EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Assessment report

Humenza

Common Name: pandemic influenza vaccine (h1n1) (split virion, inactivated, adjuvanted)

Procedure No. EMEA/H/C/001202

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

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1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant Sanofi Pasteur SA submitted on 13 January 2010 an application for Marketing Authorisation to the European Medicines Agency for Humenza, through the centralised procedure under Article 3 (2) (a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMEA/CHMP on 25 June 2009.
The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application

The application submitted is a complete dossier:

composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

The applicant applied for the following indication:
Prophylaxis of influenza in an officially declared pandemic situation.
Pandemic influenza vaccine should be used in accordance with Official Guidance.

Information on Paediatric requirements
Pursuant to Article 7 the application included an EMEA Decision P196/2009 for the following conditions:

- Influenza

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

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

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

Rapporteur: Dr. Christian Schneider Co-Rapporteur: Dr. Ian Hudson

1.2 Steps taken for the assessment of the product

- The applicant submitted five rolling review applications on the quality, non clinical and clinical data to support the marketing authorization application. The rolling reviews were submitted on 23 June 2009, 16 October 2009, 30 October 2009, 13 November 2009 and 22 December 2009.
- The application was formally received by the EMEA on 13 January 2010 together with a request for conditional Marketing Authorisation in accordance with Articles 2(2) and 4 of Council Regulation (EC) No 507/2006.
- The procedure started on 15 January 2010.
- On 14th July an interim Opinion on a rolling review (RR/01) was adopted by the EMEA Task Force (ETF)/CHMP.
- On 23 November 2009 an interim Opinion on a rolling review (RR/02/03) was adopted by the EMEA Task Force (ETF)/CHMP.
- On 16 December 2009 an interim Opinion on a rolling review (RR/04) was adopted by the EMEA Task Force (ETF)/CHMP.
- On 13 January 2010 an interim Opinion on a rolling review (RR/05) was adopted by the EMEA Task Force (ETF)/CHMP.
- During the meeting on 18-20 January 2010, the CHMP agreed on List of questions to be answered in writing and in an oral explanation.
- During the CHMP meeting on 15-18 February 2010, responses to the list of questions were addressed by the applicant during an oral explanation before the CHMP.

- During the meeting on 15-18 February 2010, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a conditional Marketing Authorisation to Humenza on 18 February 2010. The applicant provided the letter of undertaking on the specific obligations and follow-up measures to be fulfilled post-authorisation on 18 February 2010

2 SCIENTIFIC DISCUSSION

2.1 Introduction

An influenza pandemic is a global outbreak of influenza disease that occurs when a type A influenza strain to which a high proportion of the world's population is immunologically naïve emerges. In April 2009, a new strain of human influenza A(H1N1)v was identified and characterised. On 11 June 2009 the WHO declared Phase 6 of the influenza pandemic. The declaration reflected sustained transmission of the virus from person to person in several WHO regions. WHO and other international agencies are calling the disease pandemic (H1N1)v 2009. For the virus the nomenclature influenza A(H1N1)v (where v indicates variant) has been chosen.

Estimates for the attack rates associated with the influenza A(H1N1)v virus have varied from approximately 10-50 % in different geographical areas. The actual numbers of clinically apparent infections, cases that require hospitalisation and deaths in the pandemic period is expected to be higher than in recent years for seasonal influenza. These estimates may change (upwards or downwards) during the further course of the pandemic. Hospitalisation and deaths have occurred in previously healthy subjects as well as in those with underlying conditions or pregnancy that would predispose them to complications of influenza. For more information about the known clinical features of the disease caused by influenza A(H1N1)v virus please see the Risk Assessment report from ECDC under:
http://ecdc.europa.eu/en/healthtopics/Documents/0908_Influenza_AH1N1_Risk_Assessment.pdf

Humenza is based on the proposed new strain A/California/7/2009 (H1N1)v like strain (X-179A), which complies with the WHO[1] and CHMP[2] recommendations for the emergent novel A(H1N1)v influenza vaccine composition. The candidate vaccine contains a novel adjuvant AF03 (oil-in-water emulsion, squalene based).

The development programme/Compliance with CHMP Guidance/Scientific Advice

Before the current pandemic was declared, two clinical programs were independently developed for Europe and the US for investigational vaccines containing H5N1 inactivated split influenza virus produced in eggs according to the seasonal influenza vaccine production process either established in France (Vaxigrip) or in the USA (Fluzone). Both investigational vaccines contained the novel AF03 adjuvant. A series of non-clinical pharmacological and toxicological studies were conducted with the AF03 adjuvanted H5N1 vaccine.

Subsequently the applicant initiated non-clinical and clinical studies with a candidate H1N1v/AF03 vaccine. As laid down in interim guidance (EMEA/CHMP/323452/2009) the H1N1/AF03 candidate vaccine was considered as new vaccine containing an adjuvant which is not yet approved in any EU authorised vaccine. Although a full clinical data package including safety studies would ultimately have to be provided the guidance made provision for the data to be assessed in an emergency rolling review procedure.

ETF Scientific Advice on the non-clinical and clinical development of the candidate H1N1v/AF03 vaccine was provided on 12.06.2009. Based on the briefing document submitted by Sanofi Pasteur on 22 May 2009 prior to obtaining this advice the CHMP advised the company to provide all available non-clinical

[1] http://www.who.int/csr/resources/publications/swineflu/vaccine_recommendations/en/index.html
[2] EU recommendation for the emergent novel H1N1 influenza vaccine composition (EMEA/CHMP/BWP/3408312009 Rev 1) http://www.emea.europa.eu/pdfs/human/bwp/34083109enrev1.pdf

and clinical data generated for the H5N1 AF03 adjuvanted vaccine since these data would be considered supportive for the candidate H1N1v/AF03 vaccine.

General comments on compliance with GMP, GLP, GCP
The clinical trials were performed in accordance with the quality standards of the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP) and reflected the requirements of the EMEA guidance.

2.2 Quality aspects

Introduction

The A/H1N1 Pandemic Influenza Vaccine is indicated for prophylaxis of influenza in an officially declared pandemic situation. The active substance consists of an A/California/7/2009 (NYMC X-179A) (H1N1) strain cultivated on embryonated hens' eggs, then purified, split with octoxynol-9, and inactivated with formaldehyde. The A/H1N1 Pandemic Influenza Vaccine is a suspension and emulsion for injection: extemporaneous mixture of A/H1N1 Pandemic Influenza Vaccine with squalene based AF03 Adjuvant to be administered by intramuscular route. The dosage obtained is 3.8 µg of hemagglutinin per human dose of 0.5 mL. Since the final product is a 10 dose type I glass vial, thiomersal has been included in the formulation as a preservative.

About the product:
The manufacturing process for the A/H5N1 drug substance monovalent bulk as well as for the A/H1N1 antigen rely on the process licensed for seasonal influenza vaccine Vaxigrip. AF03 is a novel squalene based adjuvant, which is currently not approved as an integral part for any vaccine or any other medicinal product in Europe. Hence, the AF03 adjuvant and its formulation into the final vaccine is also a prominent new aspect to be dealt with for vaccine registration, and full quality data for all vaccine components is required.

Humenza is a 2-component vaccine consisting of inactivated split virion (H1N1) antigens (suspension) presented in a type I glass vial (10 doses) and of the AF03 adjuvant (emulsion) presented in a type I amber glass vial (10 doses). At the time of vaccine administration, the content of the antigen vial is withdrawn with a syringe and is injected into the AF03 adjuvant vial. The mixed vaccine is a whitish emulsion containing 10 individual vaccine doses of 0.5mL. As the final product is a multidose vial, thiomersal has been included in the formulation as a preservative in the antigen vial.

Humenza is indicated for the prophylaxis of pandemic influenza caused by the H1N1 strain and should be used in accordance with official guidance.

Composition:
HUMENZA consists of two vials: one vial containing the antigen (suspension) and one vial containing the adjuvant (emulsion), which are mixed prior to administration.

After mixing, 1 dose (0.5ml) contains:

Split influenza virus*, inactivated containing antigen equivalent to:
A/California/7/2009 (H1N1)-like strain (NYMC X-179A).....3.8 micrograms**

* propagated in eggs
** expressed in microgram haemagglutinin

This vaccine complies with the WHO recommendation and EU decision for the pandemic.

AF03 adjuvant composed of squalene (12.4 milligrams), sorbitan oleate (1.9 milligrams), polyoxyethylene cetostearyl ether (2.4 milligrams) and mannitol (2.3 milligrams)

The suspension and emulsion, once mixed, form a multidose vaccine in a vial.
Excipients:
The vaccine contains 11.3 micrograms thiomersal.

List of Excipients:

Antigen vial:
Thiomersal
Sodium chloride
Potassium chloride
Disodium phosphate dihydrate
Potassium dihydrogen phosphate
Water for injections

Adjuvant vial:
Sodium chloride
Potassium chloride
Disodium phosphate dihydrate
Potassium dihydrogen phosphate
Water for injections

Chemical composition of AF03

AF03 is a squalene based oil-in-water emulsion. There are other squalene oil-in-water adjuvanting emulsions licensed in other influenza vaccines (e.g. AS03 and MF59).

Squalene is a natural component of cell membranes, an intermediate in the human steroid hormone biosynthetic pathway and a precursor of cholesterol. Consequently, squalene is both biodegradable and biocompatible, since it is a normal component of the human body.

The nature of the AF03 Adjuvant with other known squalene-based adjuvants has been discussed at the BWP. Although they all are squalene based oil in water emulsions it has been pointed out that from a quality perspective there are differences in the manufacturing procedure, the composition and the resulting particle size. It is not known to what extent extrapolation of safety based on these quality data can or cannot be made.

Active Substance

H1N1 Antigen

The monovalent bulk is an aqueous suspension of inactivated, split viral particles that were propagated in embryonated eggs and purified by zonal centrifugation. The reference virus described in the current dossier is A/California/7/2009 (NYMC X-179A) which was developed by NYMC (New York Medical College) using reassortment between A/California/7/2009 and A/New York/55/2004 (NYMC X-157) (H3N2), generated by NYMC and supplied by CDC (Centers for Disease Control and Prevention). The reassortant strain combines the H1 and N1 segments of A/California/7/2009 with the X-157 backbone.

- Manufacture

The manufacturing process of the monovalent bulk is almost identical to the manufacturing process of Sanofi Pasteur's seasonal influenza vaccine and can be divided into the following steps:

-Propagation of the A/H1N1 influenza viral strain in embryonated eggs, harvesting and pooling of the allantoic fluids
-Purification of the whole virus bulk by several zonal centrifugation steps
-Splitting of the monovalent with octoxynol-9
-Inactivation of the monovalent split virus with formaldehyde, followed by sterile filtration

In general, the manufacturing process is well known from the seasonal influenza vaccine.

The production process for monovalent bulks is adequately described. Control of starting materials (virus seed lots, eggs and raw materials) is on the whole acceptable. Routine in-process tests conducted are described.

Data from 3 production batches demonstrates the capability of the purification process to reduce levels of ovalbumin and retain the HA antigen. The reduction of neomycin sulphate to acceptable levels has

been shown through process validation studies. Data from validation of the splitting step have been provided and are acceptable.
Data have been provided to show the capability of the inactivation process to inactivate X-179A produced from 2 working seed lots, ALV and mycoplasmas.
The Company has shown that inactivation kinetics provided for A/Brisbane/59/2007 represents a worse-case and thus are relevant for A/California/7/09 and other A strain influenza viruses. The data shows that all strains were inactivated after 25 hours following splitting and thus that the inactivation step is sufficient to ensure that all A/California/7/09 (NYMC X179A) is inactivated following standard manufacturing conditions. The inactivation process was assessed on six monovalent bulk industrial batches A/California/7/2009 (H1N1).
In addition, the Applicant has provided data from a third batch of MVB to demonstrate consistency of the inactivation process.

All characterization of the antigen drug substance is carried out through release tests on MSL, WSL or monovalent bulk. The tests performed to characterize seed lots and monovalent bulks focus on the HA and NA proteins and tests for impurities. The characterization tests proposed are acceptable. The Applicant has shown that the RT-PCR assay for HA and NA applied to MSL and WSL is valid for use on X-179A batches and is specific for A/California/7/2009.

Materials of synthetic or biological origin (fertilized SPF eggs, embryonated eggs) are adequately controlled. Control of the A/California/7/2009 (NYMC X-179A) (H1N1) reference virus is performed by the WHO collaborative centers which provides the virus. The virus seed lot system is well described and includes the process for generation of new working seed lots. Purification, splitting and inactivation have been identified as critical steps and are well controlled.

Process validation data have been provided to demonstrate removal of the product-related impurity, ovalbumin. Ovalbumin levels are also checked in release testing of antigen final bulk. Octoxynol-9 is a process related impurity which arises from splitting. Formaldehyde is a process-related impurity arising from inactivation. Levels of formaldehyde are controlled on release testing of final bulk product.
Ovalbumin, octoxynol-9 and formaldehyde have been identified as the major product related or process related impurities and specifications have been set where appropriate. The Applicant has provided details of impurities and the proposed testing strategy for each impurity. The impurity testing proposed is appropriate.

- Specification

Release specifications for the antigen monovalent bulk comply with Ph. Eur. 0158. Tests are conducted for HA content and identity, neuraminidase antigenicity and identity (first 3 batches only), sterility, residual infectious viruses, and octoxynol-9 content. The Applicant has provided suitable justification for the specifications. Methods of analysis are acceptable overall. The biological properties of the monovalent bulk are mainly linked to the hemagglutinin and secondarily to the neuraminidase antigens which originate from the influenza strain. The antigenic property of the monovalent bulk is supported mainly by the hemagglutinin antigen. The hemagglutinin content of each monovalent bulk is assayed by Single Radial Diffusion (SRD) in compliance with European Pharmacopoeia monograph 0158.
Data from 3 batches of H1N1 drug substance have been provided. The batches met the acceptance criteria.

Sufficient validation data is provided regarding the following procedures: Hemagglutinin content, Hemagglutinin and neuraminidase identification and octoxynol-9 content. The applicant shows batch analysis results for three consecutive industrial scale batches of the monovalent bulk. Test results for all the batches meet the specifications.

The Applicant provides brief details of the stainless steel containers or polypropylene containers used to store MVB antigen. The container/closure system is identical to that used for the MVB storage of season influenza vaccine. Evidence of compliance with CPMP/QWP/4359/03 (Guideline on plastic immediate packaging materials) has been provided with representative certificates of analysis demonstrating compliance with specifications.

- Stability

The Applicant provided information on stability studies to be conducted. Batches will be tested for appearance, pH and HA content after storage for 24 months at 5°C±3°C or 30 days at +25°C±2°C °C. Acceptance criteria have been set for these parameters and are acceptable. Assurance has been provided that stability studies will be conducted with both stainless steel and plastic carboys. The Applicant has provided a commitment to complete the ongoing stability studies and has additionally committed to provide data to the Regulatory Authorities as it becomes available and inform the Regulatory Authorities of any out-of-specification results.
Data for 3 batches of MVB stored at +5°C ± 3°C for 3 months are presented. Further data from seasonal batches of influenza vaccine stored for 12 months at 5°C±3°C to support the proposed shelf-life for H1N1 antigen filled product of 12 months at 5°C±3°C have been provided. A shelf-life of 12 months at 5°C±3°C °C is acceptable for H1N1 antigen filled product.

AF03 Adjuvant Bulk

The Adjuvant AF03 Bulk is a concentrated oil-in-water emulsion containing 32.5 % (w/w) of squalene. The adjuvant is prepared by a Phase Inversion Temperature (PIT) process used to form a stable emulsion with a mean droplet size below 100 nm. The emulsion is stabilized by two non-ionic surfactants, SO (sorbitan oleate) (hydrophobic surfactant) and PCE (polyoxyethylene cetostearyl ether) (hydrophilic surfactant).

- Manufacture of adjuvant bulk

The Applicant has indicated which sites are used to manufacture AF03 adjuvant bulk and GMP certificates have been provided.

The manufacturing process consists of three main parts:
-Preparation of the aqueous phase;
-Preparation of the oily phase;
-Emulsification by phase inversion temperature process.

The Applicant has conducted some characterization of the two surfactants sorbitan oleate and polyoxyethylene cetosteayl either by HPLC and mass spectrometry. The characterization of surfactants using mass spectrometry was predicted and subsequently observed and thus can be considered confirmatory. Major peaks highlighted corresponded with the expected mass of the relevant ions.

Details of the production process of squalene have been provided. The Applicant states that production of squalene is conducted according to GMP, and a GMP certificate for the squalene manufacturer is provided. Tests conducted by the squalene manufacturer and those conducted by sanofi pasteur are listed. Information has been supplied regarding microbial quality controls applied to all raw materials. Full information regarding the manufacture and characterization of squalene has been supplied and provides assurance regarding extraneous agents and the removal of shark proteins. The specifications for squalene are considered adequate. A revised CoA has been supplied for squalene tested on receipt by sanofi pasteur.

The applicant has identified critical steps and consequently the parameters used to monitor the process. Data from robustness studies have been provided which identify the adequate parameters to monitor

The Applicant has provided data to demonstrate that:
-process parameters during PIT emulsification were met for the 3 consistency batches,
-the batches were homogenous for squalene content,
-the batches met specifications for squalene content, size distribution sterility and acetone content.
-mannitol, SO and PCE content of these batches were within the target range.
Process validation of AF03 bulk concentrate can be considered to have been demonstrated.

Changes made to the AF03 bulk concentrate manufacturing process during development are adequately described. No quality controls were performed for release of Phase I and II AF03 bulk and

that therefore comparability of commercial batches of AF03 bulk with Phase I can not be established. Comparability has been demonstrated for AF03 final bulk product and AF03 filled product.

Data have been provided from robustness studies to show the effects of changes in composition, stirring of oily phase, stirring and temperature during PIT and shearing on the process, and quality of AF03 bulk concentrate.

Characterization has been carried out on AF03 final bulk product. Full data from these studies have been provided.

Impurities arise from the degradation of squalene, SO and PCE. Production of acetone as a degradation product of squalene is judged to be the most sensitive determinant of AF03 degradation and surfactant degradation is thought to be detectable through pH measurement. The justification relating to toxicity and the proposed acceptance limit for acetone content is acceptable.

- Specifications

Specifications for the Adjuvant AF03 Bulk:

Specifications include bacterial and fungal sterility, pH, size distribution, squalene content and identification, PCE content, SO content, mannitol content, phosphate content and acetone content.

The proposed release tests for AF03 bulk concentrate are adequate to determine the quality of AF03 bulk and have been based on industrial scale batches.

Details of the non-Ph Eur method for determining squalene content have been provided. Data have been provided to show that the method for determining squalene content of AF03 bulk concentrate has been suitably validated. Sterility testing and size distribution testing are conducted according to Ph Eur monographs. As two QC sites are listed for AF03 bulk concentrate, confirmation is has been provided that the SOP for the analytical techniques is the same at both QC sites.

The Applicant has provided data on 3 industrial batches of AF03 bulk concentrate. The data show that specifications were met and that product is consistent.

Descriptions of the use of reference standards in AF03 bulk concentrate release testing have been provided. Example certificates of analysis for each of the reference standards have been provided. Information has been supplied on storage conditions for the squalene reference standard. The justification for the use of the reference standard to measure particles of approximately 100 nm is acceptable.

A stainless steel shipper vessel is used for the storage of the AF03 bulk concentrate. Clarification regarding the maintenance of the low oxygen atmosphere in this container remains has been provided.

- Stability

Data from stability studies of 3 batches of AF03 bulk concentrate have been provided. All quality parameters remained within specification after storage for 3 months at +5°C ± 3°C. Data have also been supplied for batches stored for 3 months at +25°C ± 2°C and show an increase in acetone content for 2 of the 3 batches. Based on the 3 month data, a 6 month shelf-life at +5°C ± 3°C for AF03 bulk concentrate is acceptable.

Medicinal Product

H1N1 vial

The A/H1N1 Pandemic Influenza Vaccine is presented in 7 mL vials with 1.5 mL of the Final Bulk Product at 30µg HA/mL. The vaccine complies with the recommendations of The World Health Organization and European Pharmacopoeia (Ph. Eur.), Monograph 0158.
3.8 µg HA/dose: The product reconstitution is performed by transferring 1.5 mL of the Filled Product at 30 µg HA/mL in the amber 10-dose vial containing 4.5 mL of Adjuvant AF03 Final Bulk Product. The adjuvanted A/H1N1 Pandemic Influenza Vaccine dosage obtained is 3.8 µg HA/dose vaccine for a 0.5 mL dose volume.

The composition includes the preservative thiomersal at 11.3 ug per dose. Antigen will be added to the adjuvant vial such that the final vaccine is presented in the Type I amber vial with chlorobutyl stopper that the AF03 Adjuvant drug product is presented in.

- Pharmaceutical Development

The Final Bulk Product (FBP) is a sterile suspension of the split inactivated monovalent bulk(s) formulated in a Phosphate Buffered Saline (PBS) solution, including thiomersal as a preservative.The FBP is aseptically filtered and filled into 10-dose vials to obtain the final filled product. The target composition of active ingredients in one final dose of 0.5 ml is 3.8 µg HA.
The applicant has supplied details of antigen doses used in clinical trial, including non-adjuvanted H1N1 vaccine. All excipients used in antigen formulation comply with Ph Eur monographs.

Dosing is based on previous clinical results with H5N1 strains and seasonal influenza vaccine. An overage is included in the H1N1 antigen vial reportedly based on historical stability data.
Information and in-use stability data have been supplied to support the amount of thiomersal included in the antigen formulation.
The primary change to the manufacturing process of H1N1 antigen final bulk product during development was a scale up. Batch data have been provided for Phase II H1N1 antigen final bulk product, although quality control tests were limited to stability, thiomersal content and HA content.
Stability data have been provided which indicate that Phase II Final bulk product batches are stable for appearance, pH, HA content and sterility when stored at +5°C ± 3°C for 2 months.

No changes have been made to the H1N1 antigen drug product manufacturing process during development. Batch data are supplied for 4 batches of Phase II H1N1 antigen drug product.
The Applicant has adequately explained the choice of vial and stopper for H1N1 antigen drug product. Data are presented to demonstrate that the stoppers have been tested according to Ph. Eur. 3.2.9 and met acceptance criteria. Cytotoxicity studies were conducted according to USP 87.

Justification for the level of thiomersal used has been provided. Size distribution data indicate that there is no physicochemical interaction between H1N1 antigen and AF03 adjuvant when the components are mixed. Animal studies indicate that an interaction between antigen and adjuvant may not be critical for development of an immune response to the vaccine.

- Adventitious Agents

Details of the SPF and healthy flock eggs used to manufacture H1N1 antigen drug substance are provided with a list of manufacturing steps responsible for reduction of bioburden.
The Applicant has demonstrated the ability of the manufacturing process for monovalent bulk antigen to inactivate ALV, mycoplasmas and H1N1 influenza viruses (data in process validation). The studies showed greater than 4 log reduction in titre, in compliance with CPMP/BWP/268/95.

Squalene, a major component of the AF03 adjuvant is purified from shark liver. Bioburden is controlled and sharks are not a relevant TSE species. Whilst no data have been provided to demonstrate the capability of the squalene distillation process to inactivate/remove viruses and no testing for viruses is conducted on the squalene raw material, given the species barrier and the squalene manufacturing process, the risk to humans from any shark virus in the raw material appears to be negligible. Indeed,

no virally associated pathology has been described for sharks with the exception of a herpesvirus infected captive smooth dogfish.

- Manufacture of the Product

The sites used in manufacture of H1N1 antigen drug product and their roles have been listed. GMP certificates issued by the inspectorates of Members states have been provided for each manufacturing site.
The batch formula for both 3.8 ug HA/dose H1N1 antigen drug product has been provided.

Basically, the manufacturing process consists of three major steps: the formulation with excipients, the aseptic filling process, and the labelling and packaging operations. The experimental formulation development that has been conducted is limited to the addition of the adjuvant AF03.

The thiomersal concentration employed is 11.3 µg per single dose.
The steps critical in the drug product manufacturing process are filtration of the antigen final bulk product and aseptic filling of the antigen final bulk product.

The description of manufacture of antigen final bulk product is acceptable.

A description of secondary packaging process has been provided and is acceptable.
The specifications for antigen final bulk product have been provided and comply with Ph. Eur. 0158.
The analytical techniques proposed for use in release testing of H1N1 antigen final bulk product have been appropriately described.
Data have been provided for batches of H1N1 antigen final bulk product used in clinical studies, showing that specifications for sterility and thiomersal content were met. Data have been provided for 3 batches of FBP manufactured at industrial scale. A shelf-life of 3 months at 5°C±3°C °C is proposed for H1N1 antigen final bulk product. Currently data following 3 months storage of batches of FBP are available and show FBP to be stable when stored at 5°C±3°C °C.

Data are provided from 2 media fill studies. No contaminated vials were found in media fill studies conducted on approximately 10,000 vials. Details of studies proposed to validate consistency of filling and reproducibility of filling at each site have been provided. The Applicant has provided process validation data for manufacture of H1N1 FBP and FP. The data shows that there was a significant difference in HA content between FBP and FP and thus the Applicant has increased the FBP overage. Preliminary data from batches filled following the overage increase show that this change has had the desired effect and that batches produced with the increased overage are well within specification for HA content. Further confirmatory data are awaited as a follow-up measure with the precise details of the overage proposed for X-179A batches.

- Product Specification

<u>Specifications for the H1N1 Final Bulk Product</u>

The controls of the Final Bulk Product have been set in accordance to existing pharmacopoeias and guidance. They include sterility, haemagglutinin content, osmolality measurement, thiomersal content, formaldehyde content, protein content, total protein/HA ratio, ovalbumine content.

<u>Specifications for the H1N1 Drug Product (in Vial):</u>

The finished product specification list as presently set by the applicant includes sterility, endotoxin content, appearance, pH, extractable volume, thiomersal content, haemagglutinin content and identification.

The panel of product specifications has been established on the basis of the Ph. Eur. monograph 0158 and is acceptable.
The specifications for H1N1 antigen drug product comply with Ph. Eur. 0158. H1N1 antigen drug product is tested according to Ph. Eur. monographs.
Validation of the Hemagglutinin (HA) identification/content is performed by Single Radial Diffusion (SRD).

The specifications for H1N1 antigen drug product comply with Ph. Eur. 0158. H1N1 antigen drug product is tested according to Ph. Eur. monographs. The Applicant presents justifications for the acceptance criteria used for sterility, endotoxin content, appearance, pH, extractable volume, thiomersal content and HA content. Information on the reference standards used for endotoxin and thiomersal content testing have been supplied. The Company has provided data to demonstrate that the SRD assay is valid for use with H1N1 final bulk product and H1N1 filled product.

Batch analysis is available for 2 clinical batches and shows that all acceptance criteria were met. The Applicant has provided data from 2 batches of H1N1 filled product at industrial scale which demonstrates consistency of filling, and that acceptance criteria were met for sterility, endotoxin content, appearance, pH, extractable volume, thiomersal content and HA content. Data are required from further batches following the change to FBP HA overage, however this may be addressed as a FUM.

No new impurities are expected as a result of the H1N1 antigen drug product manufacturing process. All impurities arise from H1N1 antigen drug substance.

The container closure system for antigen filled product consists of a Type I glass vial with chlorobutyl stopper and aluminium cap. The proposed specifications for the container closure system are acceptable.

- Stability of the Product

Details of a stability study to be conducted using batches from each of the manufacturing sites, stored at 5°C±3°C °C for 12 months have been provided. Parameters to be tested are appearance, pH, extractable volume, thiomersal content, integrity, endotoxin, HA content, sterility and toxicity and the proposed acceptance criteria are acceptable.

An in-use stability study of mixed antigen and adjuvant products is proposed for mixed product stored at 5°C±3°C for up to 7 days. Parameters to be tested include appearance, pH, osmolality, extractable volume, size distribution, preservative efficacy, and HA content. The data provided show that all acceptance criteria were met. Details of the techniques used to assess container closure integrity and abnormal toxicity have been provided. Justification for the removal of only 2 doses in this study has been provided with assurance that future in-use studies conducted on product stored for 12 months prior to mixing will include simulation of paediatric half-dose removal.

Additional commitments to provide the results of the H1N1 antigen drug product stability study when they become available and to inform the regulatory authorities of any out-of-specification results have been provided.

AF03 adjuvant vial

AF03 is an oil-in water emulsion composed of 3.3% of squalene. The adjuvant is prepared with a Phase Inversion Temperature (PIT) process to form a stable emulsion with a mean droplet size below 100 nm. This adjuvant AF03 filled product is presented in 7 mL amber glass vials containing 4.5 mL of emulsion (10-dose vials). The adjuvant AF03 filled product should be extemporaneously mixed with the pandemic influenza vaccine before its administration via the intramuscular route.

- Manufacture

After dilution of the AF03 bulk in phosphate-buffered saline (PBS), the AF03 drug product consists of an oil-in-water emulsion of 3.3 % of squalene. It also contains 0.49 % of sorbitan oleate, 0.63 % of polyoxyethylene cetostearyl ether and 0.61 % of mannitol in a PBS solution. It is presented in a 7 mL multidose amber glass vial containing a nominal volume of 4.5 mL of emulsion. Instructions for administration include visual inspection of mixed vials and the syringe for particles.

The composition of adjuvant vials has been provided and includes information on the content per dose. The components of AF03; squalene, SO, PCE, mannitol and PBS are adequately described. The components of PBS are tested according to Ph. Eur. monographs.
Clinical studies have been conducted with a range of adjuvant concentrations (3.3-5 % squalene) but all vaccine administered in the trials contained adjuvant at 2.5% squalene.

Manufacture of AF03 adjuvant drug product consists of the dilution of the AF03 bulk concentrate followed by filling into glass vials. AF03 bulk concentrate (32 % squalene) is diluted with PBS to form AF03 bulk product containing 3.3 % squalene.

The Company has provided data from animal studies to show that injection of AF03 induces an influx of inflammatory cells to the injection site. Data from *in vitro* studies with AF03 and H5N1 antigen show immuno-stimulating effects on human blood cells.

Process development for the adjuvant system has included change in raw materials suppliers and concentration, manufacturing site and the scale of production. The Applicant has compared the process used to manufacture Phase II AF03 product and that used to manufacture industrial scale batches. The study included an evaluation of equipment changes on critical steps, and a comparison of conductivity and temperature during PIT. Differences seen in conductivity and temperature profiles during PIT are considered by the Applicant to be due to differences in the heating/cooling kinetics due to the increased volume. Data from robustness studies have been provided to show that heating and cooling rates do not impact product quality.

Batch data from Phase II and industrial batches are provided to demonstrate comparability of AF03 drug substance (AF03 bulk concentrate), AF03 bulk product and AF03 filled product and satisfactory explanation was provided when variability occurs.

The Applicant has conducted small scales studies to demonstrate that the filtration step for final bulk product is capable of removing high concentrations of bacteria and that the product is not bactericidal. A study of filter extractables has also been conducted under worst-case conditions. Non-volatile residue levels were assessed. Analysis of the extractables found that they are non-toxic according to USP criteria.

The Applicant has conducted photostability studies to select the vial used for AF03 drug product. The data demonstrate that exposure to light leads to an increase in the squalene degradation product, acetone, and an increase in peroxide levels and thus the use of an amber vial for filling of AF03 adjuvant product is proposed. Silicon levels seen in the photostability studies are well within the MAD and derive from the silicon coating of the stoppers.

The amber glass vials and siliconized stoppers chosen for AF03 filled product have been described. A compatibility study has been conducted to study interactions between AF03 and different stoppers. Descriptions of the interaction testing and extractables and cytotoxicity studies conducted to choose the stopper and confirm acceptability of the stopper have been provided. The interaction study was not conducted using the proposed commercial filling process or commercial scale AF03 drug product but the results remain relevant. Confirmation that glass vials conform to Ph Eur 3.2.1 has been provided. Data have been provided on stopper composition and from cytotoxicity studies.

The Applicant has provided a list of the manufacturing sites involved in AF03 drug product production. GMP certificates have been provided for all manufacturing sites. The lot size has been clarified and the batch formula has been supplied.

A flow diagram and brief description of each step of production of AF03 final bulk product have been provided. The only in-process controls applied are filter integrity and bioburden testing before filtration. Essentially the manufacturing process consists of dilution and mixing of AF03 bulk concentrate with PBS. Details of the vessels used to collect and store AF03 final bulk product have been provided.

A flow diagram for filling of AF03 bulk product has been provided. The in-process controls applied to this process are fill volume and sterilization parameters for the container closure materials. Fill volume is checked by weighing the volume withdrawn from a vial or by weighing the volume filled into the vial.

A flow diagram for the secondary packaging of AF03 drug product vials has been provided. In-process controls are applied to test conformity of the labels and boxes, the clarity and accuracy of label printing and the integrity of the product. Confirmation regarding pack sizes and secondary packaging has been provided.

The critical process steps, controls and acceptance criteria used for production of AF03 drug product are adequately described. Controls are imposed for squalene content, sterility and filled volume. The acceptance criteria for size distribution, and squalene content have been revised and are acceptable.

The Applicant has provided data to demonstrate that process parameters, and in-process control limits were met during dilution steps for 3 batches of AF03 bulk product. The Applicant has also demonstrated that these batches were homogenous for squalene content and were consistent to specifications. Data are provided to demonstrate that operations performed during dilution provided a consistent product batches. The sterile filtration of final bulk product has been shown for 3 batches. The Applicant has provided data to demonstrate consistency of the filling process which includes batch analysis and consistency of filled volume and squalene content. Clarification has been provided regarding maximum filling batch size, and the fill volume and container/closure system used in the media fill simulations.

- Specifications

Specifications for the Adjuvant AF03 Filled Product (in Vial):

The Applicant has provided a list of the tests and specifications proposed for AF03 drug product. The proposed specifications for AF03 filled product are adequate to control the quality of the product, consisting of sterility, endotoxin, pH, fill volume, squalene content, squalene identity, acetone content and size distribution. The non-Ph Eur methods are appropriately described and validated.

Batch data have been provided for Phase II clinical batches. Batch data have been provided for 3 batches of AF03 filled product manufactured at industrial scale. The data show that all specifications were met.

The Applicant assumes that the manufacturing process for AF03 filled product does not introduce any additional impurities. Given that the manufacturing process of AF03 Drug Product essentially consists of dilution and filling, the Applicant's assumption has been considered acceptable.

The container closure system for AF03 filled product consists of an amber Type I glass vial with siliconized Ultrapur chlorobutyl stopper and aluminium cap. The proposed specifications for these components are acceptable.

- Stability

Data have been provided from 3 batches of AF03 filled product manufactured by the proposed commercial process and filling conditions. Stability data have been provided from the batches stored at 5°C +/- 2°C for 3 months and show no significant deterioration of key quality parameters. Data have also been provided from these batches stored at 25°C +/-2°C for 3 months, with no apparent significant change in key quality parameters. Data from these batches stored at 37°C +/- 2°C indicate an increase in acetone content after 2-3 months. A shelf-life for filled adjuvant product of 6 months at +5°C ± 3°C is acceptable, based on both industrial scale batches and Phase I & II data.

Drug Product (mixed H1N1 and AF03 adjuvant vial)

The Company has provided data to demonstrate that the SRD assay is valid for use with H1N1 final bulk product and H1N1 filled product. The Company has also provided data to demonstrate that the SRD assay is suitable for use with mixed antigen and adjuvant.

The Company has provided data from in-use stability studies of mixed antigen & adjuvant. These data indicate that the product is stable when mixed and stored at +5°C ± 3°C for 7 days. After mixing, Humenza should be stored in a refrigerator (2- 8°C) and should be used within 24 hours.

Facilities & Equipment
GMP certificates issued by the inspectorates of members states have been provided for all manufacturing sites.
The Applicant has provided comprehensive details for the location of each manufacturing step and the movement details for personnel and materials, equipment and location for each site which have been summarized above. All critical operations are performed under Class A area or laminar air flow in Class B rooms.

GMP status: The sites of manufacture have been inspected and accepted.

2.3 Non-clinical aspects

Introduction

The non-clinical studies provided in support of H1N1/AF03 candidate vaccine consists of pharmacology/toxicology data being generated using AF03-adjuvanted H5N1 or seasonal H1N1 vaccine, and AF03 alone. Pharmacology and Toxicology studies which refer to candidate vaccine consisting of the new H1N1 strain have also been submitted.

A non-clinical Toxicological testing programme which refers to candidate vaccine consisting of the new H1N1 strain (non-adjuvanted or AF03-adjuvanted) was submitted during the rolling review RR#05. Its primary focus was on the evaluation of 1) Reproductive and Developmental toxicities of AF03 and AF03-adjuvanted A/H1N1 vaccines, and 2) Acinar Cell Toxicity of the AF03 and AF03-adjuvanted A/H1N1 vaccine. At present, final reports of all these studies are not available but will be submitted as soon as available under follow-up measures. In addition, supportive data on lachrymal glands and pancreas obtained from studies with another adjuvant containing AF03 + an immunomodulator and with vaccines adjuvanted with this other adjuvant were also provided.

There were deviations from GLP in some studies of the non-clinical programme. However, the deviations concerning the rabbit general toxicity study described below can be accepted as there has been intense regulatory oversight of the study with the protocol discussed with assessors prior to the study being conducted. Regarding reproductive toxicity studies, GLP compliance was not claimed as only draft reports are available. This can be accepted in the rolling review process and no objections are raised in respect of GLP. However, ultimately, finished, signed study reports will be provided by the applicant.

Pharmacology

- Primary pharmacodynamics

Primary pharmacodynamics with AF03-adjuvanted A/H5N1 and seasonal vaccines

AF03 adjuvant has been tested in the context of influenza vaccines that were based either on A/H5N1 (different clades and subclades) or seasonal influenza antigens. The adjuvant effect of AF03 on immune responses elicited by vaccines was shown across studies and its dose-sparing effect was documented in mice and ferrets. It was shown that the extent of an AF03 immunopotentiating effect could be affected by such factors as age of animals (e.g., young versus old mice), priming status and primary immunization dose. In general, and as expected, the adjuvanting effect of AF03 was stronger in naïve than in primed mice in both age groups, and was greater in young animals than in old ones, and when a vaccine formulation contained low and suboptimal vaccine dose level.

In A/H5N1 challenge studies using monkey (infection) and ferret (disease) models, the protective efficacy of AF03-adjuvanted vaccines was demonstrated.

Noteworthy is that a mouse study was conducted to compare immunogenicity of a A/H5N1 monovalent vaccine (at a suboptimal dose of 0.3 µg of H5N1), formulated either with AF03, MF59-like, AS03-like, or other adjuvants (e.g. AlOOH, AF03+immunomodulator). AF03 has similar adjuvanting effects as the two other squalene-in-water emulsion (MF59-like, AS03-like), both in potentiating vaccine-specific humoral (HI titres) and cellular (IFN-gamma, IL-5) immune responses.

Primary pharmacodynamics with AF03-adjuvanted A/H1N1 vaccine

The ability of AF03-adjuvanted A/H1N1/California/07/2009 vaccine to induce specific humoral and cellular immune responses and to protect animals from viral challenge has been investigated in three immunogenicity studies in BALB/c mice and one ferret challenge study. Results of these studies provide proof of principle for immunogenicity and efficacy of the candidate vaccine.

The ferret study presented so far is with interim results only, and the final report of this study will be submitted as a follow-up measure.

Of note, all pharmacological data obtained in mice and ferrets have been generated using material made at the scale used to generate clinical trials material. In addition, two additional mouse studies are performed to address batch consistency using commercial lots and will be submitted as follow-up measures.

- Secondary pharmacodynamics

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

- Safety pharmacology programme

No safety pharmacology studies were performed with Humenza vaccine.

- Pharmacodynamic drug interactions

No studies were performed.

Pharmacology / AF03

With respect to the adjuvant mode of action of AF03, in vivo and in vitro studies demonstrated that monocytes and granulocytes (mostly neutrophils) were important target cells of the emulsion.
These cell populations were rapidly recruited at the injection site and the adjuvant was shown to favor monocyte differentiation towards Dendritic Cells. All these events should create an immunocompetent environment at the vaccination site that would enhance the triggering of an immune response to the vaccine antigens. The response profiles elicited by AF03 were in most aspects comparable to those induced by MF59-like, suggesting that these two oil-in-water emulsions share common features of mechanism of action.

Pharmacokinetics

In line with the relevant guidelines CPMP/SWP/465/95 and CPMP/VEG/4717/03, no pharmacokinetics studies have been performed.

Pharmacokinetics / AF03

No pharmacokinetics studies have been performed with AF03 alone.

Toxicology

- Single-dose Toxicity

No general single dose toxicity studies were carried out apart from the studies performed to address specific findings (see Other Toxicity Studies). The safety evaluation was assessed in the repeat dose toxicity studies.

- Repeat dose Toxicity

The systemic and local tolerance of AF03 alone was assessed in both a rat and a rabbit repeat dose toxicity study (see section Toxicology / AF03). The rabbit study also included the assessment of the A/H5N1 vaccine adjuvanted with AF03.
The dose, dosing route and regimen were relevant and tested rabbits showed to be immunologically responsive to the vaccine used in toxicity testing. In these studies with AF03 and H5N1-AF03 adjuvanted vaccine, toxic findings seen were confined to local reactogenicity, with systemic effects consistent with an immune response to vaccination. No specific concerns were raised.

- Genotoxicity

As a novel unlicensed adjuvant, AF03 has been studied for genotoxicity in a standard battery of tests including Ames test, Mouse Lymphoma assay and an *in vivo* micronucleus test. No specific safety concerns were raised.

- Carcinogenicity

No carcinogenicity studies were conducted which is in line with the Note for Guidance on Preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95)

- Reproduction Toxicity

The effect of AF03 and AF03-adjuvanted A/H1N1 vaccines on Reproductive system and on Pre- and Post-natal development has been investigated in 4 studies, including 1 rat study and 3 rabbit studies.

- 2 studies evaluating the adjuvant alone in rats and rabbits, and
- 2 rabbit studies assessing the AF03 adjuvanted Swine A/H1N1 Influenza Vaccines manufactured by the Applicant in Europe or in US

The applicant provided further details on the US and the EU vaccines used in these studies and showed that the differences between the two are minor. Data from the study conducted with the US vaccine are thus considered as supportive.

Under test conditions of 1) 6x I.M. injections (D-21, D-7, D6, D8, D11, and D17 (rats) or D27 (rabbits)) with AF03 alone at concentrations of 1.25-2.5% (rats) or 2.5-5% (rabbits), or 2) 5x I.M. injections (D-21, D-7, D6, D11, D27) in rabbits with 7.5 µgHA + 2.5% AF03, there were no embryo-fetal toxicity effects noted and also no treatment related effects were noted on pup weights as well as physical, functional and neurological development (D0: first day of gestation). Immunogenicity data generated in these studies demonstrated transfer of vaccine-specific maternal antibodies to fetuses and pups.

- Local tolerance

Local tolerance was assessed in two repeat toxicity studies in rats and rabbits with AF03-adjuvanted H5N1 vaccine. No specific safety concerns were raised.

- Other toxicity studies

Other general toxicity studies presented by the applicant were intended to address a specific finding of toxicity to the lachrymal glands which arose from studies with an adjuvant that is based on AF03 but additionally includes an immunomodulator. In brief, with H1N1 AF03-adjuvanted vaccine, slightly increased apoptosis and necrosis of acinar cells in the lachrymal glands was found in some rabbits given twice the human dose, as a single intramuscular injection in male rabbits, but not at the human dose. This effect was reproducible across several studies conducted with AF03 or with AF03+immunomodulator associated or not with an antigen and was found on microscopic but not macroscopic examination. Data from studies conducted with the AF03+immunomodulator adjuvant showed that this change was mostly found in males, was not found in mice, was reversible, even where adjuvanted vaccine was given on four occasions and was not found to be associated with an ocular abnormality during the in-life stages of these studies. However, there is no mechanistic explanation at present.

Toxicity Studies with AF03 and AF03-adjuvanted A/H1N1 vaccine

The effect of acinar cell toxicity of AF03 and AF03/H1N1 vaccine was sequentially assessed in two investigative studies, which were conducted in rabbit species but included only one single I.M. dose, one gender of animals (i.e. male rabbits), a short observation period (2 days after dosing), and with histopathological examinations primarily focused on specified tissues [eyes, lachrymal tissues (lachrymal gland, nictitating membrane, Harderian gland and eyelids) and pancreas].

<u>Investigative Toxicity Study after a Single IM Injection of AF03/H1N1 vaccine in male NZW Rabbits (Study no. KH06001 IS0914)</u>

No GLP status was claimed for this study.

The objectives were to investigate the potential effects of AF03 adjuvanted-A/H1N1 vaccine at three different dose-levels (twice the human dose, the human dose, and half the human dose) on the lachrymal tissues and the pancreas. The aim was to establish a NOEL for these organs in NZW male rabbits following a single I.M. injection.

A total of 40 NZW male rabbits were allocated to the study. Ten males were given one IM injection on day 1 of either AF03 adjuvanted-A/H1N1 vaccine at 0.5-, 1- or 2-fold the human dose or saline.

Summary of acinar cell necrosis/apoptosis findings

Group Dose-level Number of males	1 0 10	2 0.5xHuD 10	3 HuD 10	4 2xHuD 10
Main body of the lachrymal gland	-	-	-	1 (bilateral)
Subconjunctival part of the lachrymal gland	1 (unilateral)	1 (unilateral)	1 (unilateral)	4 (1 bilateral and 3 unilateral)

-: not observed

At 2x human dose, one single I.M. injection of AF03/H1N1 vaccine increased the incidence of a minimal necrotic/apoptotic process in acinar cells of lachrymal glands, although in this case other lachrymal tissues like nictitating membranes and eyelids were not affected.
Notably, at the human dose or half the human dose, one single I.M. injection of AF03/H1N1 vaccine did not cause such an effect, because the microscopic observation of unilateral acinar cell necrotic/apoptotic process were consistent with the controls, thus establishing the human dose of AF03/H1N1 vaccine as a NOEL. This one human dose level in rabbit species corresponds to a safety margin of about 17x for a 50 kg body weight human and is considered quite acceptable for toxicological testing of the vaccine class for human use.

Investigative Toxicity Study After a Single IM Injection of AF03 alone in male Rabbits (Study no. KH06001 IS0912)

No GLP status was claimed for this study.
The objectives were to investigate the acinar cell toxicity (apoptosis/necrosis), induced by an adjuvant composed of AF03 + an immunomodulator previously observed in a study after 4 intramuscular injections to NZW rabbits, and to determine whether this finding could be induced after one dose, to provide quick investigative model for follow-up studies. In addition, AF03 was evaluated as it is a component of the concerned adjuvant.
A total of 30 male NZW rabbits were allocated to 2 treated groups and one control group. Groups of 10 male rabbits received one IM injection of either the mixed adjuvant AF03+ immunomodulator or AF03 at 2-fold the human dose (5% squalene + 20 µg immunomodulator and 5% squalene alone, respectively).
At 2x human dose, an exposure level higher than that to be administered in man, one single I.M. injection of AF03 alone induced a higher incidence of minimal histological change (apoptosis/necrosis of acinar cells) in the lachrymal tissues (lachrymal glands, nictitating membranes and eyelids, but not in Harderian gland), but not in pancreas in contrast to AF03+immunomodulator adjuvant.

Acinar Cell Apoptosis/Necrosis in / AF03 - Single Dose Investigative Study

Organs	**Control**		**AF03 (2xHuD)**	
	Incidence	**Maximum grade**	**Incidence**	**Maximum grade**
Pancreas	0/10	-	0/10	-
Main body of Lachrymal glands	0/10	-	2/10 (1 bilateral, 1	1

Organs	Control		AF03 (2xHuD)	
	Incidence	Maximum grade	Incidence	Maximum grade
			unilateral)	
Subconjunctival part of the Lachrymal glands	3/10 (unilateral)	1	4/10 (3 bilateral, 1 unilateral)	1
Nictitating membrane	0/10	-	2/10 (unilateral)	1
Eyelids	0/10	-	1/10	1

HuD: Human dose - not applicable

The significance of these minimal histological findings observed for AF03 or AF03/H1N1 vaccine at 2x human dose level is unknown yet, in considering that:

- These minimal histological findings in lachrymal tissues were not accompanied by microscopical structure changes in the eyes and were not associated with any ophthalmologic dysfunctions, as assessed extensively both by standard in-life ophthalmologic examinations and by several functional tests including blink rate, tear flow and corneal surface examinations. This even holds for AF03+immunomodulator adjuvant associated or not with an antigen, which were shown to induce histological changes at a higher frequency and severity in rabbit species tested
- A complete recovery of the histological changes, as revealed in two independent repeat-dose toxicity studies (one standard with AF03+immunomodulator associated with an antigen, one investigative with AF03+immunomodulator alone or with an antigen) where a recovery group was included. In these studies, no findings were observed 14 days after the last injection. Furthermore, a comparison of results across studies revealed that repeated dosing caused similar effect as did one single dose, suggesting a full recovery during dosing intervals
- AF03+immunomodulator associated with another antigen has also been tested in a standard repeat-dose toxicity study in mice with no findings in pancreas observed, in contrast to observation made in rabbits. This, together with the fact of species-species difference in lachrymal gland overall size, tissue morphology, expression of biochemical markers and abundance of gland tissue on a body weight basis, necessitates a careful interpretation of the clinical relevance of histological finding observed for AF03 and AF03/H1N1 vaccine.

Toxicology / AF03

Dedicated studies evaluated the nonclinical safety profile of the adjuvant, which was considered as a new chemical entity composed of squalene, polyoxyethylene 12 cetostearyl ether, sorbitan oleate and mannitol. These studies included three genotoxicity studies, two *in-vitro*: an Ames test (AA32696) and a mouse lymphoma assay (AA32697), and one *in-vivo*: a micronucleus test in mice after intravenous injection of a maximum tolerated dose (AA33728). Two repeat dose toxicity studies, one in rodents (in rats: AA32695) and one in non rodents (in rabbits: AA33212), assessed the systemic toxicity and local tolerance of AF03. In the rat repeat dose and in the reproductive toxicity studies, several dose levels of AF03 were assessed (from 1.25% to 10% squalene, including the human dose 2.5% squalene) in order to establish a dose effect relationship and determine the no observed adverse effect level (NOAEL). In the rabbit repeat dose toxicity study, the human dose of AF03 used in the vaccine was evaluated.
No specific safety concerns were raised.

Ecotoxicity/environmental risk assessment

No environmental risk assessment was included in the application. According to the guideline EMEA/CHMP/SWP/4447/00 "Environmental Risk Assessment of Medicinal Products fro Human Use" vaccines due to the nature of their constituents are exempted from the requirement to provide an environmental risk assessment in the application for a marketing authorisation for a medicinal product for human use.

Discussion on non-clinical aspects

Primary pharmacodynamics study performed with AF03-adjuvanted A/H1N1 vaccine provide proof of principle for immunogenicity and efficacy of the candidate vaccine. However, what immune mechanism(s) of defense can render protection against new A/H1N1 is currently unknown.

Regarding reproduction toxicity studies, the study design used did not result in exposure in early pregnancy. Doses were given 7 days before mating and from day 6 of gestation and therefore a period, corresponding to the first 6 days of pregnancy is not covered. The applicant provided further justification behind the choice of the study design. However, this did not consider the effect of dosing with vaccine and exposure to adjuvant just before implantation. The theoretical risk is that an immune effect arising from vaccination in the first trimester of pregnancy might be associated with effects leading to a failure of implantation. There is insufficient evidence of a hazard from the studies conducted. On balance, it is reasonable to conclude that the data presented show that the constituents of the adjuvant are not expected to have an adverse effect in early pregnancy; the studies are sufficient to indicate no effect is expected from immunogenic responses to the antigen.

A reproducible non-clinical finding of necrosis and apoptotis was identified in lachrymal glands in rabbit studies. The understanding of this phenomenon with new adjuvant is currently poor and requires further research and non-clinical studies. However, this finding of toxicity to the lachrymal gland did not cause clinically or macroscopically identifiable effects in animals.These findings have been adequately reflected in the SPC.

2.4 Clinical aspects

Introduction

GCP

The applicant provided a statement to the effect that clinical trials conducted within and outside the European community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Pharmacokinetics

Pharmacokinetic studies were not performed in accordance with the note for guidance on clinical evaluation of new vaccines (CPMP/EWP/463/97) and the Guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application (CPMP/VEG/4717/03).

Pharmacodynamics

In relation to vaccines, the pharmacodynamic studies consist of assessments of the immune responses.
The data on the immunological response to Humenza obtained during clinical studies are described and discussed below.

Clinical efficacy

The clinical dossier consists of three main European clinical trials conducted with the AF03/H1N1 vaccine:

- **GPF07**: a phase II, multicenter, randomised, open label trial in healthy adults aged 18 to 60 years and elderly subjects aged >60 years conducted in France.

- **GPF 08**: a phase II, multicenter, randomised, open label trial in healthy paediatric subjects aged 3 to 17 years conducted in Finland.
- **GPF 09**: a phase II, multicentre, randomised, open trial, in healthy paediatric subjects aged 6 to 35 months conducted in Finland.

In addition, the two supportive studies conducted with an AF03/H5N1 vaccines were submitted:

- **FUF04** (US): a phase I, observer-blinded, randomised, formulation/dose ranging study in healthy adults aged 18-40 years.
- **GPF01** (Belgium): a phase I (two-step) dose/formulation finding study in healthy adults aged 18-40 years.

Of note, there is a difference in manufacturing process between the four European studies and the US trial supportive study FUF04.
The design and implementation of the main and supportive studies is summarised below:

Table 1. Summary of Clinical Studies Conducted with the A/H1N1 Strain

	GPF07	GPF08	GPF09i
Phase	II	II	II
Sample size	450 subjects planned 300 adult subjects (18 to 60 years) 150 elderly subjects (over 60 years) 3 vaccine groups Adjuvanted vaccine groups - 3.8 µg HA + AF03: 99 adult and 54 elderly subjects - 7.5 µg HA + AF03: 100 adult and 51 elderly subjects Non-adjuvanted group: - 15 µg HA: 100 adult and 50 elderly subjects	300 subjects planned 150 subjects aged 9-17 years 150 subjects aged 3-8 years 3 vaccine groups Adjuvanted vaccine groups - 3.8 µg HA + AF03: 50 subjects aged 3-8 years, 49 subjects aged 9-17 years - 7.5 µg HA + AF03: 50 subjects per age group Non-adjuvanted group: - 15 µg HA: 50 subjects per age group	400 subjects planned: 200 aged 6 to 11 months 200 subjects aged 12 to 35 months. 4 vaccine groups Adjuvanted vaccine groups - 1.9 µg HA + ½ AF03: 48 subjects per age group - 3.8 µg HA + ½ AF03: 50 subjects per age group - 3.8 µg HA + AF03: 52 subjects per age group Non-adjuvanted group: - 7.5 µg HA: 50 subjects aged 12 to 35 months, 51 aged 6 to 11 months
Design	Randomised, multicenter, open trial	Randomised, multicenter, open trial	Randomised, multicenter, open trial
Countries and Number of Investigational Sites	France, 12 centers	Finland, 15 centers	Finland, 15 centers
Objective	Description of safety and immunogenicity of different A/H1N1 formulations with or without adjuvant following two vaccinations at a 21-day interval		Description of safety and immunogenicity of different A/H1N1 formulations with or without adjuvant, given as half or full doses, following two vaccinations at a 21-day interval
Immune Response Assessments	All subjects: anti-HA antibodies by HAI, neutralising antibodies by SN, antibodies to the A/H1N1 strain by SRH		
Follow-up Duration	8-month antibody persistence 12-month safety follow-up	8-month antibody persistence 12-month safety follow-up	8-month antibody persistence 12-month safety follow-up
Presentation of Vaccine	Antigen presented in multidose vials to be extemporaneously mixed with AF03 (adjuvanted formulation) antigen presented in multidose vials (non adjuvanted formulation)		

Table 2: Summary of Clinical Studies Conducted with the A/H5N1 Strain

Supportive STUDIES	GPF01	FUF04
Design	Phase I, two steps: **Step 1**: open, monocenter, uncontrolled **Step 2**: *Primary series*: blind-observer,	Phase I, randomised, blind-observer, multicenter, controlled

	multicenter, randomised, controlled *Booster*: open, controlled	
Country and No. of study sites **Trial Period presented in this report**	Belgium (3 centers, 3 investigators in step 2) Step 1 from D0 to D42 Step 2 from D0 to D386 (21 days after booster at month 12)	USA (six centers, 6 investigators) 19 April 2008 to 30 January 2009 (6 month follow-up after the primary series)
Sample size and grouping	Step 1: 15 subjects aged 18-40 years - 15µgHA+AF03 single group, N = 15 Step 2: 250 subjects aged 18-40 years planned (recruited: 251), divided into 5 groups: **For primary series:** Adjuvanted vaccine groups: - **Group 1**: 1.9µgHA+AF03, N = 50 (51 recruited) **- Group 2**: 3.75µgHA+AF03, N = 50 **- Group 3**: 7.5µg HA+AF03, N = 50 **- Group 4**: 15µg HA+AF03, N = 50 Control group: **- Group 5**: 7.5µgHA, N = 50 **For booster vaccination in Step 2 only at Month-12*:*** Groups 1 to 4 received the 3.75µgHA+AF03; Group 5 received the 7.5µgHA control vaccine	375 subjects aged 18 to 40 years included 375 randomised in 9 groups: **For primary series:** Adjuvanted vaccine groups: - **Group 1**: 2.5 µg HA + AF03 at 0.5%, N = 50 - **Group 2**: 2.5 µg HA + AF03 at 1%, N = 50 - **Group 3**: 2.5 µg HA + AF03 at 2.5%, N = 52 - **Group 4**: 6 µg HA + AF03 at 0.5%, N = 49 - **Group 5**: 6 µg HA + AF03 at 1%, N = 49 - **Group 6**: 6 µg HA + AF03 at 2.5%, N = 50 Control groups: - **Group 7**: 2.5 µg HA, N = 25 - **Group 8**: 6 µg HA, N = 26 - **Group 9**: placebo, N = 24 **For booster vaccination in a subset at Month 15**: Group 3 received the 3.8µgHA+2.5%AF03 Group 6 received the 7.5µgHA+2.5%AF03 Group 8 received the 7.5µgHA only.
Study Objectives	Description of safety and immunogenicity of different A/H5N1 formulations with adjuvant compared to one non-adjuvanted formulation following a two vaccination primary series vaccination and a booster vaccination	Description of safety and immunogenicity of different A/H5N1 adjuvanted and non-adjuvanted formulations following a two vaccination primary series vaccination and a booster vaccination
Immune Response Assessments	All subjects: anti-HA antibodies by HAI and neutralising antibodies by SN Subset of subjects: CMI assessment	All subjects: anti-HA antibodies by HAI neutralising antibodies by SN
Vaccination schedule **Study duration**	Primary series: Two I.M. injections (*A/Vietnam/1194/2004/NIBRG-14 strain*) separated by 21 days Booster: one I.M. injection (***A/Indonesia strain***) at month 12. Approx. 19 months Antibody persistence: M3, M6, M12 6-month follow-up after the last	Primary series: Two I.M. injections (*A/Indonesia/05/2005/PR8-IBCDC-RG2)* separated by 21 days Booster: one I.M. injection (*A/Bar-headed goose/Qinghai/ 1A/2005*) at month 15. Approx. 22 months Antibody persistence: M6, M12, M15,

Follow-up Duration	vaccination (booster at 12 months).	and M21 6-month follow-up for a subset of subjects boosted at Month 15. 12-month follow-up for subjects with no booster

All these studies are still ongoing. In all these studies, AF03-adjuvanted vaccine formulations are compared with non-adjuvanted vaccines using a two-dose schedule with a dose interval of 21 days.

Data following the two-dose priming schedule were provided for the three studies with the H1N1v/AF03 candidate vaccine and for the two supportive H5N1/AF03 studies.
Date up to Month 6 – Month 12 post-primary were provided for the two H5N1/AF03 supportive studies.

Assays

For a potential pandemic vaccine candidate based on an A/H5N1 strain, such as the one used in GPF01 and FUF04, an haemagglutination inhibition (HAI) assay using horse erythrocytes has been shown to be more sensitive than the standard HAI assay with turkey erythrocytes or chicken erythrocytes, which is used in the evaluation of seasonal vaccines. The HAI assay with horse erythrocytes was therefore used for evaluation of the immune response to the A/H5N1 vaccine. The HAI assay with turkey erythrocytes was used for evaluation of the immune response to the adjuvanted A/H1N1 pandemic influenza candidate vaccine.

In all studies conducted with the A/H5N1 prototype pandemic influenza vaccine and with the adjuvanted A/H1N1 pandemic influenza candidate vaccine all sera were also analysed by a seroneutralisation (SN) assay.

HAI and SN assays were carried out at GCI laboratory in USA. Control samples were included in the assays. Validation reports were provided that indicated a robust performance of both assays (for H5N1 and H1N1).

In addition, antibody titres against the swine-origin A/H1N1 influenza virus were measured in sera obtained during the three studies with the A/H1N1 adjuvanted vaccine using single radial haemolysis (SRH) performed at the laboratory of Dr. Emanuele Montomoli, University of Siena, Italy.

The HAI and SRH data, together with the SN data, provide a surrogate marker for efficacy in this application, although, as for all pandemic influenza vaccines, no direct correlation has been demonstrated between the immune response and clinical efficacy.

- Dose-Response Studies

Study GPF01 investigated AF03 adjuvanted H5N1 (strain A/Vietnam) vaccine formulations with varying antigen doses. The antigen was produced according to the Vaxigrip process (European manufacturing site). **Study FUF04** was conducted in the USA evaluating different adjuvant concentrations and employing H5N1 (strain: A/Indonesia) antigen produced according to the Fluzone process (US manufacturing site).

The immunogenicity results from GPF01 and FUF04 demonstrated that a single dose of H5N1/AF03 formulations was not sufficient. All 3 CHMP criteria were met only following two doses of vaccine formulations containing 3.75µg to 15µg HA antigen adjuvanted with 2.5% AF03. A dose-response effect was observed using different antigen and different AF03 concentrations.

There were increases in GMTR, SCRs and SPRs following the second dose of H5N1/AF03 formulations compared to values observed after the first dose. At 6 months post-vaccination 15-19.5% of subjects were still HIH seroprotected after receipt of 2.5-6 µg/2.5% AF03 formulations while none of the subjects in the unadjuvanted vaccine control groups were seroprotected. High SN titres were observed in groups vaccinated with 2.5-6 µg/2.5% AF03 formulations (85-99.5% with titres >=40 at day 42 and 47-79% with titres >=40 at Month 6). Induction of cross-reactive neutralising antibodies was demonstrated against A/Indonesia although titres were considerably less than against the homologous virus. There was a limited vaccine specific Th1 response seen in CMI assays with the AF03 adjuvant. However this area remains largely exploratory in influenza vaccine development.

Overall it was considered that the choice of a regimen consisting of two doses of either 3.75µg or 7.5µg HA and 2.5% AF03 for the planned H1N1/AF03 clinical trials in adults and children was justified.

- Main studies

Study GPF07 and GPF08 (subjects of >3 years of age)

METHODS (GENERAL)

Study Participants
The inclusion and exclusion criteria were in general identical for studies GPF01, FUF04, GPF07, GPF08 and GPF09.
Specific exclusion criteria included:
- Previous participation in a clinical trial involving an investigational flu pandemic vaccine
- Vaccination with an influenza vaccine during the past 6 months (GPF01)
- Confirmed infection with the swine-origin A/H1N1 influenza strain in 2009 (GPF07, GPF08 and GPF09)

Treatments and Objectives
This is described in table 1.

Outcomes/endpoints
The **primary immunogenicity endpoints** were defined as described below.
HAI antibody (Ab) titres, Neutralising Ab titres and Ab titres measured by SRH method were obtained on D0, D21 and D42.

HAI assay:
HAI Ab titres were obtained in duplicate assays; the geometric mean of these two values was used.
The following endpoints were derived:
- Geometric Mean Titre (GMT) ratios
- Seroprotection rate defined as HAI Ab titre ≥40 (1/dilution [dil])
- Seroconversion rate defined as the proportion that was either seronegative prior to vaccination and had a post-vaccination titre ≥40 (1/dil) or seropositive prior to vaccination and had at least a 4-fold increase in titre post-vaccination.

The results were compared against the CHMP criteria applied to the assessment of seasonal influenza vaccines for age groups 18-60 years and > 60 years.

SN assay:
- GMT ratios
- Subjects with two- and four-fold increase of titres
- Subjects with neutralising Abs titre ≥20, ≥40, and ≥80 (1/dil)

Randomisation
A randomisation list stratified by centre and age group was created using the block permutation method, stratified by age group within centres. This guaranteed, at any time, a similar number of subjects between the three investigational medicinal products inside each centre and each age group.

Blinding/Masking
The studies were open-label but serology testing was performed in a blinded manner.

Statistical methods
No statistical hypothesis was tested. All analyses were descriptive.

RESULTS

Participant Flow

Study GPF07

A total of 450 subjects (300 adults [aged 18 to 60 years], and 150 elderly subjects [aged over 60 years]) were included in the study and randomised to one of the three vaccine groups. All subjects included at D0 were included in the Full Analysis Set (FAS) for immunogenicity analysis and in the Safety Analysis set (SafAS) for safety assessment after the first vaccination. All but two subjects (withdrawals after the first vaccination) were included in the SafAS for safety assessment after the second vaccination.

Study GPF08

A total of 303 subjects, i.e. 152 in the 3 to 8 years group and 151 in the 9 to 17 years group were included in the study. All subjects were included in the FAS and in the SafAS after the first vaccination. Four subjects discontinued the study within 21 days after the first vaccination and were not included in the Safety Analysis set after the second vaccination.

Recruitment

Subjects were enrolled in study GPF07 between 18 and 22 August 2009.
In study GPF08, subjects were included between 18 and 25 August 2009.

Baseline data

A high proportion (%) of adult and elderly subjects had evidence of pre-existing immunity to the H1N1 virus. Values shown represent %. In () are CIs.

BASELINE SEROPOSITIVITY (HAI or SN ≥ 10 1/dil)

GPF07	adult **18-60 y**	elderly **> 60y**
3.8µgHA+AF03	HI:44 (35; 55); SN: 60 (49; 69)	HI: 59 (45; 72); SN: 63 (49; 76)
7.5µgHA+AF03	HI:45 (35; 55); SN: 55 (45; 65)	HI: 49 (34; 64); SN: 71 (56; 83)
15µgHA	HI:41 (31; 51); SN: 47 (37; 57)	HI: 45 (30; 61); SN: 58 (42, 72)
GPF08	children **3-8 y**	children **9-17 y**
3.8µgHA+AF03	HI: 0 (0; 7); SN: 2 (0; 11)	HI: 23 (12; 37); SN: 20 (10; 34)
7.5µgHA+AF03	HI: 2 (0; 11); SN: 2 (0; 11)	HI: 31 (18; 45); SN: 36 (23; 51)
15µgHA	HI: 0 (0; 7); SN: 4 (1; 13)	HI: 12 (4; 23); SN: 17 (8; 30)

BASELINE SEROPROTECTION (HAI ≥ 40 1/dil, SN ≥ 80 1/dil)

GPF07	adult **18-60 y**	elderly **> 60y**
3.8µgHA+AF03	HI: 14 (8; 23); SN: 23 (15; 33)	HI: 15 (7; 27); SN: 17 (8; 29)
7.5µgHA+AF03	HI: 10 (5; 18); SN: 14 (8, 22)	HI: 10 (3; 22); SN: 18 (8, 31)
15µgHA	HI: 15 (9; 23); SN: 17 (10; 26)	HI: 9 (3; 22); SN: 18 (8; 32)
GPF08	children **3-8 y**	children **9-17 y**
3.8µgHA+AF03	HI: 0 (0; 7); SN: 0 (0; 7)	HI: 4 (1; 14); SN: 4 (1; 14)
7.5µgHA+AF03	HI: 0 (0; 7); SN: 0 (0; 7)	HI: 10 (3; 22); SN: 16 (7; 29)
15µgHA	HI: 0 (0; 7); SN: 0 (0; 7)	HI: 8 (2; 19); SN: 8 (2; 19)

Beyond serostatus, other baseline characteristics including age, sex, race, influenza history / pre-vaccination with influenza vaccines are also well balanced across study groups in each study.

Outcomes and estimation

Summary of HAI data following vaccination with the 3.8 µg HA pandemic A/H1N1 influenza vaccine

The results of the HAI and SN assays showed similar trends after both vaccinations.
Twenty-one days after one vaccination, the HAI data demonstrated that almost all adult, elderly and 3 to 17-year old subjects had detectable anti-HA Ab (titre ≥ 10 [1/dil]) and that 97.0% of adults, 83.3% of elderly subjects, and 100% of subjects aged 3 to 17 years had seroprotective titres (≥ 40 [1/dil]) to the A/H1N1 influenza strain. Neutralising antibody titres ≥ 40 (1/dil) were observed for more than 85% of subjects in GPF07 and in all subjects in GPF08 21 days after the first vaccination.

The benefit of the second dose was in terms of level of response, particularly in 3 to 8-year-old subjects and in subjects aged 60 years and over (a single dose was clearly insufficient in persons of >70 years of age). Clinical data in subjects of >80 years age were extremely limited. After the second vaccination, almost all subjects reached seroprotective HAI Ab titres to the A/H1N1 strain and had neutralising Ab titres ≥ 80 (1/dil).

The three CHMP criteria were reached in all age groups after the first and after the second vaccination.

Table 3: GPF07 and GPF08 - Summary of Immunogenicity Data Following the First (D21) and the Second (D42) Vaccinations (3.8 µg HA Pandemic A/H1N1 Influenza Vaccine) - Full Analysis Set (FAS)

		GPF07		GPF08	
Assay	**Parameter**	**18 to 60 years (N=99)**	**>60 years (N=54)**	**3 to 8 years (N=50)**	**9 to 17 years (N=49)**
HAI	**Geometric mean titre (1/dil) D21**	826	184	631	1208
	D42	1205	298	4476	3595
	Subjects with titres ≥ 40 (1/dil) (%) D21	97.0	83.3	100.0	100.0
	D42	100.0	96.3	100.0	100.0
	Seroconversion* or significant increase† from D0 (%) D21	93.9	74.1	100.0	100.0
	D42	99.0	90.7	100.0	100.0
	Geometric mean of titre ratio D21/D0	76.0	16.2	124	177
	D42/D0	115	26.2	883	527
SN	**Geometric mean titre (1/dil) D21**	2972	536	2094	3661
	D42	4316	884	9932	9172
	Subjects with titres ≥ 80 (1/dil) (%) D21	99.0	85.2	100.0	100.0
	D42	100.0	96.3	100.0	100.0
	4-fold increase from D0 (%) D21	94.9	85.2	100.0	100.0
	D42	95.9	88.9	100.0	100.0

*Seroconversion for subjects with pre-vaccination titre <10 (1/dil) on D0, post-vaccination titre ≥40 (1/dil)

† Significant increase for subjects with pre-vaccination titre ≥10 (1/dil), ≥4-fold increase of the titre after vaccination (post/pre)

Detailed HAI immune response observed following vaccination with the 3.8 µg HA and 7.5 µg HA formulations

The key data are summarised in Tables 3 and 4.

HAI

Adults (18 to 60 years of age)
Twenty-one days after the first vaccination, 100% of subjects had a detectable antibody titre, with GMTRs of 76.0 and 82.9 in the 3.8 µg HA + AF03 and 7.5 µg HA + AF03 groups, respectively and seroconversion rates ≥ 93% in each group. A second dose of of adjuvanted vaccines did not elicit a further significant increase of immune response.

Elderly (>60 years of age)
At D21, GMTs of 184 (1/dil) in the 3.8 µg HA + AF03 group and of 271 (1/dil) in the 7.5 µg HA + AF03 group were observed with GMTRs of 16.2 and 25.0 in the 3.8 µg HA + AF03 and 7.5 µg HA + AF03 groups, respectively, and with seroconversion rates ≥ 74% in each group. A second dose of both adjuvanted vaccines did not elicit a further significant increase of immune response.

9-17 years group
At D21 all subjects had detectable antibody.

- Geometric mean of titres ratio (GMTRs) D21/D0 reached 177 in the 3.8 µg HA + AF03 group and 190 in the 7.5 µg HA + AF03 group.
- The seroconversion rate was 100% in the two adjuvanted vaccine groups.
- GMTs increased from 6.82 (1/dil) at D0 to 1208 (1/dil) at D21 in the 3.8 µg HA + AF03 group and from 8.38 (1/dil) at D0 to 1544 (1/dil) at D21 in the 7.5 µg HA + AF03 group.

At D42 the immune response to the vaccine was increased compared to D21:

- GMTRs D42/D21 were 2.98 in the 3.8 µg HA + AF03 group and 2.93 in the 7.5 µg HA + AF03 group.
- GMTs increased from 1208 (1/dil) at D21 to 3595 (1/dil) at D42 in the 3.8 µg HA + AF03 group and from 1544 (1/dil) at D21 to 4476 (1/dil) at D42 in the 7.5 µg HA + AF03 group.

3 to 8 years group
At D21 all children had detectable antibody.

- GMTRs D21/D0 reached 124 in the 3.8 µg HA + AF03 group and 152 in the 7.5 µg HA + AF03 group
- Seroconversion rate or significant increase was 100% in the two adjuvanted vaccine groups.
- GMTs increased from 5.07 (1/dil) at D0 to 631 (1/dil) at D21 in the 3.8 µg HA + AF03 group and from 5.07 (1/dil) at D0 to 772 (1/dil) at D21 in the 7.5 µg HA + AF03 group.

At D42 the immune response to the vaccine was increased compared to D21:

- GMTRs D42/D21 were 7.09 in the 3.8 µg HA + AF03 group and 6.12 in the 7.5 µg HA + AF03 group.
- GMTs increased from 631 (1/dil) at D21 to 4476 (1/dil) at D42 in the 3.8 µg HA + AF03 group and from 772 (1/dil) at D21 to 4729 (1/dil) at D42 in the 7.5 µg HA + AF03 group.

Table 4: GPF07 and GPF08 - Summary of Immunogenicity Data Following the First (D21) and the Second (D42) Vaccinations (7.5 µg HA Pandemic A/H1N1 Influenza Vaccine) - Full Analysis Set (FAS)

		GPF07		GPF08	
Assay	**Parameter**	**18 to 60 years (N=99)**	**>60 years (N=54)**	**3 to 8 years (N=50)**	**9 to 17 years (N=49)**
HAI	**Geometric mean titre (1/dil) D21**	787	271	772	1544
	D42	1198	414	4729	4476
	Subjects with titres ≥ 40 (1/dil) (%) D21	99.0	89.6	100.0	100.0
	D42	100.0	96.0	100.0	100.0
	Seroconversion* or significant increase† from D0 (%) D21	98.0	85.4	100.0	100.0
	D42	100.0	93.8	100.0	100.0
	Geometric mean of titre ratio D21/D0	82.9	25.0	152	190
	D42/D0	126	41.5	932	558
SN	**Geometric mean titre (1/dil) D21**	2580	788	2791	4492
	D42	4203	1315	9791	10109
	Subjects with titres ≥ 80 (1/dil) (%) D21	99.0	90.2	100.0	100.0
	D42	99.0	98.0	100.0	100.0
	4-fold increase from D0 (%) D21	98.0	86.3	100.0	100.0
	D42	99.0	96.0	100.0	100.0

*Seroconversion for subjects with pre-vaccination titre <10 (1/dil) on D0, post-vaccination titre ≥40 (1/dil)
† Significant increase for subjects with pre-vaccination titre ≥10 (1/dil), ≥4-fold increase of the titre after vaccination (post/pre)

Neutralising antibody

Adults (18 to 60 years of age)
After the first vaccination, 99.0% of subjects in both adjuvanted groups had a titre ≥ 80 (1/dil). Around 95% of subjects in the 3.8 µg HA + AF03 group and 98.0% of subjects in the 7.5 µg HA + AF03 group had a four-fold increase in titre from D0 to D21. A second dose of both adjuvanted vaccines did not elicit further significant increase of immune response. The GMTR D42/D21 were equal to 1.46 in the 3.8 µg HA + AF03 group and to 1.63 in the 7.5 µg HA + AF03 group. Twenty-one days after the second vaccination, between 99.0% and 100% of subjects had neutralising antibodies at a titre ≥ 80 (1/dil).

Elderly (>60 years of age)
After the first vaccination, more than 85% of subjects in both adjuvanted groups had a titre ≥ 80 (1/dil) and had a four-fold increase in titre from D0 to D21. As observed for the adult subjects, a second dose of both adjuvanted vaccines did not elicit further significant increase of immune response. Twenty-one days after the second vaccination, 96.3% of subjects in the 3.8 µg HA + AF03 group and 98.0% of subjects in the 7.5 µg HA + AF03 group had neutralising antibodies at a titre ≥ 80 (1/dil). Between 88.9% and 96.0% of subjects had a four-fold increase in titre from D0 to D42 in the two adjuvanted groups.

9-17 years group

Overall, 20.4% in the 3.8 µg HA + AF03 group and 36.0% in the 7.5 µg HA + AF03 group had detectable SN titres (≥ 10 1/dil) prior to the first vaccination. Twenty one days after the first vaccination, 100% of the subjects in the two adjuvanted vaccine groups had SN titres ≥ 80 (1/dil) and a four-fold rise of SN titres from D0. GMTs and GMTR further increased after the second vaccination. GMT of SN at D42 were >9 000 (1/dil.).

3 to 8 years group
Only one subject (2.0%) in each of the two adjuvanted vaccine groups had detectable antibody titres prior to the first vaccination. Twenty one days after the first vaccination, 100% of the subjects in the two adjuvanted vaccine groups had SN titres ≥ 80 (1/dil) and a four-fold rise of SN titres from D0. GMTs and GMTR further increased after the second vaccination. GMT of SN at D42 were >9 000 (1/dil.).

Ancillary Analysis

Effect of baseline titre, previous influenza vaccination and gender on the immune response

In GPF07, a baseline titre (prior to the first vaccination) was detected in around 45% of adult subjects and 60% of elderly subjects using the HAI assay. In GPF08, no subjects aged 3 to 8 years had detectable anti-HA titres prior to the first vaccination. A few subjects (22.9%) from 9 to 17 years of age had pre-existing antibodies at baseline. Subjects with detectable titres at baseline tended to have higher GMTs compared to subjects with an undetectable titre after the first vaccination, regardless of the age group, and after the second vaccination only in adult and elderly subjects. All CHMP criteria were fulfilled following both vaccinations in all age groups. The pre-existing titres observed in GPF07 are consistent with the published literature and other licensed pandemic vaccines. Whether this represents cross reactivity from previous vaccination or exposures to previous seasonal strains or exposure to the pandemic A/HINI strain remains speculative. The clinical significance of these observations remains unknown.

In GPF07, subjects who were not previously vaccinated against seasonal influenza tended to develop higher GMTs than the subjects who received seasonal vaccine before. This was observed after the first and after the second vaccinations, in adult and elderly subjects. Of note, due to the small number of elderly subjects without previous vaccination, the data from this age group have to be interpreted with caution. In GPF08, after the second vaccination, GMTs tended to be slightly higher in 3- to 8-year-old subjects who did not receive previous seasonal vaccination than in other subjects, whereas a similar immune response was observed in all subsets of subjects analyzed in 9-to 17-year-old subjects.

The proportion of males and females in each study was similar (except in the elderly subgroup in which there were more female subjects than male subjects) and there was not considered to be a clinically important effect of gender on the immune response. Regardless of the studied covariates, the three CHMP criteria were met in all subgroups 21 days after the first and the second vaccinations, with high seroprotection rates.

Analysis of immunogenicity at more narrow age strata

Additional analyses of immunogenicity Post dose 2 as well as Post dose 1 were also performed at more narrow age strata with adjustment to baseline seropositivity and prior vaccination status and were presented in Responses to LOQ of RR#02 and RR#03.

- All 3 CHMP criteria were met since the first vaccination and were still kept so after the second dose at strata of 3 – 5 y, 6 – 8 y, 9 – 12 y, 13 – 17 y, 18 – 40 y, 41 – 50 y, 51 – 60 y, 61 – 70 y, independent of the age, pre-vaccination status, and antibody titre status at baseline.
    - Pre-vaccination status has no significant influence on GMTs at 3 – 5 y and 6 – 8 y strata (all subjects seronegative);
    - There is a trend for seropositive subjects at baseline to develop higher GMTs after first dose at 9 – 12 y and 13 – 17 y age strata, but such trend of difference between seropositive and seronegative subjects disappeared after the second dose; no influence of pre-vaccination status at these age strata;
    - There is a trend for seropositive subjects at baseline to develop higher GMTs compared to seronegative subjects after first dose at 18 – 40 y, 41 – 50 y, 51 – 60 y, and 61 – 70 y age strata; the second vaccination has little impact on GMTs in seropositive subjects and GMTs in such subjects still trended higher compared to seronegative subjects in these age strata; no influence of pre-vaccination status at these age strata;
- At 71 – 80 y age stratum (all has pre-vaccination history), all 3 CHMP criteria were met in

seropositive subjects after the first dose while seronegative subjects did not achieve SPR criterum (i.e. < 60%), however, after the second dose, all these criteria were fulfilled in all subjects of this age stratum whatever the serostatus; the impact of seropositivity on GMTs observed in this age stratum is the same as observed in age strata of 18 – 40 y, 41 – 50 y, 51 – 60 y, and 61 – 70 y;

- At ≥ 81 y age stratum (all has pre-vaccination history), only 1 out of 7 subjects was seronegative at baseline and did not develop HI antibodies after the first and second doses of HUMENZA; in the remaining subjects of this age stratum, all CHMP criteria were met.

Study GPF09 (younger children 6-35 months of age)

METHOD

The method has been mainly described in table 2 (objective, sample size, treatments) and within the section on study GP07 and GP08.

RESULTS

Participants Flow
A total of 401 subjects, i.e. 201 aged 6 to 11 months and 200 aged 12 to 35 months, were included in the study. All subjects received the first vaccine injection. A total of 14 subjects did not complete the D0-D42 period [13 did not complete the D0-D21 period: 11 aged 6 to 11 months and 2 aged 12 to 35 months; 1 subject aged 12 to 35 months (3.8 µg HA + AF03 group) did not complete the D21-D42 period]. All but 14 subjects (withdrawals of the study) received the second vaccine injection.

All subjects included at D0 were included in the full analysis set (FAS) for immunogenicity analysis and in the safety analysis set (SafAS) for safety assessment after the first vaccination. All but 14 subjects (withdrawals of the study) were included in the SafAS for safety assessment after the second vaccination.

Recruitment
Subjects were included in the study between 16 September and 07 October 2009.

Baseline data
Regarding the baseline serostatus, all subjects (except one) were seronegative to the A/H1N1 influenza strain prior to vaccination.
Demographic and baseline characteristics (age, gender, ethnic origin, history of influenza vaccination) were similar across groups.
Overall, in the 6 to 11 months group, the age of subjects ranged from 6.1 to 12.0 months and the average age was 9.1 months. In the 12 to 35 months group, the age of subjects ranged from 13.0 to 36.0 months and the average age was 27.1 months.

Outcomes and Estimation

HAI

Subjects Aged 6 to 11 Months
In each adjuvanted group, no subjects had a detectable antibody titre, i.e. ≥ 10 (1/dil), prior to the first vaccination. Twenty-one days after the first vaccination, at least 97% of subjects had a detectable antibody titre. At D21, in the 1.9 µg HA + ½ AF03, 3.8 µg HA + ½ AF03 and 3.8 µg HA + AF03 groups respectively:

- GMTs were equal to 200, 256, and 290 (1/dil), corresponding to geometric mean titre ratios (GMTRs) D21/D0 of 39.9, 51.3, and 58.0 respectively.
- The seroconversion rates or significant increases, as well as the seroprotection rates reached 95.7%, 97.9%, and 100.0%

Subjects Aged 12 to 35 Months
Prior to the first vaccination, all subjects in the three adjuvanted groups were seronegative to the A/H1N1 influenza strain (antibody titre <10 [1/dil]), except one subject in the 3.8 µg HA + ½AF03 group. Twenty-one days after the first vaccination, at least 98% of subjects had a detectable antibody titre. A strong immune response was induced by the A/H1N1 pandemic influenza vaccines adjuvanted with AF03.

At D21, in the 1.9 µg HA + ½ AF03, 3.8 µg HA + ½ AF03 and 3.8 µg HA + AF03 groups respectively:

- GMTs were equal to 253, 220, and 409 (1/dil), corresponding to GMTRs D21/D0 of 50.7, 42.2, and 81.9 respectively.
- The seroconversion rates or significant increases, as well as the seroprotection rates reached 97.8%, 98.0%, and 100.0%.

For both age groups, twenty-one days after the first vaccination, the three CHMP criteria were fulfilled, 95% CIs inclusive, in the three adjuvanted groups, with very high estimates: at least 95% of subjects raised seroprotective titres of Ab against the A/H1N1 strain in the 6-11 months group and at least 97% in the 12-35 months group.
Twenty-one days after the second vaccination (D42), the immune response to the vaccine was increased compared to that observed after the first one. In the 1.9 µg HA + ½ AF03, 3.8 µg HA + ½ AF03 and 3.8 µg HA + AF03 groups respectively, GMTs reached 3008, 2500, and 3241 (1/dil) for the 6-11 months group and 2736, 2524, and 3748 (1/dil) for the 12-35 months group, corresponding to GMTRs D42/D21 of 13.3, 9.90, and 11.0 and 10.8, 11.2, and 9.51, respectively.
The seroconversion rates or significant increases from D0, as well as the seroprotection rates were equal to 100.0% in the three adjuvanted groups.
Twenty-one days after the second vaccination, as seen following the first injection in the three adjuvanted vaccine groups, the three CHMP criteria were met, 95%CI inclusive, with 100.0% subjects having developed seroprotective levels of antibodies against the A/H1N1 strain. Similar results were observed in the PP population.

Neutralising Antibody

Subjects Aged 6 to 11 Months
Prior to the first vaccination, all subjects were seronegative to the A/H1N1 strain (neutralising antibody titre <10 [1/dil]), except one subject in the 1.9 µg HA + ½ AF03 group. After the first vaccination, as observed with the HAI method, at least 97% of subjects had detectable neutralising antibodies. At D21, at least 97% of subjects in the three adjuvanted groups had neutralising antibodies at a titre ≥80 (1/dil) and a four-fold increase in titre from D0 to D21. GMTs further increased after the second vaccination to reach values >9 000 (1/dil.). At D42, 100% of subjects in the three adjuvanted groups had neutralising antibodies at a titre ≥ 80 (1/dil) and had a four-fold increase in titre from D0 to D42. The trends for the SN data reflect those described for the HAI data.

Subjects Aged 12 to 35 Months
In subjects aged 12 to 35 months, antibodies were not detectable (neutralising antibody titre <10 [1/dil]) prior to the first vaccination, except in two subjects (one subject in the 1.9 µg HA + ½AF03 group and one subject in the 3.8 µg HA + AF03 group). After the first vaccination, as observed with the HAI method, at least 98% of subjects had detectable neutralising antibodies. At D21, at least 98% of subjects in the three adjuvanted groups had neutralising antibodies at a titre ≥80 (1/dil) and a four-fold increase in titre from D0 to D21. GMTs further increased after the second vaccination to reach values ranging from 8539 to 9761 (1/dil.). After the second vaccination, 100% of subjects in the three adjuvanted groups had neutralising antibodies at a titre ≥ 80 (1/dil) and had a four-fold increase in titre from D0 to D42. The trends for the SN data reflect those described for the HAI data.

The key data are summarised in Table 5

Table 5: GPF09 - Summary of Immunogenicity Data Following the First Vaccination (D21) (half dose vs full dose of 3.8 µg HA Pandemic A/H1N1 Influenza Vaccine) - Full Analysis Set (FAS)

		Half dose		Full dose	
		1.9 µg HA + ½ AF03		3.8 µg HA + AF03	
Assay	**Parameter**	**6 to 11 months (N=48)**	**12 to 35 months (N=48)**	**6 to 11 months (N=52)**	**12 to 35 months (N=52)**
HAI	**Geometric mean titre (1/dil)**	200	253	290	409
	Subjects with titres ≥ 40 (1/dil) (%)	95.7	97.8	100.0	100.0
	Seroconversion* or significant increase† from D0 (%)	95.7	97.8	100.0	100.0
	Geometric mean of titre ratio	39.9	50.7	58	81.9
SN	**Geometric mean titre (1/dil)**	817	1113	1179	1875
	Subjects with titres ≥ 80 (1/dil) (%)	97.9	100.0	97.8	100.0
	4-fold increase from D0 (%)	97.9	100.0	97.8	100.0

*Seroconversion for subjects with pre-vaccination titre <10 (1/dil) on D0, post-vaccination titre ≥40 (1/dil)
† Significant increase for subjects with pre-vaccination titre ≥ 10 (1/dil), ≥ 4-fold increase of the titre after vaccination (post/pre)

Twenty-one days after the first vaccination, there was a trend towards better titres with the full dose compared to half dose, but there was no difference in terms of seroprotection. Therefore, half dose was selected for dose recommendation in 6 to 35-month-old subjects.
A single vaccination with half dose of the candidate vaccine induced a strong immune response in both age groups. This immune response was further increased after the second vaccination, with GMTs ≥ 2500 (1/dil) with HAI and ≥ 8500 (1/dil) with SN in both vaccine and age groups. The seroconversion or significant increase rate, as well as the seroprotection rate were equal to 100% in both age groups, regardless of the vaccine dose given (half or full dose). The analysis of the immune response based on covariates will be provided in a next submission as part of a follow-up measure according to the action plan.

- Supportive studies

Studies GPF01 and FUF04 also provided supportive data on antibody persistence and boosting data.

Three, six and twelve months after primary series vaccination, a decrease in GMTs was observed in all groups. By month 3, Ab titres declined but substantial wane of the immunity became apparent by month 6. However, all adjuvanted groups maintained a higher proportion of subjects with detectable Ab titres. Within adjuvanted groups, Group 2 (3.75µgHA+AF03), Group 3 (7.5µgHA+AF03) and Group 4 (15µgHA+AF03) were comparable, whereas the antibody persistence in Group 1 (1.9µgHA+AF03) was lower. The same trend was observed with the SN method.

Upon boosting with A/Indonesia strain at month 12 (Step 2 only of GPF01 study), a higher immune response was induced as soon as 7 days (D372) after booster vaccination, and at 21 days (D386) after booster vaccination, all three CHMP criteria were met for all the adjuvanted groups, whereas none of these criteria were met for the non-adjuvanted control group.

Booster immune responses were also demonstrated able to cross-inhibit A/Vietnam strain in HI as well as SN assay, with all three CHMP criteria met when assayed against this strain. Similarly, none of these criteria were met for the non-adjuvanted control group even 21 days after booster.

Interestingly, even though the primary vaccination series and the booster vaccination increased the influenza specific lymphoproliferative response in GPF01 study, the IFN-γ and IL-13 cytokine secretion and the multi-cytokine producing CD4 T-cell frequencies in all groups, no clear antigen dose-effect or significant difference between the four adjuvanted groups was observed after the primary or booster

vaccinations. The booster vaccination did not improve the cellular response compared to the primary vaccination series. The Th1/Th2 balance was not significantly impacted by vaccination.

In FUF04 study, only the Month 6 Ab persistence data are presented so far, and data at M12 (D365) and at M15 (D456) and 6 months postbooster (M21 [D636] for a subset of subjects are still in collection. Similar to study GPF01, the antibody titres after a 2-dose vaccination series decreased over time (at M6) for all study groups, and responses for all endpoints (GMTs, SCR, SPR) were higher for the adjuvanted groups than for the control groups. In FUF04, H5N1 vaccine material was produced in the US using a manufacturing process distinct from the one constantly used for GPF01, GPF07, and GPF08).

- Discussion on clinical efficacy

In main studies in subjects aged 3 years and above the adjuvantation and antigen-sparing effect of AF03 is reassured for the actual H1N1 vaccine antigen.

The HI and SN data at 21 days post dose 1 clearly showed adequacy for one dose of 3.8 µg HA/AF03 H1N1 vaccine for each age group: 18 – 60 y, > 60 y, 9 -17 y, and 3 – 8 y, independent of pre-existing immunity status of subjects (children 3 – 8 y were all immune näive for H1N1 in GPF08). Similar to what was previously seen for the first dose, after the second dose of HUMENZA (3.8 µg HA/AF03 H1N1 vaccine), the fulfilment of all three immunogenicity criteria [GMTR >2.5/2.0, SCR > 40%/30%, SPR > 70%/60% in HAI method, defined by CHMP for 18 – 60 y adults / > 60 y elderly] is maintained in any age group: 3 – 8 y, 9 -17 y, 18 – 60 y, and > 60 y. These HI data are well supported by the SN data.
The most notable chance among three parameters after the second dose is the substantial increase of GMTs and GMTRs in 3 – 8 y subgroup. Whereas the gain of GMTs and GMTRs from the second dose is low in the rest age groups: increase of GMTR (D42/D21, HAI method) is: 7.09 for 3 – 8 y, 2.98 for 9 - 17 y, 1.48 for 18 -60 y, and 1.62 for > 60 y subgroups.

Analyses of immunogenicity performed at more narrow age strata showed that all 3 CHMP criteria were met in seropositive subjects aged 71-80 years old after the first dose while seronegative subjects in this strata did not achieve SPR criterum. Also, in the age group 61-70 years who were seronegative at baseline the seroprotection rate after one dose (78%) was much lower than in younger subjects seronegative at baseline (90%+).

In the 6 – 35 months age group and in 6 – 11 months and 12 – 35 months subgroups, one single dose of either half dose or full dose (3.8 µg HA + 2.5% AF03) of HUMENZA elicited strong HI and SN antibody response. All 3 CHMP criteria defined for 18 – 60 years of age adults were met after one single dose, whereas non-adjuvanted vaccine is less immunogenic and needs a 2-dose schedule in order to fulfil 3 CHMP criteria in 6 – 35 months children. The second dose vaccination further increased the antibody response both in HI and SN assays. This is a reminiscence of the observation made in GPF08 study (3 – 17 y children/adolescent study), in that great GMT titre increase by the second dose was evidenced only in 3 – 8 y subgroup with almost all subjects sero-negative at baseline. However, as indicated for other pandemic vaccines, the benefit of this apparent GMT titre increase is unknown.

The CHMP laid down 3 immunological criteria as a basis for considering licensure of pandemic vaccines. All these criteria were well fulfilled by Humenza (a AF03-adjuvanted H1N1 vaccine) after a single dose administration in healthy subjects aged 6 months and above in three European studies. This has been shown by two independent assays – HAI and SN assays. The reliability of these immunogenicity data will be substantiated independently via the analysis of randomised sera submitted to independent laboratories. For future confirmation by a re-test, clinical trial sera need to be stored as requested by the CHMP for all pandemic vaccines.

The antibody persistence data after one single dose of HUMENZA are not available as yet, which could somewhat be regarded as a limitation to HUMENZA's clinical immunogenicity data package. However, this limitation is not HUMENZA-specific, but was common to other pandemic vaccines at the time of their licensure. In order to address the issue of antibody persistence after single dose of HUMENZA, the antibody persistence after one dose will be monitored in the on-going US study FUF16, which assesses the 3.8 µg HA + AF03 vaccine manufactured by using the US process in adults and elderly subjects. In addition, antibody persistence after two doses of HUMENZA will be assessed in on-going GPF07, GPF08 and GPF09 clinical trials.

Clinical safety

- Patient exposure

Overall, 1020 subjects received at least one injection of an A/H1N1 or A/H5N1 pandemic influenza vaccine adjuvanted with AF03 at 2.5%, given as half (196 subjects) or full (824 subjects) dose, during the Phase I study (GPF01) and the three Phase II studies (GPF07, GPF08 and GPF09) conducted in Europe in subjects from 6 months of age. All were healthy subjects. In addition to these subjects, 125 subjects were included in a Phase II study conducted in the USA (FUF16) to receive a full dose of the 3.8 µg HA pandemic influenza vaccine adjuvanted with AF03; and 9000 subjects are planned to be enrolled in a post-authorization safety study (GPF11) to further increase the safety database (Also, 5000 will be vaccinated with unadjuvanted Panenza vaccine).
Overall, around 1500 doses of adjuvanted A/H1N1 pandemic influenza vaccine formulations were administered, either as half (386) or full (1208) doses, and 2214 doses of A/H5N1 or A/H1N1 influenza vaccines adjuvanted with AF03 were given, either as half or full dose.

Limitation of the safety database

Population
The safety data are generated from a healthy population: the clinical trials excluded subjects with congenital or acquired immunodeficiency, receiving immunosuppressive therapy, with a chronic illness that could interfere with trial conduct or completion, with current alcohol or drug abuse, or those having received blood-derived products within 3 months prior to the first vaccination.
The three Phase II clinical studies are conducted in Western Europe, with a predominantly Caucasian population in both studies, with insufficient data to conduct an analysis of the safety profile by ethnicity. Again, based on the safety profile of the seasonal vaccine, it is expected that the clinical safety data described for the adjuvanted A/H1N1 pandemic influenza vaccine in this application would be similar in diverse ethnic populations.

Size of the safety database

With a safety database of this size, the frequency of uncommon and rare adverse events cannot be accurately predicted.
The safety database of 1 020 subjects that received the EU pandemic influenza vaccine adjuvanted with AF03 provides a probability of 95% to observe at least one event which occurs with an incidence of 1 / 340 (0.29%), i.e. if no event is observed in this 1 020 subjects cohort, we can reject, with an alpha level of 5%, incidences greater than 0.29%.
In GPF07, in each vaccine group (N=150), there is a probability of approximately 95% of observing any AE with a true incidence of 2%. For each vaccine formulation, there is a probability of approximately 95% of observing any adverse event (AE) with a true incidence of 3% in adult subjects (18 to 60 years) (N=100) and of 5.8% in elderly subjects (>60 years) (N=50).
In GPF08, in each vaccine group (N=100), there is a probability of approximately 95% of observing any AE with a true incidence of 3%. For each vaccine formulation, there is a probability of approximately 95% of observing any AE with a true incidence of 5.8% in each stratum of age (N=50).
In GPF09, for each vaccine formulation (half dose and full dose) tested (N=100 per formulation), there is a probability of approximately 95% of observing any AE with a true incidence of 3%. For each group (vaccine formulation in each of the age strata, 6-11 months subjects and 12-35 months subjects, N=50), there is a probability of approximately 95% of observing any AE with a true incidence of 5.8%.

- Adverse events

Adult and Elderly Subjects

In adults, the overall incidence of solicited adverse events was similar across adjuvanted groups, whereas in the elderly, the overall incidence of solicited adverse events tended to increase as the dosage of HA increased. The incidence of unsolicited adverse events was similar across groups in adult and elderly subjects.

In GPF07, the incidence of solicited and unsolicited adverse events was slightly lower in the elderly population compared to adult subjects aged 18 to 60 years; such an observation is common in seasonal influenza clinical trials. These effects were not considered to be clinically important and overall, in all age groups and vaccine groups, the adjuvanted A/H1N1 pandemic influenza candidate

vaccine was considered to be well tolerated. Solicited and unsolicited reactions were generally less frequently reported after the second vaccination than after the first, in both adjuvanted vaccine groups, but the safety profile was similar after the first and after the second vaccinations.

There were no findings of clinical concern in the solicited injection site and solicited systemic adverse events within 7 days after any vaccination. For the 3.8 µg HA adjuvanted A/H1N1 pandemic influenza vaccine, in the 7 days following any vaccination in adult and elderly subjects, injection site reactions such as injection site pain occurred with an incidence of 69.7% in adults and of 29.6% in the elderly. Other injection site reactions, such as, injection site induration, injection site erythema, injection site swelling and injection site ecchymosis occurred with an incidence of <10%. For solicited systemic adverse events within 7 days after any vaccination with the 3.8 µg HA adjuvanted A/H1N1 pandemic influenza vaccine, headache, myalgia and malaise were very common (>14%) in adults, and fever and shivering (<10%) were common. In elderly subjects, headache was very common (>14%), and malaise and myalgia were common (<8%). Overall, most of the injection site reactions occurred within 3 days after vaccination, lasted 3 days or less and were of Grade 1.

Table 6: GPF07 - Incidence of Solicited Reactions in the 7 Days after Any Vaccination (3.8 µg HA) - Safety Analysis Set

	18-60 years of age	**Over 60 years of age**
Injection site pain	69.7 %	29.6 %
Injection site erythema	7.1 %	5.6 %
Injection site swelling	6.1 %	5.6 %
Injection site induration	7.1 %	3.7 %
Injection site ecchymosis	2.0 %	1.9 %
Fever	5.1 %	1.9 %
Headache	36.4 %	14.8 %
Malaise	14.1 %	5.6 %
Myalgia	40.4 %	7.4 %
Shivering	9.1 %	1.9 %

Children Aged 3 to 17 Years

In children from 3 to 17 years of age, the overall incidence of solicited adverse events was similar across adjuvanted groups. In both age groups, solicited injection site reactions were more frequent than solicited systemic reactions particularly in younger subjects. The incidence of unsolicited adverse events was similar across adjuvanted groups in subjects from 9 to 17 years of age. In younger subjects from 3 to 8 years of age, the incidence of unsolicited adverse events increased with the strength of antigen. Overall, solicited reactions and unsolicited events were less frequent after the second vaccination than after the first one in both age groups.

The incidence of solicited reactions in the 7 days after any vaccination is presented in Table 7.

Table 7: GPF08 - Incidence of Solicited Reactions in the 7 Days after Any Vaccination (3.8 µg HA) - Safety Analysis Set

	3 to 8 years of age	**9 to 17 years of age**
Injection site pain	88.0 %	89.8 %
Injection site erythema	50.0 %	38.8 %
Injection site swelling	28.0 %	20.4 %
Injection site induration	22.0 %	18.4 %
Injection site ecchymosis	26.0 %	6.1 %
Fever	24.0 %	12.2 %
Headache	42.0 %	69.4 %

	3 to 8 years of age	9 to 17 years of age
Malaise	44.0 %	57.1 %
Myalgia	44.0 %	49.0 %
Shivering	28.0 %	46.9 %

In GPF08, the incidence of solicited reactions was similar between 3 to 8-year-old subjects and 9 to 17-year-old subjects. The incidence of unsolicited adverse events was higher in 9 to 17-year-old subjects than in 3 to 8-year-old subjects in subjects having received the 3.8 µg HA adjuvanted A/H1N1 pandemic influenza vaccine. Overall, solicited adverse events (except injection site pain) were more frequent in children from 3 to 17 years of age than in adult and elderly subjects, as is common with seasonal influenza vaccines. These effects were not considered to be clinically important and overall, in all age groups and vaccine groups, the vaccine was considered to be well tolerated. It is to be noted that the severity grading scale was different between 3 to 11-year-old subjects and subjects from 12 years of age.

For the 3.8 µg HA adjuvanted A/H1N1 pandemic influenza vaccine, in the 7 days following any vaccination, injection site pain occurred with an incidence of 89.8% in 9 to 17-year-old subjects and of 88.0% in 3 to 8-year-old subjects. Other injection site reactions such as injection site erythema, injection site induration, and injection site swelling occurred with an incidence >18%. Injection site ecchymosis occurred with an incidence of 6.1% in 9 to 17-year-old subjects and 26.0% in 3 to 8-year-old subjects. For solicited systemic adverse events within 7 days after any vaccination with the 3.8 µg HA adjuvanted A/H1N1 pandemic influenza vaccine, malaise (>44%), headache (>42%), myalgia (>44%), shivering (>28%), and fever (>12%) were very common. Overall, most of the solicited reactions occurred within 3 days after vaccination, lasted 3 days or less and were of Grade 1. The incidence of Grade 3 solicited reactions was low except the increase of grade 3 fever and injection site erythema especially following second dose of the vaccine.

Subjects from 6 to 35 Months of Age
In subjects from 6 to 35 months of age, the overall incidence of solicited adverse events was similar across adjuvanted groups receiving half dose of adjuvant, and was slightly higher in subjects receiving a full dose of adjuvant. In 6 to 11-month subjects, solicited injection site reactions were less frequent than solicited systemic reactions, whereas in 12 to 35-month subjects solicited injection site reactions were more frequent than solicited systemic reactions. The incidence of unsolicited adverse events was similar across adjuvanted groups.

The incidence of solicited reactions in the 7 days after the first vaccination is presented in each age group in Table 8.

Table 8: GPF09 - Incidence of Solicited Reactions in the 7 Days After the First and the Second Vaccination – 1.9 µg HA + ½ AF03 Vaccine (Half Dose of 3.8 µg HA + AF03 vaccine) - Safety Analysis Set

		Children 6 to 11 months of age	Children 12 to 35 months of age	
			12 to 23 months	24 to 35 months
Injection site pain/tenderness	1st injection	18.8 %	50 %	
	2nd injection	**28.3 %**	29.2 %	
Injection site erythema	1st injection	10.4 %	14.6 %	
	2nd injection	**19.6 %**	**33.3 %**	
Injection site swelling	1st injection	8.3 %	2.1 %	
	2nd injection	6.5 %	**12.5 %**	
Injection site induration	1st injection	8.3 %	12.5 %	
	2nd injection	**21.7 %**	12.5 %	

		Children 6 to 11 months of age	Children 12 to 35 months of age	
			12 to 23 months	24 to 35 months
Injection site ecchymosis	1st injection	2.1 %	6.3%	
	2nd injection	4.3 %	6.3 %	
Fever	1st injection	8.3 %	28.6 %	0.0 %
	2nd injection	**32.6 %**	7.1 %	**11.8 %**
Headache	1st injection	-	-	2.9 %
	2nd injection			5.9 %
Malaise	1st injection	-	-	17.6 %
	2nd injection			17.6 %
Myalgia	1st injection	-	-	11.8 %
	2nd injection			17.6 %
Shivering	1st injection	-	-	5.9 %
	2nd injection			17.6 %
Vomiting	1st injection	25.0 %	7.1 %	-
	2nd injection	23.9 %	0.0 %	
Abnormal crying	1st injection	39.6 %	14.3 %	-
	2nd injection	37 %	14.3 %	
Drowsiness	1st injection	22.9 %	14.3 %	-
	2nd injection	**30.4 %**	**28.6 %**	
Appetite lost	1st injection	33.3 %	42.9 %	-
	2nd injection	30.4 %	21.4 %	
Irritability	1st injection	45.8 %	28.6 %	-
	2nd injection	**50.0 %**	28.6 %	

In GPF09, the incidence of solicited injection site reactions was lower in 6 to 11-month-old subjects than in 12 to 35-month-old subjects, whereas the opposite was observed for solicited systemic reactions. It is to be noted that the solicited systemic reactions were different between 6 to 23-month-old subjects and subjects from 24 months of age. The incidence of unsolicited adverse events was similar between age groups. In all age groups and vaccine groups, the vaccine was considered to be well tolerated.

For the 3.8 µg HA adjuvanted A/H1N1 pandemic influenza vaccine given as a full dose, in the 7 days following the first vaccination, injection site pain/tenderness occurred with an incidence of 28.8% in 6 to 11-month-old subjects and of 50.0% in 12 to 35-month-old subjects. When the vaccine was given as half dose, the proportion of subjects with pain/tenderness decreased to 18.8% in younger subjects, and remained similar in older subjects. When the 3.8 µg HA pandemic A/H1N1 influenza vaccine adjuvanted with AF03 was given as a full dose, injection site erythema and induration were very common in all subjects, injection site swelling was very common in 12 to 35-month old subjects (>10%) and the remaining solicited injection site reactions were common (>5%). When the vaccine was given as half dose, injection site erythema was very common in all subjects (>10%), injection site induration was very common in 12 to 35-month old subjects, and the remaining solicited injection site reactions were common (>2%).

For solicited systemic adverse events within 7 days after the first vaccination with the 3.8 µg HA adjuvanted A/H1N1 pandemic influenza vaccine given as full dose, all solicited systemic reactions, i.e. fever, vomiting, crying abnormal, drowsiness, appetite lost and irritability, were very common in 6 to 11-month old subjects, and all solicited systemic reactions but vomiting were very common in 12 to 23-month old subjects. In 24 to 35-month old subjects, myalgia and shivering were very common (>10%), the remaining reactions, i.e. fever, headache and malaise, being common (>2%).

When the 3.8 µg HA pandemic A/H1N1 influenza vaccine was given as half dose, the following solicited systemic reactions were very common (>14%):

- Vomiting, crying abnormal, drowsiness, appetite lost and irritability in 6 to 11-month subjects
- Fever, crying abnormal, drowsiness, appetite lost and irritability in 12 to 23-month subjects
- Malaise and myalgia in 24 to 35-month old subjects

The following solicited systemic reactions were common:

- Fever in 6 to 11-month subjects
- Vomiting in 12 to 23-month subjects
- Shivering and headache in 24 to 35-month old subjects.

- Serious adverse event/deaths/other significant events

A total of two SAEs were reported within 21 days after the first vaccination with the 7.5 µg HA adjuvanted A/H1N1 pandemic influenza vaccine, one in GPF07 in an adult subject and one in GPF08 in a 5–year-old subject. No SAEs were reported in GPF09.
Two additional SAEs were reported within 21 days after the second vaccination in GPF07: one SAE was reported after vaccination with the 3.8 µg HA adjuvanted A/H1N1 pandemic influenza vaccine and one after vaccination with the 7.5 µg HA adjuvanted A/H1N1 pandemic influenza vaccine.
All SAEs were assessed as not related to vaccination.
Line listings with all the SAEs that have been reported to the Pharmacovigilance Department in GPF07 were provided. So far, none of the SAEs taking place in any of the adjuvanted groups of both studies was assessed as related by the Sponsor or Investigator.
No deaths occurred in either study. At the time of this application, no safety concern resulting from the SAE data has been identified during the clinical trials that are still on-going.

AEs of special interest (AESIs)

Anaphylaxis, Guillain-Barré syndrome, encephalitis, Bell's palsy, neuritis, convulsions, vasculitis, demyelinating disorders and laboratory-confirmed vaccination failure have been identified as being of special interest for pandemic influenza vaccines. For all groups in all studies conducted during the Clinical Development of the vaccine, none of these events of special interest was reported.
Furthermore, based on key data obtained within the 21 days following the first and second vaccinations in GPF07 and GPF08, and in the 21 days following the first vaccination in GPF09, no safety signal was detected from all related adverse events reported from the three Phase II studies by system organ class.

In GPF01 study, one syncopal episode took place in GPF01 in the primary vaccination series, after the administration of the first dose of vaccine. This AESI involved a patient with a relevant history of syncope occurring at the time of injections and was assessed as not related to the study vaccine on the basis on subject's previous medical history. The subject was withdrawn from the trial after this event.

- Safety in special populations

Pregnancies

At the time when the FUF04 report was prepared, a total of 12 pregnancies were reported to the Company (7 pregnancies were reported during the 6 month follow-up period after Dose 1). Pregnancy outcomes were reported for 4 of the 12 cases: 3 resulted in the delivery of healthy babies and one resulted in a spontaneous abortion assessed as unrelated by the Investigator (5 months after vaccination with 2.5% AF03 vaccine). The female subjects who delivered healthy babies did not experience any AEs related to vaccination; no congenital anomalies were reported. Outcomes were not reported for 8 subjects due to the following reasons: ongoing pregnancies (n=5) and pending follow-up (n=3). There were no SAEs reported for subjects with unknown outcomes at the time this report was prepared. Follow-up information about delivery and newborn or pregnancy termination will be completed. No cases of pregnancy were reported within 21 days after the first vaccination in GPF07 and GPF08 studies.

- Safety related to drug-drug interactions and other interactions

The co-administration of Humenza with other vaccines or drug products was not formally investigated.

- Discontinuation due to adverse events

In GPF01 study, two subjects who had received one of the adjuvanted formulations discontinued because of an AE (moderate nausea and mild malaise for one subject, myalgia and injection site pain for the other subject). One subject discontinued because of an SAE (vasovagal syncope).

In the FUF04 study, a total of eight subjects discontinued following the occurrence of an AE. AEs that led to discontinuation were related to vaccination in five subjects. Four of them received one of the adjuvanted formulations. AEs reported were the following:

- allergic reaction due to vaccine
- severe muscle pain and fever
- fever, dry throat, intermittent fatigue, sweats, and decrease of appetite
- weakness

One subject who received placebo reported a flu-like syndrome.

In GPF07 study, no AE or SAE led to study discontinuation of subjects who received the any of the adjuvanted vaccines. In GPF08 study, one SAE led to study discontinuation of 3 to 8 year-old subject who received the 7.5 µg HA adjuvanted vaccine. No other AE led to study discontinuation during the D0-D42 period in these two studies.

In GPF09 study amongst 14 discontinuations, only 2 were due to AEs unrelated to vaccine administration (skin rash and gastroesophageal reflux).

- Post marketing experience

There is no post-marketing experience with Humenza at present.

- Discussion on clinical safety

Based on a consideration of safety data collected within 21 days after any vaccination with the adjuvanted A/H1N1 pandemic influenza vaccines, both candidate vaccines were safe and well tolerated.
There was no specific safety signal detected with AF03 adjuvanted vaccine during H5N1 and H1N1 studies completed so far. Reactogenicity was observed in form of injection pain, erythema, injection site swelling, fever, headache, malaise, myalgia and shivering. There was some trend in reduction of adverse reaction rates following second dose especially in adults and elderly. The incidence of shivering and malaise was especially high in children of 9-17 years of age with almost 50% of children affected.

The incidence of fever (>38°C for at least 1 day) was higher in smaller 12 month-8 year old children than in children 9-17 years and especially in those vaccinated with two doses of full dose 3.8 and 7.5 µg+AF03 vaccine (up to 26%). However GPF08 and GPF09 studies were relatively small and therefore limited to make sound safety comparisons between different age sub-groups. In terms of unsolicited reactions, there were no cases for specific concern. Clearly the incidence of fevers ≥ 38°C was reaching up to 20% after second dose of 1.9-3.8 µg HA in children of 6 months to 8 years of age. Therefore the safety profile following second dose does not allow concluding on sufficient benefits of second dose. Relevant warning on use of antipyretics and adequate monitoring of fever in children have been implemented in the SPC. In this context a theoretical risk of febrile convulsions in exposed population cannot be ruled out.

Safety in pregnancy and lactation period was not investigated. However, the information unintentionally obtained during clinical studies with H1N1 will be continuously monitored and reported in a cumulative way. The MAH has also provided an outline of the planned registry to follow-up pregnant woman vaccinated with HUMENZA. The RMP has been updated accordingly.

The Applicant was also asked to justify that the safety database was sufficient to adequately characterize the safety profile of the vaccine and sufficient to support the safe us in each age group.
The current safety database can be considered sufficient to adequately characterize the common and most common adverse events, as well as those uncommon AEs occurring at a frequency of ≥ 0.29% but does not allow an uncommon AE or any rare AE with an incidence of < 0.29% ~ ≥ 0.1% to be detected, as is otherwise demanded by new vaccine Guideline. In order to consolidate the current safety profile, the MAH committed to perform a large post-authorization safety study (GPF011) in at

least 9,000 patients in different age groups, including immunocompromised subjects. Furthermore, the MAH committed to implement a post-licensure prospective clinical safety study (N=3 000 subjects).

A common observation associated with the use of squalene-based adjuvant is the increased incidence of injection site reactions, particularly pain, compared to the non-adjuvanted formulation. This observation is expected, as with the use of any other emulsion adjuvant, and may be related to the increase of inflammation at the injection site, corroborating with the stimulation of the immune response. This difference is not considered to be of clinical concern. Regarding systemic events, the addition of a squalene-based adjuvant is not generally associated with an increased incidence of systemic events.

The data from GPF01, GPF07, GPF08 and GPF09 suggest that, in accordance with the predicted effect of adding squalene-based adjuvant as described above, the incidence of injection site reactions after the first vaccination was slightly higher in the presence of the adjuvant; there appeared to be no major effect regarding systemic reactions.

Finally, in relation to the reproductible finding of acinar cells necrosis and apoptosis observed in non-clinical studies, the possibility of an autoimmune syndrome development following AF03 administration has been considered. However, although the reason for the changes in the lachrymal glands is unclear, an overview of the data and literature provides supporting evidence that the lesions are less likely to indicate such a pathologic process. The time and the dose-dependent relationship between AF03 necrosis/apoptosis in animals and any potential theoretical risks in humans is difficult to appreciate at this moment. The time required for an autoimmune condition to manifest can be as long as several months to several years. Therefore the company has committed to monitor eye symptoms in GPF11 postmarketing study as well as in the post-licensure prospective clinical safety study planned in 3000 subjects and to refer any found patients for further screening of autoimmune antibodies and long-term follow-up.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

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

Risk Management Plan

The MAH submitted a risk management plan, which included a risk minimisation plan.

In non clinical studies AF03 was shown to induce a higher incidence of minimal apoptosis/necrosis in the lachrymal tissues of rabbits at twice the human dose than in controls, which was reversible within 14 days. The evidence to date suggests that the apoptosis/necrosis in the toxicology studies is not related to autoimmunity, but the exact mechanism is not known. A study conducted with the H1N1 AF03 adjuvanted vaccine established the no observed effect dose level in relation to apoptotis/necrosis induction in the lachrymal tissues at one human dose. No signal that would correlate with an ophthalmic dysfunction was identified to this date and the applicant commits to monitor in the post-marketing setting visual and ocular adverse events which might relate to the findings in the non clinical studies for AF03.
Pivotal trials GPF07, GPF08 and GPF09 exploring the safety of an AF03-adjuvanted swine A/H1N1 influenza vaccine are currently ongoing. From the safety data obtained so far for these trials and from the SAEs reported to the Global Pharmacovigilance Department (GPVD) until the 20th February 2010, no major safety concern is to be underlined. Injection site reactions, headache, myalgia and malaise were nevertheless considered as important safety risks due to the observed frequency of occurrence in GPF07, GPF08 and GPF09 clinical trials. In addition, injection site reactions, fever, vomiting, crying abnormal, drowsiness, appetite lost and irritability in 6 to 23 months population are also considered as important identified risks considering their high frequency of occurrence in GPF09.
Additionally, from supportive trials GPF01 and FUF04 testing a H5N1 AF03-Adjuvanted Pandemic Influenza Vaccine in 216 and 297 healthy adult subjects respectively, no important identified risk which could have a major negative impact on the benefit/ risk assessment of HUMENZA is to be identified.

Additionally, a group of adverse events of special interest were considered as potential risks for HUMENZA. This list of events was developed using as reference the known safety profile of seasonal influenza vaccines, which includes:

- Neuritis;
- Convulsions;
- Anaphylaxis;
- Encephalitis;
- Guillain-Barré syndrome;
- Bell's palsy;
- Demyelinating disorders;
- Vasculitis;
- Laboratory Confirmed Vaccination failure.

These potential risks occur very rarely after seasonal influenza vaccination, an aspect which could justify why none of these events occurred during HUMENZA clinical development phase as of today. In this line of thought, the RMP proposes 4 main pharmacovigilance activities which have per aim to further explore and guarantee the safety usage of HUMENZA as soon as it starts being more widely used in the post-marketing setting:

1. Routine Pharmacovigilance activities: as soon as HUMENZA moves from a pre to post-marketing setting, a new set of pharmacovigilance activities which qualify as routine activities (collection and analysis of AE spontaneous reports, aggregate reports and signal detection activities) will start and be conducted on an ongoing basis. These have per aim to detect and characterize (qualitative and quantitative data, reporting rate trend) all relevant adverse events which occur in biggest possible pool of patients, *i.e.*, the post-marketing setting;
2. Routine Risk Minimization activities: activities which will have per aim to minimize some of the identified or potential risks presented in this RMP, such as potential contamination of vials, vaccination errors and other risks linked to adverse events. In addition, the Applicant proposes an additional risk minimization activity, linked to the fact that a mixing procedure prior to vaccine administration is needed;
3. Continue the clinical development program assessing the safety of HUMENZA (still ongoing). The MAH commits to implement an additional post-licensure prospective clinical safety study (N=3 000 subjects from 6 months of age). While the use of adjuvanted H1N1 vaccine remains the preferred choice, adjuvanted H5N1 vaccine can be considered as a realistic option for the study given the current epidemiology, feasibility and risk-benefit to study volunteers.

4. Post-authorization safety studies: A prospective non-interventional post-marketing cohort safety study involving 9 000 subjects (GPF11) will be conducted in order to further consolidate the known safety profile of HUMENZA. In addition, sanofi pasteur participates in a H1N1 pandemic influenza vaccine post-marketing GBS surveillance study.

   In regards to studies contributing with additional safety data in pregnant women, the applicant will implement a prospective pregnancy registry for HUMENZA, which will combine data on pregnant women exposed to HUMENZA from the following sources: clinical trials; spontaneously reported case reports; literature reports; post-marketing studies; and through a toll-free call-in phone number. Follow-up of pregnancy outcome and adverse events will be proactively collected. Results will be reported on a monthly-basis, as part of the S-PSUR.

In regards to studies contributing with additional safety data in pregnant women, the applicant will implement a Prospective pregnancy registry for HUMENZA, which will combine data on pregnant women exposed to HUMENZA from the following sources: clinical trials; spontaneously reported case reports; literature reports; post-marketing studies; and through a toll-free call-in phone number. Follow-up of pregnancy outcome and adverse events will be proactively collected. Results will be reported on a monthly-basis, as part of the S-PSUR.

The Risk Management Plan will be reassessed periodically for revision based on information arising from the activities comprised within the Pharmacovigilance plan.

Table-Summary of the Risk Management Plan

Identified/potential safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimization activities (routine and additional)
Important identified risk		
Injection site reactions, headache, myalgia and malaise. Injection site reactions, fever, vomiting, crying abnormal, drowsiness, appetite lost and irritability in 6 to 23 months population	Routine Pharmacovigilance activities (ICSRs, s PSURs, monitoring safety profile, safety signal detection)	These events are described in SPC section 4.8, Clinical trials data
Important potential risk		
Neuritis	- Routine Pharmacovigilance activities (ICSRs, s-PSURs, monitoring safety profile, safety signal detection); - 3 000 subjects clinical trial - Post-marketing safety cohort study to investigate the incidence of AEs in different age groups.	- SPC section 4.8, Post-Marketing surveillance includes: *- Nervous system disorders:* *Neuralgia, paraesthesia, febrile convulsions, neurological disorders, such as encephalomyelitis, neuritis and Guillain-Barré syndrome*
Convulsions	- Routine Pharmacovigilance activities (ICSRs, s-PSURs, monitoring safety profile, safety signal detection); - 3 000 subjects clinical trial - Post-marketing safety cohort study to investigate the incidence of AEs in different age groups.	- SPC section 4.8, Post-Marketing surveillance includes: *- Nervous system disorders:* *Neuralgia, paraesthesia, febrile convulsions, neurological disorders, such as encephalomyelitis, neuritis and Guillain-Barré syndrome*
Anaphylaxis/severe allergic reactions	- Routine Pharmacovigilance activities (ICSRs, s-PSURs, monitoring safety profile, safety signal detection); - 3 000 subjects clinical trial - Post-marketing safety cohort study to investigate the incidence of AEs in different age groups.	- SPC section 4.8, Post-Marketing surveillance includes: *- Immune system disorders:* *Allergic reactions, in rare cases leading to shock, angioedema* *- Skin and subcutaneous tissue disorders:* *Generalized skin reactions including pruritus, urticaria or non-specific rash.* - Contraindication for history of anaphylactic reaction to any constituent of the vaccine is included in the labeling. - The patient leaflet will use a language adapted to individuals with no medical education.
Encephalitis	- Routine Pharmacovigilance activities (ICSRs, s-PSURs, monitoring safety profile, safety	- SPC section 4.8, Post-Marketing surveillance includes:

Identified/potential safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimization activities (routine and additional)
	signal detection); - 3 000 subjects clinical trial - Post-marketing safety cohort study to investigate the incidence of AEs in different age groups.	*- Nervous system disorders:* *Neuralgia, paraesthesia, febrile convulsions, neurological disorders, such as encephalomyelitis, neuritis and Guillain-Barré syndrome*
Guillain-Barré Syndrome	- Routine Pharmacovigilance activities (ICSRs, s-PSURs, monitoring safety profile, safety signal detection); - 3 000 subjects clinical trial - Post-marketing safety cohort study to investigate the incidence of AEs in different age groups; - Post-marketing study of enhanced surveillance of Guillain-Barré Syndrome (PGRx).	- SPC section 4.8, Post-Marketing surveillance includes: *- Nervous system disorders:* *Neuralgia, paraesthesia, febrile convulsions, neurological disorders, such as encephalomyelitis, neuritis and Guillain-Barré syndrome*
Bell's palsy	- Routine Pharmacovigilance activities (ICSRs, s-PSURs, monitoring safety profile, safety signal detection); - 3 000 subjects clinical trial - Post-marketing safety cohort study to investigate the incidence of AEs in different age groups.	- SPC section 4.8, Post-Marketing surveillance includes: *- Nervous system disorders:* *Neuralgia, paraesthesia, febrile convulsions, neurological disorders, such as encephalomyelitis, neuritis and Guillain-Barré syndrome*
Demyelinating disorders	- Routine Pharmacovigilance activities (ICSRs, s-PSURs, monitoring safety profile, safety signal detection); - 3 000 subjects clinical trial - Post-marketing safety cohort study to investigate the incidence of AEs in different age groups.	- SPC section 4.8, Post-Marketing surveillance includes: *- Nervous system disorders:* *Neuralgia, paraesthesia, febrile convulsions, neurological disorders, such as encephalomyelitis, neuritis and Guillain-Barré syndrome*
Vasculitis	- Routine Pharmacovigilance activities (ICSRs, s-PSURs, monitoring safety profile, safety signal detection); - 3 000 subjects clinical trial - Post-marketing safety cohort study to investigate the incidence of AEs in different age groups.	- SPC section 4.8, Post-Marketing surveillance includes: *- Vascular disorders:* *Vasculitis associated in very rare cases with transient renal involvement*
Laboratory Confirmed Vaccination failure	- Routine Pharmacovigilance activities (ICSRs, s-PSURs, monitoring safety profile, safety signal detection); - 3 000 subjects clinical trial - Post-marketing safety cohort study to investigate the incidence of AEs in different age groups.	- SPC section 4.4, Post-Marketing surveillance includes: *A protective immune response may not be elicited in all vaccinees (see section 5.1).*

Vision and ocular events	- Routine Pharmacovigilance activities (ICSRs, s-PSURs, monitoring safety profile, safety signal detection); - 3 000 subjects clinical trial - Post-marketing safety cohort study to investigate the incidence of AEs in different age groups.	No action proposed at the time of this RMP.
Important missing information		
Vaccine effectiveness	ECDC vaccine effectiveness studies including a pooled analysis for case-control studies will permit the evaluation of the effectiveness of H1N1 pandemic influenza vaccines which will be used in Europe. Sanofi pasteur clinical trials have scheduled immuno-logical tests for cross-reactivity in case potential drifted variants of the A/H1N1 virus occur.	SPC section 4.2: *HUMENZA is currently not recommended in children less than 6 months of age.*
Very rare AEs (other than AE of special interest) which could not be identified during the clinical development	-the 3 000 subjects clinical trial and the post-marketing safety cohort study GPF11 will increase the opportunity to detect rare AEs which would not be detected during the clinical development.	Not Applicable
Data on pregnant or lactating women	- Post-marketing safety cohort study GPF11 - Prospective pregnancy registry containing data from various sources on pregnant women exposed to HUMENZA. Data will be communicated to Health Agencies on a monthly basis.	SPC section 4.6: *No data have been generated in pregnant or lactating women with the vaccine HUMENZA or with any other vaccine containing adjuvant AF03.* *A reproductive and developmental toxicity study conducted in rabbits with HUMENZA showed no effects on embryo fetal development.* *The use of HUMENZA may be considered during pregnancy and lactation if this is thought to be necessary, taking into account official recommendations.*
Data on immuno-compromized individuals	- Post-marketing safety cohort study GPF11	SPC section 4.4: *Antibody response in patients with endogenous or iatrogenic immuno-suppression may be insufficient*.

Data on children	Clinical studies in pediatric population are ongoing 3 000 subjects clinical trial Post-marketing safety cohort study GPF11.	- SPC section 4.2: *HUMENZA is currently not recommended in children less than 6 months of age.* - Safety and immuno-genicity data of H1N1 pandemic vaccine in children and adolescents are presented in SPC sections 4.8 and 5.1.
Medication errors/misidentification of vaccine	Review of cases of medication error; Review of spontaneous adverse event reports containing vaccine Tradename and batch number.	- Section 4.2 of SPC contains the following sentence: "*It is recommended that subjects who receive a first dose of HUMENZA, complete the vaccination course with HUMENZA (see section 4.4)".* - Section 4.4 of SPC contains the special warning: "*There are no safety, immuno-genicity or efficacy data to support interchangeability of HUMENZA with other H1N1 pandemic vaccines*". - SPC for HUMENZA includes a sentence to instruct Healthcare professionals to record the Tradename of the vaccine and the lot number by using the stickers provided in the package containing both the antigen and adjuvant vials. This will allow for vaccinators to know, at the time of second vaccination, what vaccine had been received before. - Section 6.6 of SPC Special precautions for disposal and other handling provide detailed instructions for mixing vaccine. - Labeling and characteristics of the vials (color and volume) of vials enables distinction between antigen vial and adjuvant vial.

Contamination with multi-dose vials	Adverse event reports describing events which could be suggestive of contamination of the injected vaccine volume such as injection site cellulitis or other infectious event will be closely monitored.	- Section 6.6 of SPC Special precautions for disposal and other handling provides detailed instructions for mixing and/or administration of the vaccine with instructions to discard the vaccine for any variation in appearance. - Shelf-life section 6.3 of SPC states, "After mixing, the vaccine should be stored in a refrigerator (2°C to 8°C) and should be used within 24 hours". - Section 4.4 Special precautions for storage " Do not freeze" - Vaccine contains thiomersal as a preservative. - Additional stand-alone instructional materials (pictogram) to demonstrate proper mixing.

The CHMP, having considered the data submitted in the MA application is of the opinion that the above risk minimisation activities are necessary for the safe and effective use of the medicinal product: see as detailed in section 2.3 of this CHMP Assessment Report.

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The manufacture of the H1N1 antigen, the H1N1 formulated vial and the AFO3 (adjuvant) vial are appropriately controlled. Adequate release and shelf life specifications have been set. Commitments are made by the applicant to update some missing information, which does not impact on the risk/benefit assessment of this vaccine.

Non-clinical pharmacology and toxicology

The non-clinical programme consisted in supportive pharmacology/toxicology data generated with AF03-adjuvanted H5N1 or seasonal H1N1 or with AF03 alone, and in pharmacology/toxicity studies performed with AF03-adjuvanted H1N1 pandemic vaccine including reproduction toxicity and single-dose toxicity addressing specific findings to the lachrymal glands.
The data provided from reproduction toxicity studies, based on draft reports, showed that 6x I.M. injections in rats or rabbits with AF03 alone at concentrations of 1.25-2.5% (rats) or 2.5-5% (rabbits), or 5x I.M. injections in rabbits with 7.5 µgHA + 2.5% AF03, did not cause toxic effects on foetus or on post-natal development.
The data on lachrymal glands of male rabbits have been provided in provisional report and showed that I.M. injection with one human dose of AF03/H1N1 vaccine induced histological changes that were comparable to the background. This dose level tested in rabbits ensures a safety margin which is well acceptable for the toxicological testing of a vaccine in this species.

Efficacy

Clinical immunogenicity data derived from healthy subjects, which are routinely taken as a means of efficacy assessment for other pandemic vaccines, are available for HUMENZA for all age groups to which the CHMP are currently recommended, i.e., 6 – 35 months, 3 – 8 y, 9 – 17 y, 18 – 60 y, and > 60 y healthy subjects.
In adults from the age of 18 years the administration of a single dose of 3.8 µg HA elicited antibody responses that exceeded the CHMP criteria except that subjects aged > 70 years who were seronegative at baseline required two doses. In subjects aged 3-8 years and 9-17 years a single dose of 3.8 µg HA/AF03 elicited antibody responses that exceeded the CHMP criteria applied to young adults

and these data were supported by the NA titres. In children ages 3-8 years, there was a very marked increment in GMT after the second dose but it is not known whether this would result in a clinical benefit. In subjects aged 6 to 35 months a single half adult dose elicited antibody responses that exceeded the CHMP criteria applied to young adults and all except one subject achieved a NA titre of at least 1:80.
Antibody persistence information will be collected post-approval but relies mainly on A/H5N1 data pre-authorization.

Safety

The safety profile of Humenza is based on three main studies performed in subjects aged 6 – 35 months, 3 – 17 years and ≥ 18 years from which no safety concerns were raised during a D0 – D42 period after one or two doses of vaccines. This safety profile is further supported by 6 months safety follow-up data from two supportive studies GPF01 and FUF04 where the safety of two doses of AF03/H5N1 vaccines was investigated.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

- User consultation

A user consultation test has been performed and was considered acceptable.

Risk-benefit assessment

Benefits

The real benefits of Humenza can only be assessed by its use during a pandemic. At present the potential benefit can only be evaluated based on detailed characterisation of immunological responses to vaccination with AF03/H1N1 obtained in the three Phase II clinical studies and with supportive data obtained with AF03/H5N1

A single dose of 3.8 µg HA/AF03 has been shown to be suitably immunogenic in children aged 3-17 years old and adults 18-60 years old. Similarly, in subjects aged 6-35 months, it was found that one half adult dose will be adequately immunogenic. Based on narrow age strata immunogenicity analyses, only two-dose schedule can be advocated in elderly aged > 60 years old.

Data from the three main studies show that the vaccine induces a strong immune response in all subjects.
Therefore the expected benefit of Humenza is to provide some protection against clinically-apparent infection due to A(H1N1)v.

Risks

The safety and reactogenicity profile of the vaccine resembles what was seen for other pandemic vaccines that contain an adjuvant like MF59 and AS03.

However, safety data package of Humenza submitted so far suffers from similar limitations as do other pandemic vaccines, including lack of data from high-risk groups and diseased subjects, lack of data on concurrent use with other vaccines or drugs, and small database unable to detect rare adverse events. A sound RMP and PhVS has been put in place in order to minimise the potential risks. Although unspecific for Humenza, the occurrence of erythema, fever, shivering and malaise in a significant proportion of vaccinees following Humenza (especially following second dose) highlights the identified risk.

Cases of pregnancy with favourable outcomes were noted in supportive study, but the number of cases was very small and no firm conclusion can be drawn. The recommendation will be updated as soon as data on use of Humenza in pregnancy become available.

AF03 is regarded as a new adjuvant and licensure of Humenza does not build on an approved mock-up vaccine. In this respect, supportive safety follow-up and booster data with AF03/H5N1 vaccines are considered very helpful. Overall, no safety concern was identified following the booster vaccination, and within the time period of up to 12 month follow up after primary series and 6 month follow-up after booster vaccination.

The specific commitments include collection of safety, immunogenicity and effectiveness data from the ongoing and planned clinical studies.

Balance

Based on all the quality safety and efficacy data specific to the pandemic influenza A(H1N1)v strain and on supportive data provided with the H5N1 strain, it is considered that in the current pandemic situation the benefits outweigh the risks that may be associated with the use of the vaccine in accordance with the SPC.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns.
- the following additional risk minimisation activities were required: see as detailed in section 2.5

The CHMP considers that the eligibility in accordance with Article 2(2) of Council Regulation (EC) No 507/2006 together with the criteria of conditional Marketing Authorisation in accordance with and 4 of Council Regulation (EC) No 507/2006 are fulfilled.

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

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by majority decision that the risk-benefit balance of Humenza in the "Prophylaxis of influenza in an officially declared pandemic situation" was favourable and therefore recommended the granting of the conditional marketing authorisation.