre-pandemic use in children also, who might be not only most at risk of infection, but also responsible for the spread of the disease. The present study is therefore designed to evaluate the immunogenicity, safety and tolerability of two doses of FLUAD-H5N1 vaccine administered 3 weeks apart in subjects aged 6 months to 17 years.
The study is well planned and all the requirements described in the CHMP guidelines are fulfilled. This study could collect important information about planning PIP. Since the vaccine is intended pre-pandemic use it could be used by children in the pre-pandemic period if a strategy of population priming is adopted. Therefore a PIP is requested although not required for the current application of use limited to adult subjects (aged 18 years or more).
The use of vaccine in children cannot be excluded completely, depending on the development of the influenza. The applicant should describe how the off-label use in paediatric area will be monitoring. Any cases of paediatric use should be documented and followed up. The applicant is also requested to describe the methods to be implemented to collect all information about pregnant or lactating women and other not-studied population.