WITHDRAWAL ASSESSMENT REPORT

EMERFLU

Common name: pandemic influenza vaccine (H5N1) (split virion, inactivated, adjuvanted) A/Vietnam/1194/2004 NIBRG-14

Procedure No. EMEA/H/C/000859

Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.
This should be read in conjunction with the "Question and Answer" document on the withdrawal of the application.

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1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1. Submission of the dossier

The applicant Sanofi Pasteur SA submitted on 27 April 2007 an application for Marketing Authorisation to the European Medicines Agency (EMEA) for Emerflu, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The legal basis for this application referred to:

A - Centralised / Article 8(3) / New active substance.

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

The application submitted was a complete dossier composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies.

The applicant applied for the following indication:

Prophylaxis of influenza in an officially declared Pandemic situation.

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 were:

Rapporteur: Christian Schneider **Co-Rapporteur :** Ian Hudson

1.2. Steps taken for the assessment of the product

- The application was received by the EMEA on 27 April 2007.
- The procedure started on 23 May 2007.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 10 August 2007. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 10 August 2007.
- During the meeting on 17 - 20 September 2007, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 20 September 2007.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 03 December 2007.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 01 February 2008.
- During the meetings on 18-21 February 2008 and 27 – 30 May 2008 the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- During the meeting on 27-30 May 2008 the CHMP agreed on the request from the applicant on the extension of timeframe in order to answer to the List of outstanding issues.
- The applicant submitted the responses to the CHMP List of Outstanding issues on 16 January 2009.

- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 4 February 2009.
- During the CHMP meeting on 16 - 19 February 2009, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 16 – 19 March 2009 the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a Marketing Authorisation under exceptional circumstances to Emerflu.
- On 25 June 2009, the European Commission in view of the pandemic situation at the time, requested the CHMP to evaluate new data submitted by the applicant and to consider the revision of the negative CHMP opinion.
- On 6 October 2009, the CHMP answered to the European Commission that the additional data presented do not allow to conclude on a positive benefit/risk balance and, based on a public health ground, recommended the European Commission to suspend the decision-making process related to the core dossier application as long as the pandemic phase 6 remains.
- On 1 December 2010, the applicant submitted to the European Commission and EMA a letter of withdrawal of the application.

2. SCIENTIFIC DISCUSSION

2.1. Introduction

An influenza pandemic is a global outbreak of influenza disease that occurs when a new type A influenza strain emerges in the human population, causes serious illness, and then spreads easily from person to person worldwide. Pandemics are different from seasonal outbreaks of influenza, as the latter are caused by subtypes of influenza viruses that are already present among people, whereas pandemic outbreaks are caused by new subtypes or by subtypes that have not circulated among people for a long time. In recent history three influenza pandemics, the 1918–1919 "Spanish Flu", the "Asian Flu" in 1957 and the "Hong Kong Flu" in 1968 were reported. Pandemics can cause high levels of mortality, with the Spanish flu being responsible for the deaths of over 50 million people. In contrast to the regular seasonal epidemics of influenza, these pandemics occur irregularly. The widespread occurrence of H5N1 infections in birds and the recent H5N1 outbreaks in humans following transmission from birds by close contact however has raised concerns of a pandemic in the next few years. Vaccines will form the main prophylactic measure against pandemic influenza.

For obtaining pandemic influenza vaccines marketing authorisations, CHMP established the core dossier/mock-up vaccine procedure (CPMP/VEG/4986/03). Further, a guideline addressing the dossier requirements for a pandemic vaccine application was developed (CPMP/VEG/4717/03) and forms the basis for evaluation of the Emerflu dossier submitted by Sanofi Pasteur.

As virtually all people are immunologically naïve for a pandemic strain, pandemic vaccines clearly will differ from seasonal vaccines in that a single dose may not be sufficient to protect individuals from infection and severe illness caused by a new influenza strain. Moreover vaccination recommendations may not be limited to defined risk or age groups. Since the influenza virus strain, which will cause a pandemic, is currently unknown, a prototype vaccine using a potentially pandemic 'novel' virus strain has to be evaluated in preclinical studies using appropriate animal models and in clinical trials obtained from healthy adults of various age groups to support a vaccination strategy that is likely to be used for a pandemic. The ferret model of influenza is well established and is believed to correlate with human disease using fever and virus load in lung washes as indicators of infection. Evidence on the vaccine efficacy should be provided by vaccination of ferrets or other animals by a single or two-dose regimen of a prototype vaccine and protection against a challenge with wild type virus either of the same strain as used for vaccine manufacture or against a further evolved heterologous strain. To predict the efficacy of a prototype pandemic vaccine in humans the antibody response should be measured according to the existing standards set for seasonal vaccines (HI and/or SRH assay) although it is presently not known whether the immunological criteria applied for existing seasonal vaccines are relevant to the assessment of potential pandemic vaccines. In addition, neutralising antibody responses against homologous and heterologous virus strains should be determined as well as cell-mediated immunity. The reactogenicity/safety profile of the vaccine is determined by the purity of the vaccine as well as the nature and amount of antigen. Therefore it has to be demonstrated that the manufacture of the pandemic vaccine is based on a validated and consistent process. In addition the reactogenicity and tolerability of the prototype vaccine has to be evaluated in animals and human studies. All these data together are the basis for the proof of concept principle of the pandemic vaccine development. The immunogenicity/efficacy and safety of a pandemic vaccine however can only be verified in the pandemic situation using the actual virus strain. This has to be addressed in a post-approval clinical development program with the final pandemic vaccine or by the proposed pharmacovigilance activities.

A further important issue is the availability of sufficient vaccine doses within a short time frame. Although seasonal influenza vaccine uptake has increased in recent years, requests for a pandemic vaccine must be expected to significantly exceed the existing manufacturing capacities. Vaccine compositions that raise a satisfactory immune response using a minimum amount of antigen may be the best option in a pandemic situation.

2.2. Quality aspects

Introduction

Emerflu is a vaccine indicated for the prophylaxis of pandemic influenza and contains the inactivated split virion H5N1 antigens as active substance. The reference virus of the mock-up vaccine is A/Vietnam/1194/2004 (H5N1) NIBRG-14 and was developed by NIBSC using reverse genetics. The reassortant strain combines the H5 and N1 segments with the PR8 strain backbone. In addition the H5 was engineered to eliminate the polybasic stretch of amino-acids at the HA cleavage site that is responsible for high virulence of the original strains.The studies on A/Indonesia H5N1 strain provided are considered supportive to the main pivotal data H5N1 A/Vietnam.

The vaccine is provided in ready-to-use multidose vials containing 10x 0.5mL doses and thiomersal as preservative. One dose of vaccine is composed of 30 µg of haemagglutinin adsorbed on 0.6mg aluminium hydroxide. Unlike for the seasonal vaccine, a single immunization is expected not to be sufficient to achieve protection, since in a pandemic situation vaccinees will be most likely immunologically naïve for the pandemic influenza strain. Thus, the proposed vaccination schedule is intended to be two 0.5 ml intramuscular injections with an interval of 3 weeks for individuals from 18 years of age and older.

The drug substance is manufactured by Sanofi Pasteur in its facility at Val de Reuil, France. The manufacturing process is based on the process used for the Sanofi Pasteur's seasonal influenza vaccine, which is licensed through mutual recognition procedure throughout Europe. In contrast to the seasonal vaccine, an adjuvant was included to achieve a sufficient immune response.

Emerflu is a suspension for injection, presented in multidose vials. A 0.5 ml dose of the vaccine contains 30 µg of hemagglutinin as active substance. The total amount of aluminium (adjuvant) per dose is 0.6 mg. Thiomersal is added as a preservative. The other ingredients are PBS solution (buffering agents) composed of sodium chloride, potassium chloride, disodium phosphate dehydrate, potassium dihydrogen phosphate and water for injections (solvent).

Drug Substance

- Manufacture

The drug substance is a monovalent bulk suspension of split, inactivated virus particles. The reference virus described is A/Vietnam/1194/2004 NIBRG-14 (H5N1). The reassortant strain combines the H5 and N1 segments to the PR8 strain backbone. In addition the H5 was engineered to eliminate the polybasic stretch of amino-acids at the haemagglutinin cleavage site that is responsible for high virulence of the original strains.

The manufacturing process of the drug substance is based on the manufacturing process for Sanofi Pasteur's seasonal vaccine. It involves:

1. Inoculation of the working seed into embryonated eggs from healthy flocks, followed by harvest and pooling of the allantoic fluid from the eggs to give a concentrated monovalent
2. Purification using a sucrose gradient to give a purified suspension of the virus
3. Splitting of whole virus using octoxynol-9 and inactivation using formaldehyde solution, followed by filtration and filling

The production of the monovalent bulk is adequately described and all the minor questions asked in during the procedure have been satisfactorily answered by the applicant. The starting materials (virus seed lots, eggs and raw material) are adequately controlled. In-process controls are performed as appropriate, and specifications for the microbial count before sterile filtration and for residual formaldehyde in the monovalent bulk have been set as requested. Process validation has been performed at key manufacturing stages: purification, splitting and inactivation.

Two major concerns regarding the validation of the splitting and inactivation of the virus have been raised:

-Validation of virus splitting has been sufficiently demonstrated in the company's response to the CHMP LoOI. Supporting data using a new virus strain (A/Indonesia/5/05-RG2 (H5N1)) have been provided.

-The major objection regarding the validation of the inactivation by formaldehyde treatment was not resolved. The applicant planned a new validation study to demonstrate inactivation of vaccine virus and ALV/Mycoplasmas.

The requested upper limit for the protein content has been set, and successful virus inactivation up to this limit is demonstrated as part of the new validation study.

- Control of materials

The materials are adequately controlled and the seed lot system is well described. The compositions of the solutions are provided; raw materials used for preparation of the solutions for the manufacture of the seed lots are controlled according to the respective Eur. Ph. monographs.

All raw materials of synthetic origin are controlled according to the European Pharmacopoeia, except for octoxynol-9 used for viral splitting. This reagent is tested and released in compliance with the National Formulary (NF) monograph published by the US Pharmacopoeial Convention.

- Fertilized Eggs from SPF Chicken Flocks

Fertilized eggs from SPF chicken flocks are used for the preparation of the seed lots. Both the chicken flocks and the eggs are SPF. All the eggs used comply with Ph. Eur. 5.2.2. The SPF eggs are sourced from validated external suppliers guaranteeing appropriate standards: the manufacturing process and controls follow the regulatory requirements in force (above mentioned Ph. Eur. paragraph) and the provider commits to notifying the manufacturer in case of problems encountered during production.

- Embryonated Eggs from Healthy Flocks

The embryonated hen eggs from healthy flocks are used for the preparation of the concentrated monovalent harvest. Eggs are provided by several flocks, which are regularly inspected for sanitary conditions according to the European Directives in force (89/662/EC, 2004/41/EC and 91/496/EC). The animals in these breeding colonies must be healthy and immunized against Newcastle disease, infectious bronchitis, encephalomyelitis, laryngotracheitis and Marek's disease. In addition, the company states that breeding colonies should be free of *Salmonellae* of groups B and D1. In addition, flocks should be free from *Mycoplasma gallisepticum and synoviae*.

- Source, History and Generation of the Viral Vaccine Strain A/Vietnam/1194/2004/NIBRG-14 (H5N1)

The selection of the viral strains for generation of the recombinant virus was made in accordance with EMEA 'Guideline on Dossier Structure and Content for Pandemic Influenza Vaccine Marketing Authorization Application' CPMP/VEG/4717/03. The haemagglutinin and neuraminidase coding sequences were isolated from viral strain A/Vietnam/1194/2004 (H5N1), whereas the remaining six segments were derived from an influenza virus, A/PR/8/34.

The reference viral strain A/Vietnam/1194/2004/NIBRG-14 (H5N1) was generated and provided by the National Institute for Biological Standards and Control (NIBSC) and all quality control tests were performed by NIBSC.

The genetic stability of the strain A/Vietnam/1194/2004/NIBRG-14 (H5N1) obtained through reverse genetics has been satisfactorily addressed.

- Process validation

Validation information is provided for purification, splitting and inactivation which are considered to be critical phases for the vaccine quality.

For every new influenza vaccine strain, the splitting and inactivation steps will be revalidated. The purification step will only be revalidated if the new pandemic influenza strain has different migration characteristics in the sucrose gradient compared to known pandemic influenza strains.

Together with the validation of H5N1 virus inactivation by the splitting step in the presence of octoxynol-9, the results of a new study validate sanofi pasteur's manufacturing process regarding the inactivation of H5N1 virus.

The combined results of the inactivation steps of a new study validate sanofi pasteur's manufacturing process regarding the inactivation of mycoplasma, a potential contaminating agent.

- Specifications

The drug substance specification set is appropriate and complies with the relevant Ph Eur monograph 0158 with one exception: For neuraminidase only the identity of the RNA is demonstrated by RT-PCR. It has been requested in the CHMP LoQ that according to the Ph. Eur. 0158, the presence and type of neuraminidase antigen should be confirmed by a suitable enzymatic or immunological method. An enzymatic method has been applied to three monovalent bulks resulting from A/Vietnam and A/Indonesia WSLs.

The controls of the drug substance have been set in accordance to existing pharmacopoeias and guidance and include haemagglutinin content and identity, neuraminidase identification, sterility, test for inactivation and octoxynol-9 content. The analytical methods have been described and are generally adequately validated. As requested, in-process test acceptance criteria for microbial count, pH, ovalbumin content and endotoxin content have been tightened.

- Stability

The drug substance is proposed to be stored in stainless steel drums or polypropylene vessels. Stability data has only been provided in glass vessels for 18 months. This data shows a reduction in haemagglutinin over time. However, this parameter is measured during the manufacture of the finished product and therefore this trend is deemed acceptable. The applicant proposes to perform additional stability studies in stainless steel and polypropylene vessels using a different H5N1 strain for 24 months.

Drug Product

- Pharmaceutical Development

The antigen is received as a sterile bulk and manufacture of the finished product results in a sterile suspension for injection to be administered by the intramuscular route. The excipients in the product are aluminium hydroxide (as an adjuvant), thiomersal (as a preservative) and PBS solution. The container closure system is a 10-dose Type I glass vial with a chlorobutyl rubber stopper sealed with a flip-off cap.

Product dosed in Phase I studies was prepared extemporaneously an adjuvant solution with a solution of antigen while Phase II studies were conducted with vaccine product manufactured as a ready-to use formulation. To compare these two formulations, the applicant determined the rate of adsorption of the haemagglutinin antigen to the adjuvant. The results obtained by this approach raised concerns that the formulations used in phases I and II did not have completely the same characteristics. To some extent these concerns could be resolved by the applicant´s previous studies undertaken in mice which revealed that the adsorption rate has no impact on the antibody response after immunisation. Furthermore, in its recent submissions the applicant has presented additional data generated by an improved and validated methodology on vaccine batches produced from the A/Indonesia/5/05-RG2 (H5N1) strain. Adsorption rates for these batches appear to be higher and much more consistent also for large scale batches. Although these data are considered supportive in order to demonstrate the consistency of drug product manufacturing and resulting vaccine quality the applicant has so far not provided a fully convincing explanation for the different adsorption rates found for A/Vietnam batches produced at different scales.

- Adventitious Agents

Non-viral adventitious agents

Control of mycoplasma, bacteria and fungi:

The materials with potential risk of contamination are the biological materials used for the production of the Seed Lots and Monovalent Bulks, i.e.:

The Master Seed Lot is derived from A/Vietnam/1194/2004/NIBERG-14 (H5N1) virus strain provided by the NIBSC. Compliance with NIBSC specifications is certified by a certificate of analysis accompanying the virus at delivery.

Fertilized SPF eggs are used as substrate for the Seed Lot production, and

Embyonated eggs from healty flocks are used for the production of the monovalent bulks.

Several steps, including filtrations, are involved in the microbial control of the manufacturing process. Details of the manufacturing and the control of critical steps and intermediates are discussed in the respective chapters of the Drug Substance.

The capacity of the splitting and inactivation step to inactivate mycoplamas has been demonstrated according to the requirements of the Ph.Eur. for seasonal split viral influenza vaccines (Ph. Eur. 0158).

Risk of contamination with animal TSE

No material derived from animals naturally susceptible to TSE is used in the preparation of the Master Seed Lots, the Working Seed lots or the manufacturing of the monovalent bulks.

The reference strain A/Vietnam/1194/2004/NIBRG-14 (H5N1) was prepared by the NIBSC.

Adventitious viruses

According to the requirements of the Ph.Eur. (0158) for seasonal split viral influenza vaccines, the inactivation process shall have been shown to be capable of inactivating avian leucosis viruses. No virus has been detected, thus demonstrating the effective inactivation of the fowl leucosis virus.

According to the descriptions given by the applicant the adventitious agents safety of the pandemic influenza vaccine complies with the requirements of the Ph.Eur. For egg derived seasonal influenza vaccines (split virus, inactivated) additional data on the validation of the inactivation process has been provided including the data on effective inactivation of Fowl Leucosis Virus and Mycoplamas.

No novel excipient is included in the pandemic influenza vaccine.

- Manufacture of the Product

The manufacture of the finished product is simple aseptic mixing of the antigen with excipients, filling and labelling. Microbial control is ensured through sterile filtration of the excipients and formulated bulk. An in-process control to minimise bioburden prior to filtration is included. Media fill studies have been performed to validate the process. In general, the data submitted in support of the 1 month hold period do not reveal any unwanted trend in stability indicating parameters and is considered acceptable.

In order to reinforce and supplement this retained hold time period, a stability study was also performed on the new industrial FBP batch with the A/Indonesia/5/05-RG2 (H5N1) strain. Stability results of the new industrial scale FBP batch also support the 1 month hold time between formulation and filling.

- Product characterisation and Specifications

The finished product specification list as presently set by the applicant includes haemagglutinin content, sterility, appearance, pH, extractable volume, thiomersal content, formaldehyde content, protein content, aluminium content, endotoxins and abnormal toxicity.

The finished product specifications have been set in accordance with Ph. Eur. monograph 0158.

As requested and in accordance with the requirements laid down in Ph. Eur. monograph 0153 the applicant has now added a release specification for the degree of adsorption.

The procedure for HA potency determination for batch release purposes based on the HA content measurement of the non-adsorbed final bulk has already been agreed on. However, initially there was no fully validated procedure for the HA content measurement for A/Vietnam stability assessment. The applicant has outlined its strategy for the development and establishment of alternative methods for HA content determination for future testing. In general, these new techniques (HPLC and immunogenicity in mice) appear to be appropriate for the intended purposes.

In its latest response document the applicant has provided a more detailed description of the methodology applied for the HA content determination conducted for stability assessment of the final product. Also, satisfactory validation protocols for these approaches have been submitted.The applicant is therefore asked to make sure that the results obtained by SRD are really indicative of the H5N1 virus haemagglutinin content in the drug product.

For both the A/Vietnam/1194/2004/NIBRG 14 (H5N1) and the A/Indonesia/5/05-RG2 (H5N1) strains, the measurement of the total haemagglutinin (HA) content was performed using the classical Single Radial Immunodiffusion (SRD) method.

The release specifications for the H5N1 final bulk and drug product were agreed.

- Stability of the Product

Based on all available stability data observed for batches produced with the A/Vietnam/1194/2004/NIBRG-14 (H5N1) and the A/Indonesia/5/05-RG2 (H5N1) strains, and the associated statistical analysis, a 12 month at +5°C ± 3°C shelf-life is presently recommended.

These data provide a full validation and stability package for the A/Indonesia/5/05-RG2 (H5N1) strain batches. The SRD and the HPLC methods were validated. These methods and the mice immunogenicity test are included in the stability protocol of the A/Indonesia/5/05-RG2 (H5N1) strain batches since the 2007 production. The total HA content did not drop below the specification all along the stability study.

- General comments on compliance with GMP

The active substance is produced in Val-de-Reuil, the drug product is manufactured at two manufacturing sites: Val-de-Reuil and Marcy l'Etoile. Manufacturing authorisations for both manufacturing sites and a GMP certificate are provided.

2.3. Non-clinical aspects

Introduction

Pharmacology

The immunogenicity of pandemic influenza vaccine was repeatedly proven in studies using 4 species including mice, rabbits, monkeys and ferrets. In these studies, the aluminum hydroxide adjuvant effect for HA antigen at antigen dosing levels of 0.18-3 µg in mice, 7.5-30 µg in rabbits and ferrets and 30 µg in monkeys was constantly documented

for 30-60 µg aluminum in mice, 600 µg aluminum in rabbits, monkeys and ferrets. Likewise, protection against homologous viral challenge (10^6 TCID50, intratracheally) was shown in an infection model. This study demonstrated that monkey receiving adjuvanted vaccine had no detectable virus in the lungs and only transient or low levels of virus in the upper respiratory tract, despite imperfect matching of protection with HI antibody responses, as also occasionally seen in challenge studies using mouse/ferret disease model.

- Primary pharmacodynamics

The applicant developed a disease model and conducted ferret challenge studies. Two ferret studies have been conducted using the ready-to-use FLU H5N1 30µg HA + aluminum hydroxide vaccine.

In the first study, the ability of two intramuscular doses of FLU H5N1 30µgHA+aluminum hydroxide vaccine or the unadjuvanted 30µg HA formulation to protect against mortality and disease following subsequent wildtype challenge with the homologous A/H5N1/Vietnam/1194/04 strain was evaluated. Animals that received FLU H5N1 30µgHA+aluminum hydroxide vaccine mounted an HI response and after viral challenge all of these animals survived whereas no animals that received the control vaccine (either phosphate-buffered saline or aluminum hydroxide) survived. In addition, it is noted that following administration of the unadjuvanted 30µgHA formulation, all animals survived even though the HI response was low in these animals before challenge. After challenge, the HI titres were increased in all animals previously vaccinated with both the unadjuvanted and adjuvanted vaccines, but remained undetectable in animals that had received the control vaccine. This indicates that although low antibody responses were detected in ferrets that received the unadjuvanted vaccine following the primary series, both the adjuvanted and unadjuvanted vaccines have a priming effect as evidenced by the strong increase in HI titre after challenge. A comparison of the 30µg HA+aluminum hydroxide with the 30µgHA unadjuvanted dose showed additional protection against disease of the adjuvanted formulation as assessed by temperature and body weight monitoring and histopathology.

In the second study, the cross-protection in ferrets vaccinated with two doses of the FLU H5N1 30µg HA+aluminium hydroxide vaccine or the 30µg HA unadjuvanted formulation was evaluated after wild-type challenge with either homologous (A/Vietnam/1194/04, Clade 1) or heterologous (A/Indonesia/5/05, Clade 2) H5N1 strains. The results of this study demonstrated that the FLU H5N1 30µg HA +aluminum hydroxide vaccine elicited functional antibodies that were cross-reactive against the Clade 2 strain. Furthermore, ferrets immunized with the FLU H5N1 30µg HA+aluminum hydroxide vaccine were protected from lung infection after both homologous and heterologous challenge, assessed by measuring the viral load, viral shedding from throat swabs, and histopathology. Viral shedding was also decreased in animals vaccinated with the unadjuvanted vaccine after homologous challenge.

Together, the data from these two studies demonstrate complete protection against mortality in all ferrets that had been vaccinated with the FLU H5N1 30µgHA+aluminum hydroxide vaccine.

- Secondary pharmacodynamics

This section is not applicable to this vaccine.

Safety pharmacology programme

No safety pharmacology studies were conducted. The applicant justified this stance on the basis that no cardiotoxic, respiratory or neurotoxic specific risks were identified.

Pharmacodynamic drug interactions

No information regarding drug interactions has been investigated in animals. The applicant justified this stance on the basis that the vaccine should not be given at the same time as other vaccines. The assessor considers this to be acceptable.

Pharmacokinetics

N/A

Toxicology

- Single dose toxicity

No dedicated single dose toxicity study was performed. The applicant justified this stance by referring to the guidance on dossier requirements for pandemic influenza vaccine MAA (CPMP/VEG/4717/03). The assessor considers this to be acceptable.

- Repeat dose toxicity (with toxicokinetics)

No dedicated repeated dose toxicity study was performed. The applicant justified this stance by referring to the guidance on dossier requirements for pandemic influenza vaccine MAA (CPMP/VEG/4717/03). The assessor considers this to be acceptable.

- Genotoxicity

No studies on genotoxicity have been performed. Such studies are not required for vaccines.

- Carcinogenicity

No studies on carcinogenicity have been performed. Such studies are not required for vaccines.

- Reproduction Toxicity

A GLP-compliant development toxicity study has been conducted in female New Zealand White rabbits with no vaccine-related effects noted on the pre- and post-natal development of the pups or on the mating performance and fertility of the vaccinated females.

- Local tolerance

The local tolerance of the vaccine was assessed in a rabbit study which showed that single as well as three intramuscular injections (at 2-week intervals) of 7.5, 15 or 30 µg of haemagglutinin adjuvanted with aluminium hydroxide were overtly well tolerated. Histologically, the inflammatory responses observed at the injection site(s) of treated animals were considered to be treatment-related without obvious dose-relationship in the magnitude of the response and appeared to be typical observations seen following injections of aluminium hydroxide adjuvanted vaccines. The severity of the findings was reduced 14 days after injection possibly indicating partial recovery.

- Other toxicity studies

An immunopathology study following intramuscular immunisation with the vaccine and challenge with parental A/H5N1 virus was conducted in monkeys. This study showed no overt signs of toxicity or effect on body weight gain following treatment with 30 µg HA either adjuvanted or not with aluminium hydroxide. Evidence of broncho-interstitial pneumonia was seen in all animals, although the extent and the severity of this pneumonia were the highest in controls, compared to the adjuvanted and unadjuvanted vaccine group, and the lowest in the adjuvanted vaccine group. In addition, increased eosinophilic infiltrates were occasionally observed in lungs and lymph nodes of

vaccinated and non-vaccinated animals but with no appreciable difference in incidence and severity between groups. Therefore, they were considered incidental and/or related to individual variability in the inflammatory response to the viral challenge. No pneumopathy exacerbation was observed when adjuvanted pandemic influenza vaccinated monkeys were challenged with an A/H5N1/Vietnam parental wild-type strain.

- Ecotoxicity/environmental risk assessment

No environmental risk assessment is included in this application. According to the guideline EMEA/CHMP/SWP/4447/00 “Environmental Risk Assessment of Medicinal Products for 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 the non-clinical aspects

The non-clinical pharmacological investigation programme for EMERFLU focuses mainly on immunogenicity and protective efficacy, which is in line with available regulatory guidances. The immunogenicity of pandemic influenza vaccine was assessed in animal studies using 4 species including mice, rabbits, monkeys and ferrets. Protection against homologous viral challenge (106 TCID50, intratracheally) was shown in challenge studies in monkeys and in two independent ferret models: mortality and disease model, and an infection model..

Together, the data from these two studies demonstrate protection against mortality in all ferrets that had been vaccinated with the FLU H5N1 30µgHA+aluminum hydroxide vaccine.

Based on the results from non-clinical safety studies conducted with the vaccine at the dose level of 30 µg HA adjuvanted with aluminium hydroxide (600 µg Al), no major safety concerns were raised and treatment related effects appeared to be limited to only local reactions at the site of injection.

2.4. Clinical aspects

Introduction

Except where stated below the version of Emerflu administered contained antigens derived from the A/Vietnam strain (Clade 1) of influenza A subtype H5N1. The studies on A/Indonesia H5N1 (Clade 2) strain provided are considered supportive to the main pivotal data H5N1 A/Vietnam.

The clinical study data comprised the following:

GPA01 was designed as a dose-finding study and was confined to healthy adults aged 18-40 years. The initial report presented the post-primary immunogenicity data at D42 plus antibody persistence data at D180. During the procedure the applicant provided antibody persistence data to M12 and post-booster data (after a third dose of Emerflu) obtained at M12 +21 days (D386).

GPA02 is the pivotal study using the final formulation and was conducted in healthy adults aged 18-60 years or > 60 years. The initial report presented post-primary immunogenicity data up to D42. During the procedure the applicant provided antibody persistence data to M6 plus CMI and anti-neuraminidase data related to the primary series with Emerflu. Data were provided following administration of unadjuvanted 7.5 µg HA from the vaccine strain to a subset of the study population at 6 months after completing two doses of Emerflu. Subsequently data were provided on responses to one dose of Emerflu containing the A/Indonesia H5N1 strain administered to healthy adult and elderly subjects at 22 months after completing two doses of Emerflu containing the A/Vietnam strain. The applicant also

provided data from previously unvaccinated healthy subjects between 18 and 60 years of age who were given two doses of Emerflu containing the A/Indonesia strain 21 days apart.

Some results (but no formal CSRs) from two supportive studies were submitted during the procedure. GPA04 was a study conducted in Thai children and GPA11 was an Australian study in which 100 healthy adults received two doses of Emerflu.

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. All studies were performed in Europe. Written informed consent was obtained from each subject prior to entry into the study.

Pharmacokinetics

As noted in the CHMP guideline 'Note for guidance on clinical evaluation of new vaccines' (CPMP/EWP/463/97) pharmacokinetic studies are generally not required for injectable vaccines.

Pharmacodynamics

The pharmacodynamic principle of vaccines generally could be regarded as the induction of an immune response sufficient to protect from infection with or disease arising from the specific pathogen, the vaccination is directed against. Thus the immunological response to Emerflu is covered as part of the evaluation of efficacy.

Clinical efficacy / Immunogenicity

Assays

In GPA01:

- HI and SRH tests using turkey erythrocytes were performed by Dr. J. Wood at NIBSC, UK
- HI tests using horse erythrocytes and SN tests were performed by Dr. M. Zambon at HPA, UK.

In GPA02:

- HI tests using horse erythrocytes and SN tests were performed by Dr. M. Zambon at HPA, UK and by Sanofi Pasteur.

Haemagglutination Inhibition Test

A qualified HI adapted to the avian strain was used. The principle of the HI test is based on the ability of specific anti-influenza antibodies to inhibit haemagglutination of horse or turkey red blood cells (RBC) by influenza virus HA. The sera to be tested have to be previously treated to eliminate the non-specific inhibitors and the anti-species HAs. Preparation is performed simultaneously for serum obtained on D0, D21, and D42. The serum titre is equal to the highest reciprocal dilution, which induces a complete inhibition of haemagglutination. For HI assay using turkey RBCs the serum samples are serially diluted starting at 1:10 dilution whereas for the HI assay using horse RBCs the starting dilution is 1:8.

Seroneutralisation

The influenza virus microneutralisation test is a specific assay for antibodies to the avian influenza A (H5N1) virus in human serum and could potentially be used to detect antibodies to other avian subtypes. This microneutralisation test is more sensitive than the HI assay as this assay can detect H5-specific Ab in human serum at titres that can not be detected by the HI assay. Inactivated human serum samples are pre-inoculated with a standardised amount of virus

prior to the addition of Madin-Darby canine kidney (MDCK) cells. After overnight incubation, an ELISA is used to measure the viral NP protein in infected MDCK cells. Since serum antibodies to the influenza virus HA inhibit the viral infection of MDCK cells, the optical density results of the ELISA are inversely proportional to the serum Ab concentration.

The challenge virus for SN routinely used was A/Vietnam/1194/2004/NIBRG-14. Immune responses to Emerflu containing A/Indonesia were assayed using A/Indonesia/05/2005-Rg.

Wild-type virus (A/Vietnam/1194/2004/2004-WT, clade 1 and A/Turkey/Turkey/2005-WT, clade 2-2) and A/Turkey/Turkey/2005-Rg were also used in the SN assay to evaluate cross-reactivity of the response elicited by Emerflu.

Neuraminidase Inhibition Test

The methods used for the neuraminidase inhibition test were described by Aymard-Henry *et al.*

- Dose response study(ies)

GPA01 was a dose-response study in which three HA doses with or without alum were evaluated. GPA02 evaluated responses to 30µg HA with alum and 7.5µg HA without alum.

Both are discussed under main studies.

- Main studies

GPA01 was a dose-finding study confined to healthy adults aged 18-40 years.

GPA02 was the pivotal study and was conducted in healthy adults aged 18-60 years or > 60 years

METHODS

Study Participants

Eligible subjects were healthy male or female subjects aged:

- 18 and 40 years (**GPA01**)
- 18 years and over (**GPA02**)

In GPA01 and GPA02 the following populations were defined:

- The Per Protocol Analysis Set for Immunogenicity (PPAS) was used as the primary analysis and included subjects who fulfilled the conditions defined in the protocol at D21 and D42.
- The Full Analysis Set (FAS) for immunogenicity (FAS) was the subset that received at least one dose of vaccine.
- The FAS II for immunogenicity was the subset that received the booster doses as detailed below.
- The Safety Analysis Set (SafAS) consisted of all who received at least one dose of vaccine.

Treatment

In **GPA01** six groups were treated as follows:

• Group 30µg+Ad: 30 µg HA + aluminium hydroxide as adjuvant (600 µg aluminium)

• Group 15µg+Ad: 15 µg HA + aluminium hydroxide as adjuvant (600 µg aluminium)

• Group 7.5µg+Ad: 7.5 µg HA + aluminium hydroxide as adjuvant (600 µg aluminium)

• Group 30µg: 30 µg HA, no adjuvant

• Group 15µg: 15 µg HA, no adjuvant

• Group 7.5µg: 7.5 µg HA, no adjuvant

At M12 (D365) booster vaccines were:

• Group 30µg+Ad: 30 µg HA + aluminium hydroxide as adjuvant (600 µg aluminium) or

• Group 7.5µg: 7.5 µg HA, no adjuvant

Vaccines were initially administered on D0 and D21. Three of the six formulations had to be mixed before administration, i.e. for all alum adjuvanted vaccine formulations, the content of the pre-filled syringes of antigen and adjuvant had be added into the sterile vial followed by mixing for 10 seconds with a vortex shaker prior i.m. administration. All vaccines contained thiomersal as preservative.

The two dosages used for the booster vaccination were provided as ready-to-use multidose vials of ten doses. For both formulations, the volume injected was 0.5 mL.

In **GPA02** 600 subjects (300 aged 18 to 60 years and 300 aged >60 years) received Clade 1 vaccine as a primary series of either 30 µg HA with adjuvant or 7.5 µg HA without adjuvant.

- At M6, 160 were to be boosted with Clade 1 vaccine 7.5 µg HA without adjuvant.
- At M12 440 were to be boosted with Clade 2 vaccine (30 µg + Ad or 7.5 µg HA without adjuvant)
- A further 100 subjects (aged 18 to 60 years) were to be recruited at M12 to receive a primary series with Clade 2 vaccine (30 µg + Ad or 7.5 µg without adjuvant).

The vaccines for primary and booster vaccinations were presented in ready-to-use multidose vials containing 10 x 0.5 mL doses. The vaccine contained thiomersal as preservative.

Sample size

In **GPA01** for the primary vaccination phase the sample size was arbitrarily set to 50 subjects per group. For the booster vaccination phase each group of 50 subjects was randomly divided in two subgroups resulting in 12 groups of 25 subjects.

In **GPA02** the planned sample size of 300 subjects per formulation group (including the two sub-groups of 150 subjects by age in each formulation group) provided a 95% probability to observe a 1% incidence for any AE in any formulation group, and a probability of 78% to observe a 1% incidence of any AE in each formulation group.

For the additional subjects receiving a primary vaccination series of Clade 2, a sample size of 50 subjects per formulation group was sufficient to detect any difference above 0.301 for the log10 post-vaccination titre (corresponding to a ratio >3.0) based on an observed standard deviation of 0.4 for the SN assay.

Objectives

Study GPA01:

Primary Objective

To describe the immunogenicity after the first and the second injections of different formulations of an A/H5N1 inactivated split-virion influenza vaccine, administered in adults as two IM injections separated by 21 days, using haemagglutination inhibition (HI) and single radial haemolysis (SRH) methods according to the CHMP Note for Guidance for influenza vaccines (CPMP/BWP/214/96)

Secondary Objectives were:

To describe the injection site and systemic safety profiles during the 21 days following each injection (including the 12-month booster).

- To describe the antibody persistence 6 months and 1 year after the first vaccination.
- To describe the seroneutralisation (SN) response to vaccination.
- To describe the immune response 7 days and 21 days after a booster vaccination administered at 12 months after the first vaccination in primo-vaccinated subject

Objectives of Study **GPA02** included:

- To describe the immune response 21 days after each of two primary series IM injections in two age groups: subjects aged 18 to 60 years (adults) and >60 years (elderly).
- To describe the antibody persistence until the time of the booster vaccination at 6 or 12 months after the first vaccination in two age groups: subjects aged 18 to 60 years (adults) and >60 years (elderly).
- To describe the immune response 21 days after a booster vaccination administered at either 6 or 12 months after the first vaccination in two age groups: subjects aged 18 to 60 years (adults) and >60 years (elderly).
- To describe the injection site reactions and systemic safety profile during the 21 days following each of two primary series and one booster (as applicable) IM injections in two age groups: subjects aged 18 to 60 years (adults) and >60 years (elderly).

To describe any serious adverse events (SAEs) during the whole trial.

Outcomes/endpoints

In study GPA01 the primary endpoints were related to antibody measured by HI and SRH on D0, D21, and D42.

Antibody persistence measured by HI on D180 and D365 in all subjects were secondary endpoints.

Further secondary endpoints included neutralising antibody (NA) titres and antibody response after the booster vaccination at M12.

In study GPA02 endpoints were related to the HI- and SN titres on Day 0, D21, and D42.

Antibody persistence was measured by HI and SN in duplicate at M6 (D180) (if booster performed at M6) or at M6 and M12 (if booster performed at M12).

Responses to boosters were assayed by HI and SN at M6 (V04) or M12 (V05), M6+21 days (V05) or M12+21days (V06).

Anti-neuraminidase (NA) titres were measured on D0 and D42.

Randomisation

In Study GPA01 the randomisation list for the primary vaccination was created using the block method. For the booster vaccination at D365, subjects from each of the six predefined groups obtained from the first randomisation were assigned by balance block randomisation to one of the two booster formulations. This randomisation was stratified according to first randomised vaccine groups at V01 and to centre.

In Study GPA02 the randomisation list for primary vaccination was created using the block permutation method, stratified by age group within centres. Additionally the size of the blocks was adapted to handle the randomisation of blood sample aliquots for additional serological tests for 160 subjects (HI assay using turkey erythrocytes, neuraminidase, ELISA).

For the Clade 2 booster vaccination a second randomisation list was prepared, stratified by primary vaccine formulation, randomised group, age group within centre and centre (Centres 1, 3 and 4). The list will be created using the block permutation method. Additionally the size of the blocks will be adapted to handle the centre size for Centres 1, 3 and 4.

A third randomisation list for the two vaccine formulation groups was prepared using the block permutation method for the primary vaccination of Clade 2 for the 100 additional subjects enrolled after Amendment 1.

Blinding (masking)

Both studies were open label.

Statistical methods

Study GPA01:

No statistical hypothesis was tested for the main analysis.

For both the HI and the SRH methods for anti-HA antibody titration, point estimates and the associated 95% confidence intervals (CIs) were provided for the variables involved in the CHMP criteria for each of the six study vaccine groups.

In GPA02:

For HI tests using turkey and horse erythrocytes, point estimates and their associated 95% CIs were provided for the variables involved in the CHMP criteria according to vaccine formulation group and age groups. For the SN method, point estimates and their associated 95% CI were provided for the GMTs, the GMTRs and for the two- and four-fold increases of neutralising antibody.

Reverse cumulative distribution curves were plotted for each group, on the FAS population.

For each vaccine formulation group and for each age group, the analysis on the FAS population was illustrated by a plot of the geometric means and their associated 95% CI at D0, D21 and D42.

RESULTS

Recruitment

GPA01 was conducted in France (3 sites). fromMay 2005 to July 2006

GPA02 was conducted in England (1 site) and Belgium (3 sites) from May 2006 to February 2009.

Baseline data and Numbers analysed

In GPA01 299/300 subjects enrolled were included in the PPAS dose 1 and 279 in the PPAS dose 2. M6 antibody persistence data were reported from 286 using HI with horse erythrocytes and 296 using SN. The mean age at D0 was 25.08 years and the age distribution ranged between 18.5 and 40.6 years.

In the initial submission of study GPA02 all the 600 subjects were included in the FAS immunogenicity population, 590 in the PPAS after dose 1 and 582 in the PPAS after dose 2. The overall mean age was 37 years for the younger cohort and 68 years for the older cohort.

Study GPA01 – data to D42

Based on results of HI performed with horse erythrocytes and using a seroprotection threshold ≥ 40:

Pre-vaccination, only one subject was seropositive (HI titre 192) and all GMTs were around 4.

None of the six groups met all the three CHMP criteria after the first vaccination or second vaccination.

The 30µg+Ad group showed the highest GMTR (11.55), seroconversion rate (62.0%) and seroprotection rate (62.7%).

Table 1: HI (horse erythrocytes) up to D42 using a seroprotection definition of ≥40

	30µg+Ad	15µg+Ad	7.5µg+Ad	All
N analyzed (D0-D21)	50	50	50	298
N analyzed (D21-D42)	51	50	50	300
N analyzed (D0-D42)	50	50	50	298
Anti HA titers (1/dil) GMT[95% CI]				
D0	4.00[4.00;4.00]	4.00[4.00;4.00]	4.00[4.00;4.00]	4.06[3.95;4.16]
D21	14.45[8.61;24.26]	7.01[4.69;10.49]	5.43[4.00;7.37]	9.94[8.23;12.01]
D42	48.8[28.2;84.3]	17.3[10.4;28.7]	10.41[6.73;16.11]	21.2[17.1;26.2]
Anti-HA ratios of titers GMTR[95% CI]				
D21/D0	3.39[2.03;5.65]	1.75[1.17;2.62]	1.357[0.999;1.842]	2.43[2.02;2.93]
D42/D21	3.38[2.19;5.19]	2.46[1.65;3.68]	1.92[1.36;2.70]	2.13[1.83;2.48]
D42/D0	11.55[6.68;19.97]	4.32[2.59;7.18]	2.60[1.68;4.03]	5.19[4.20;6.41]
Seronegativity rate (titers <8 1/dil.) (% [95% CI])				
D0	100.0%[92.9;100.0]	100.0%[92.9;100.0]	100.0%[92.9;100.0]	99.7%[98.1;100.0]
D21	62.7%[48.1;75.9]	84.0%[70.9;92.8]	92.0%[80.8;97.8]	74.7%[69.3;79.5]
D42	33.3%[20.8;47.9]	56.0%[41.3;70.0]	68.0%[53.3;80.5]	52.0%[46.2;57.8]
Seroprotection (>=40 1/dil.) (% [95% CI])				
D21	31.4%[19.1;45.9]	12.0%[4.5;24.3]	6.0%[1.3;16.5]	22.3%[17.7;27.5]
D42	62.7%[48.1;75.9]	44.0%[30.0;58.7]	20.0%[10.0;33.7]	42.7%[37.0;48.5]
Seroconversion* or significant increase† from D0 (% [95% CI])				
D21	30.0%[17.9;44.6]	12.0%[4.5;24.3]	6.0%[1.3;16.5]	22.1%[17.6;27.3]
D42	62.0%[47.2;75.3]	44.0%[30.0;58.7]	20.0%[10.0;33.7]	42.6%[36.9;48.4]
Seroconversion* or significant increase† from D21 (% [95% CI])				
D42	37.3%[24.1;51.9]	28.0%[16.2;42.5]	12.0%[4.5;24.3]	21.0%[16.5;26.1]

Table 2: HI (horse erythrocytes) up to D42 using a seroprotection definition of ≥40

	30µg	15µg	7.5µg	All
N analyzed (D0-D21)	49	50	49	298
N analyzed (D21-D42)	50	50	49	300
N analyzed (D0-D42)	49	50	49	298
Anti HA titers (1/dil) GMT[95% CI]				
D0	4.32[3.70;5.06]	4.03[3.97;4.08]	4.00[4.00;4.00]	4.06[3.95;4.16]
D21	14.42[8.39;24.77]	9.78[6.32;15.14]	12.40[7.23;21.26]	9.94[8.23;12.01]
D42	28.2[16.5;48.4]	18.3[10.8;30.8]	19.6[11.3;34.0]	21.2[17.1;26.2]
Anti-HA ratios of titers GMTR[95% CI]				
D21/D0	3.42[2.03;5.76]	2.43[1.57;3.75]	3.10[1.81;5.32]	2.43[2.02;2.93]
D42/D21	1.96[1.36;2.82]	1.87[1.27;2.74]	1.58[1.16;2.16]	2.13[1.83;2.48]
D42/D0	6.80[4.03;11.48]	4.53[2.69;7.62]	4.91[2.83;8.51]	5.19[4.20;6.41]
Seronegativity rate (titers <8 1/dil.) (% [95% CI])				
D0	98.0%[89.1;99.9]	100.0%[92.9;100.0]	100.0%[92.7;100.0]	99.7%[98.1;100.0]
D21	66.0%[51.2;78.8]	72.0%[57.5;83.8]	71.4%[56.7;83.4]	74.7%[69.3;79.5]
D42	44.0%[30.0;58.7]	56.0%[41.3;70.0]	55.1%[40.2;69.3]	52.0%[46.2;57.8]
Seroprotection (>=40 1/dil.) (% [95% CI])				
D21	34.0%[21.2;48.8]	22.0%[11.5;36.0]	28.6%[16.6;43.3]	22.3%[17.7;27.5]
D42	50.0%[35.5;64.5]	40.0%[26.4;54.8]	38.8%[25.2;53.8]	42.7%[37.0;48.5]
Seroconversion* or significant increase† from D0 (% [95% CI])				
D21	34.7%[21.7;49.6]	22.0%[11.5;36.0]	28.6%[16.6;43.3]	22.1%[17.6;27.3]
D42	51.0%[36.3;65.6]	40.0%[26.4;54.8]	38.8%[25.2;53.8]	42.6%[36.9;48.4]
Seroconversion* or significant increase† from D21 (% [95% CI])				
D42	16.0%[7.2;29.1]	22.0%[11.5;36.0]	10.2%[3.4;22.2]	21.0%[16.5;26.1]

HI titres using turkey erythrocytes showed a pattern of findings after each dose and between groups that was essentially similar to that seen with HI using horse erythrocytes but the immune responses were much poorer across the board.

SN titres showed that:

- Only two subjects were seropositive (titre ≥20) before vaccination and pre-vaccination GMTs were similar between the six groups.
- At D21 there was only a slight immune response with a global D21/D0 GMTR of 1.36. The percentage with a 2-fold increase was higher in non-adjuvanted vaccine groups.
- At D42 60.3% remained seronegative (titre <20). However, 60.8% in the 30 µg + Ad group were seropositive, which was a much higher rate than seen in any of the other adjuvanted or non-adjuvanted dose groups. The next highest rate was 46%, observed with 30 µg without adjuvant.
- Proportions with a 2-fold increase ranged from 22.0% in the 7.5µg+Ad group to 60.8% in the 30µg+Ad group. Four-fold increases were seen in 41.2% in the 30µg+Ad group, 26.5% in the 30µg group and 22% in the 15µg group.
- Similar results were observed in the PPAS population

Table 3: SNA titres against the A/Vietnam/1194/2004 (H5N1) strain (FAS)

	30µg+Ad	15µg+Ad	7.5µg+Ad	All
N analyzed (D0-D21)	51	50	50	299
N analyzed (D21-D42)	51	50	50	300
N analyzed (D0-D42)	51	50	50	299
Antibody titers (1/dil) GMT[95% CI]				
D0	10.20[9.81;10.60]	10.0[10.0;10.0]	10.0[10.0;10.0]	10.10[9.95;10.26]
D21	14.1[11.5;17.3]	12.4[10.1;15.1]	11.55[9.83;13.58]	13.8[12.6;15.0]
D42	32.6[24.9;42.8]	20.3[15.9;26.1]	15.3[12.4;18.9]	21.4[19.3;23.8]
Ratios of antibody titers GMTR[95% CI]				
D21/D0	1.39[1.14;1.68]	1.24[1.01;1.51]	1.155[0.983;1.358]	1.36[1.26;1.48]
D42/D21	2.31[1.79;2.98]	1.64[1.36;1.98]	1.32[1.14;1.54]	1.56[1.43;1.69]
D42/D0	3.20[2.46;4.17]	2.03[1.59;2.61]	1.53[1.24;1.89]	2.12[1.92;2.35]
Seronegativity rate (titers < 20 1/dil.) (% [95% CI])				
D0	98.0 [89.6;100.0]	100.0 [92.9;100.0]	100.0 [92.9;100.0]	99.3 [97.6;99.9]
D21	84.3 [71.4;93.0]	94.0 [83.5;98.7]	94.0 [83.5;98.7]	84.7 [80.1;88.6]
D42	39.2 [25.8;53.9]	64.0 [49.2;77.1]	78.0 [64.0;88.5]	60.3 [54.6;65.9]
2-fold increase from D0 (% [95% CI])				
D21/D0	15.7 [7.0;28.6]	6.0 [1.3;16.5]	6.0 [1.3;16.5]	15.4 [11.5;20.0]
D42/D0	60.8 [46.1;74.2]	36.0 [22.9;50.8]	22.0 [11.5;36.0]	39.8 [34.2;45.6]
2-fold increase from D21 (% [95% CI])				
D42/D21	47.1 [32.9;61.5]	26.0 [14.6;40.3]	16.0 [7.2;29.1]	25.3 [20.5;30.7]
4-fold increase from D0 (% [95% CI])				
D21/D0	9.8 [3.3;21.4]	6.0 [1.3;16.5]	6.0 [1.3;16.5]	9.0 [6.0;12.9]
D42/D0	41.2 [27.6;55.8]	18.0 [8.6;31.4]	16.0 [7.2;29.1]	24.1 [19.3;29.3]
4-fold increase from D21 (% [95% CI])				
D42/D21	29.4 17.5;43.8]	12.0 [4.5;24.3]	10.0 [3.3;21.8]	13.3 [9.7;17.7]

Table 4: SNA titres against the A/Vietnam/1194/2004 (H5N1) strain (FAS)

	30µg	15µg	7.5µg	All
N analyzed (D0-D21)	49	50	49	299
N analyzed (D21-D42)	50	50	49	300
N analyzed (D0-D42)	49	50	49	299
Antibody titers (1/dil) GMT[95% CI]				
D0	10.44[9.57;11.39]	10.0[10.0;10.0]	10.0[10.0;10.0]	10.10[9.95;10.26]
D21	16.4[12.7;21.1]	13.7[11.2;16.8]	15.0[11.8;19.2]	13.8[12.6;15.0]
D42	24.2[18.6;31.3]	20.0[15.4;26.0]	19.6[15.2;25.1]	21.4[19.3;23.8]
Ratios of antibody titers GMTR[95% CI]				
D21/D0	1.59[1.26;1.99]	1.37[1.12;1.68]	1.50[1.18;1.92]	1.36[1.26;1.48]
D42/D21	1.47[1.20;1.81]	1.46[1.17;1.82]	1.30[1.13;1.51]	1.56[1.43;1.69]
D42/D0	2.34[1.85;2.96]	2.00[1.54;2.60]	1.96[1.52;2.51]	2.12[1.92;2.35]
Seronegativity rate (titers < 20 1/dil.) (% [95% CI])				
D0	98.0 [89.1;99.9]	100.0 [92.9;100.0]	100.0 [92.7;100.0]	99.3 [97.6;99.9]
D21	74.0 [59.7;85.4]	82.0 [68.6;91.4]	79.6 [65.7;89.8]	84.7 [80.1;88.6]
D42	54.0 [39.3;68.2]	64.0 [49.2;77.1]	63.3 [48.3;76.6]	60.3 [54.6;65.9]
2-fold increase from D0 (% [95% CI])				
D21/D0	26.5 [14.9;41.1]	18.0 [8.6;31.4]	20.4 [10.2;34.3]	15.4 [11.5;20.0]
D42/D0	46.9 [32.5;61.7]	36.0 [22.9;50.8]	36.7 [23.4;51.7]	39.8 [34.2;45.6]
2-fold increase from D21 (% [95% CI])				
D42/D21	24.0 13.1;38.2]	20.0 [10.0;33.7]	18.4 [8.8;32.0]	25.3 [20.5;30.7]
4-fold increase from D0 (% [95% CI])				
D21/D0	14.3 [5.9;27.2]	8.0 [2.2;19.2]	10.2 [3.4;22.2]	9.0 [6.0;12.9]
D42/D0	26.5 [14.9;41.1]	22.0 [11.5;36.0]	20.4 [10.2;34.3]	24.1 [19.3;29.3]
4-fold increase from D21 (% [95% CI])				
D42/D21	10.0 [3.3;21.8]	14.0 [5.8;26.7]	4.1 [0.5;14.0]	13.3 [9.7;17.7]

Study GPA02 – data to D42

HI titres using horse erythrocytes (table 5)

Adults (18 to 60 years of age)

- Six subjects had detectable HI titres at D0, ranging between 8 and 64.
- At D21 no CHMP criteria were met.
- At D42 two CHMP criteria (GMTR and seroconversion/significant increase) were met in the 30 µg + Ad group while the GMTR criterion was met in the 7.5 µg group. The seroprotection rate in the 30 µg + Ad group was 39.7%, which falls very short of the CHMP criterion (70%).

Elderly (>60 years of age)

- There were 47 subjects with detectable HI titres at D0, ranging between 11 and 512.
- At D21 two CHMP criteria were met in the 30 µg + Ad group (seroconversion/significant increase in 34.9% and GMTR 3.36) and one was met in the 7.5 µg group (mean geometric increase 2.71).
- At D42 two CHMP criteria (seroconversion/significant increases in 51.7% and 36.5% and GMTRs of 5.21 and 3.60) were met in the 30 µg + Ad and 7.5 µg groups, respectively.
- The seroprotection rate in the 30 µg + Ad group was 49.7% (CHMP criterion is 60%).
- Among those with HI titres ≥8 at D0 the D21 and D42 titres ranged between 32 and 1024. However, the immune response at D42 was not much increased over D21 and only 9/23 and 4/24 per vaccine group had a higher HI titre at D42 than at D21.

Table5: HI (horse erythrocytes) up to D42 by age and vaccine group (FAS)

	18-60 yrs			>60 yrs		
	CHMP threshold*	30µg+Ad	7.5µg	CHMP threshold*	30µg+Ad	7.5µg
N titers at D0 and D21		151	149		146	149
N titers at D21 and D42		146	148		146	149
N titers at D0 and D42		146	148		147	148
Anti-HA titers (1/dil) GMT [95% CI]						
D0		4.14[3.98;4.31]	4.15[3.97;4.34]		5.51[4.84;6.29]	6.17[5.20;7.32]
D21		8.61[6.92;10.72]	8.76[7.00;10.97]		18.7[14.0;25.0]	16.6[12.6;21.7]
D42		19.4[15.1;24.9]	13.0[10.3;16.4]		28.9[22.1;37.9]	21.4[16.5;27.8]
Anti-HA ratios of titers GMTR [95% CI]	>2.5			>2		
D21/D0		2.08[1.69;2.56]	2.11[1.70;2.62]		**3.36**[2.64;4.29]	**2.71**[2.20;3.35]
D42/D21		2.26[1.86;2.75]	1.48[1.29;1.69]		1.54[1.32;1.79]	1.33[1.18;1.48]
D42/D0		**4.68**[3.66;5.97]	**3.13**[2.49;3.93]		**5.21**[4.12;6.59]	**3.60**[2.91;4.46]
Subjects with titers <8 (1/dil) % [95% CI]						
D0		98.0[94.3;99.6]	98.0[94.2;99.6]		84.7[77.9;90.0]	83.9[77.0;89.4]
D21		72.2[64.3;79.2]	72.5[64.6;79.5]		50.7[42.3;59.0]	50.0[41.7;58.3]
D42		43.2[35.0;51.6]	54.1[45.7;62.3]		34.0[26.4;42.3]	38.9[31.1;47.2]
Subjects with titers >=32 (1/dil) % [95% CI]	>70%			>60%		
D0		1.3[0.2;4.7]	1.3[0.2;4.8]		6.0[2.8;11.1]	12.8[7.9;19.2]
D21		20.5[14.4;27.9]	22.1[15.8;29.7]		42.5[34.3;50.9]	36.7[29.0;44.9]
D42		45.9[37.6;54.3]	33.8[26.2;42.0]		57.1[48.7;65.3]	45.6[37.5;54.0]

Subjects with titers >=40 (1/dil) (%) [95% CI]	**>70%**			**>60%**		
D0		0.0[0.0;2.4]	0.7[0.0;3.7]		4.7[1.9;9.4]	10.1[5.7;16.1]
D21		20.5[14.4;27.9]	18.8[12.9;26.0]		37.0[29.2;45.4]	32.0[24.6;40.1]
D42		39.7[31.7;48.1]	27.7[20.7;35.7]		49.7[41.3;58.0]	37.6[29.8;45.9]
Seroconversion† or significant increase‡ from D0 % [95% CI]	**>40%**			**>30%**		
D21		20.5[14.4;27.9]	21.5[15.2;28.9]		**34.9**[27.2;43.3]	26.2[19.3;34.0]
D42		**45.9**[37.6;54.3]	33.1[25.6;41.3]		**51.7**[43.3;60.0]	**36.5**[28.7;44.8]
Seroconversion† rate from D0 % [95%CI]	**>40%**			**>30%**		
D21		18.9[13.0;26.2]	20.5[14.3;28.0]		**31.7**[23.6;40.7]	24.8[17.5;33.3]
D42		**44.8**[36.4;53.3]	32.4[24.9;40.7]		**49.2**[40.1;58.3]	**36.0**[27.6;45.1]

After removal of the 6 subjects aged 18-60 years and 47 subjects aged > 60 years with HI titres ≥ 8 at baseline it was apparent that immune responses for older persons who were seronegative at baseline (HI < 8) were generally lower at D21 and at D42 compared with the overall elderly population and with the baseline seropositive subset (table 6).

In particular, at D42 only 38.5% in the 18-60 years group and 40.3% in the > 60 years group that received the 30 µg + Ad vaccine were seroprotected. However, immune responses in subjects seronegative at baseline were still slightly better in the cohort aged > 60 years compared to the cohort aged 18-60 years. This suggests that despite having titres < 8 a slightly higher proportion of the older subjects had experienced some degree of natural priming with respect to the H5 antigen.

Table 6: HI (horse erythrocytes) up to D42 by age and vaccine group (FAS seronegative at D0 ONLY)

	[18-60 yrs]			**>60 yrs**		
	CHMP threshold*	**30µg+Ad**	**7.5µg**	**CHMP threshold***	**30µg+Ad**	**7.5µg**
N titers at D0 and D21		148	146		123	125
N titers at D21 and D42		143	145		123	125
N titers at D0 and D42		143	145		124	125
Anti-HA titers (1/dil) GMT[95% CI]						
D0		4.00[4.00;4.00]	4.00[4.00;4.00]		4.03[4.00;4.07]	4.00[4.00;4.00]
D21		8.06[6.54;9.92]	8.27[6.64;10.30]		12.14[9.27;15.89]	10.64[8.37;13.53]
D42		18.3[14.3;23.5]	12.48[9.90;15.73]		20.0[15.4;26.0]	14.7[11.6;18.7]
Anti-HA ratios of titers GMTR[95% CI]	**>2.5**			**>2**		
D21/D0		2.01[1.64;2.48]	2.07[1.66;2.57]		**3.01**[2.30;3.93]	**2.66**[2.09;3.38]
D42/D21		2.29[1.88;2.79	1.50[1.31;1.72]		1.64[1.37;1.95]	1.38[1.21;1.58]
D42/D0		**4.58**[3.58;5.87]	**3.12**[2.47;3.93]		**4.96**[3.82;6.45]	**3.68**[2.89;4.68]
Subjects with titers <8 (1/dil) (%) [95% CI]						
D0		100.0[97.5;100.0]	100.0[97.5;100.0]		100.0[97.1;100.0]	100.0[97.1;100.0]
D21		73.6[65.8;80.5]	74.0[66.1;80.9]		60.2[50.9;68.9]	59.2[50.1;67.9]
D42		44.1[35.8;52.6]	55.2[46.7;63.4]		40.3[31.6;49.5]	45.6[36.7;54.7]
Subjects with titers >=32 (1/dil) (%) [95% CI]	**>70%**			**>60%**		
D0		0.0[0.0;2.5]	0.0[0.0;2.5]		0.0[0.0;2.9]	0.0[0.0;2.9]
D21		18.9[13.0;26.2]	20.5[14.3;28.0]		31.7[23.6;40.7]	24.8[17.5;33.3]
D42		44.8[36.4;53.3]	32.4[24.9;40.7]		49.2[40.1;58.3]	36.0[27.6;45.1]
Subjects with titers >=40 (1/dil) (%) [95% CI]						
D0		0.0[0.0;2.5]	0.0[0.0;2.5]		0.0[0.0;2.9]	0.0[0.0;2.9]
D21		18.9[13.0;26.2]	17.1[11.4;24.2]		25.2[17.8;33.8]	20.8[14.1;29.0]
D42		38.5[30.5;47.0]	26.2[19.3;34.2]		40.3[31.6;49.5]	27.2[19.6;35.9]
Seroconversion† or significant increase‡ from D0 (% [95% CI])	**>40%**			**>30%**		
D21		18.9[13.0;26.2]	20.5[14.3;28.0]		**31.7**[23.6;40.7]	24.8[17.5;33.3]
D42		**44.8**[36.4;53.3]	32.4[24.9;40.7]		**49.2**[40.1;58.3]	**36.0**[27.6;45.1]

In contrast, table 7 shows the data only for those with titres ≥8 at D0.

- The six adults (three in each of the 30 µg + Ad and 7.5 µg groups) aged 18-60 years with a detectable HI titre achieved immune responses after a single dose that met each of the three CHMP criteria. At D42 there was little or no further increase in the immune response.

- Similarly in the 47 subjects (23 in the 30µg+Ad group and 24 in the 7.5µg group) aged > 60 years with a detectable HI titre each of the three CHMP criteria was fulfilled in both vaccine groups after the first dose. At D42 there was only a slight further increase in the immune response.

Table 7: HI (horse erythrocytes) up to D42 by age and vaccine group (FAS seropositive at D0 ONLY)

	[18-60 yrs]		> 60 yrs		All	
	30µg+Ad	7.5µg	30µg+Ad	7.5µg	30µg+Ad	7.5µg
N titers at D0 and D21	3	3	23	24	26	27
N titers at D21 and D42	3	3	23	23	26	26
N titers at D0 and D42	3	3	23	23	26	26
Anti-HA titers (1/dil) GMT[95% CI]						
D0	22.63[5.09;100.52]	25.40[4.23;152.45]	31.1[21.3;45.3]	58.7[39.5;87.2]	29.9[21.3;42.1]	53.5[36.8;77.7]
D21	228.07[4.70;11068.19]	143.7[38.6;535.2]	189[118;303]	176[107;290]	193[123;303]	172[110;268]
D42	287.4[18.1;4574.4]	90.5[38.3;214.1]	210[133;334]	178[107;297]	218[143;334]	165[104;261]
Anti-HA ratios of titers GMTR[95% CI]						
D21/D0	10.079[0.726;139.867]	5.66[1.01;31.65]	6.10[3.51;10.61]	3.00[1.95;4.60]	6.46[3.90;10.70]	3.22[2.17;4.77]
D42/D21	1.260[0.210;7.563]	0.630[0.072;5.499]	1.111[0.889;1.389]	1.062[0.937;1.204]	1.127[0.911;1.395]	1.000[0.849;1.177]
D42/D0	12.70[1.45;110.85]	3.564[0.408;31.106]	6.78[3.93;11.68]	3.19[2.03;5.02]	7.29[4.45;11.94]	3.23[2.15;4.87]
Subjects with titers <8 (1/dil) (%) [95% CI]						
D0	0.0%[0.0;70.8]	0.0%[0.0;70.8]	0.0%[0.0;14.8]	0.0%[0.0;14.2]	0.0%[0.0;13.2]	0.0%[0.0;12.8]
D21	0.0%[0.0;70.8]	0.0%[0.0;70.8]	0.0%[0.0;14.8]	0.0%[0.0;14.2]	0.0%[0.0;13.2]	0.0%[0.0;12.8]
D42	0.0%[0.0;70.8]	0.0%[0.0;70.8]	0.0%[0.0;14.8]	0.0%[0.0;14.8]	0.0%[0.0;13.2]	0.0%[0.0;13.2]
Subjects with titers >=32 (1/dil) (%) [95% CI]						
D0	66.7%[9.4;99.2]	66.7%[9.4;99.2]	39.1%[19.7;61.5]	79.2%[57.8;92.9]	42.3%[23.4;63.1]	77.8%[57.7;91.4]
D21	100.0%[29.2;100.0]	100.0%[29.2;100.0]	100.0%[85.2;100.0]	100.0%[85.8;100.0]	100.0%[86.8;100.0]	100.0%[87.2;100.0]
D42	100.0%[29.2;100.0]	100.0%[29.2;100.0]	100.0%[85.2;100.0]	100.0%[85.2;100.0]	100.0%[86.8;100.0]	100.0%[86.8;100.0]
Subjects with titers >=40 (1/dil) (%) [95% CI]						
D0	0.0%[0.0;70.8]	33.3%[0.8;90.6]	30.4%[13.2;52.9]	62.5%[40.6;81.2]	26.9%[11.6;47.8]	59.3%[38.8;77.6]
D21	100.0%[29.2;100.0]	100.0%[29.2;100.0]	100.0%[85.2;100.0]	91.7%[73.0;99.0]	100.0%[86.8;100.0]	92.6%[75.7;99.1]
D42	100.0%[29.2;100.0]	100.0%[29.2;100.0]	100.0%[85.2;100.0]	95.7%[78.1;99.9]	100.0%[86.8;100.0]	96.2%[80.4;99.9]
Seroconversion* or significant increase† from D0 (% [95% CI])						
D21	100.0%[29.2;100.0]	66.7%[9.4;99.2]	52.2%[30.6;73.2]	33.3%[15.6;55.3]	57.7%[36.9;76.6]	37.0%[19.4;57.6]
D42	100.0%[29.2;100.0]	66.7%[9.4;99.2]	65.2%[42.7;83.6]	39.1%[19.7;61.5]	69.2%[48.2;85.7]	42.3%[23.4;63.1]
Seroconversion* or significant increase† from D21 (% [95% CI])						
D42	0.0%[0.0;70.8]	0.0%[0.0;70.8]	8.7%[1.1;28.0]	0.0%[0.0;14.8]	7.7%[0.9;25.1]	0.0%[0.0;13.2]

HI titres using turkey erythrocytes

The pattern of findings after each dose and between groups was essentially similar to that seen with HI using horse erythrocytes but the immune responses were much poorer across the board.

Serum Neutralising Antibody (SNA) titres (Table 8)

- Only two subjects aged 18-60 years but 16 in the older cohort were seropositive (titre ≥ 20) before vaccination. However pre-vaccination GMTs were similar between the four age/vaccine groups.

- After two doses of 30 µg + Ad 38.8% aged 18-60 years and 40% aged > 60 years were seropositive.

- Those subjects aged > 60 years who were seropositive at D0 had higher SN titres at D21 (68 to 518 across the two groups) and greater responses than seen in the overall elderly population. After the second vaccination, their SN titres remained higher than in the overall elderly population (38 to 535 across groups) but the change from D21 to D42 was small. The supplementary tables indicated that all of the six subjects aged > 60 years who were seropositive at baseline and received the 30 µg + Ad vaccine had SNA titres ≥ 80 at D42 compared to 13.2% of those in this age group who were seronegative at baseline.

Table 8: SNA titres against the A/Vietnam/1194/2004 (H5N1) strain (FAS)

	[18-60 yrs]		> 60 yrs	
	30µg+Ad	7.5µg	30µg+Ad	7.5µg
N titers at D0 and D21	151	149	150	150
N titers at D21 and D42	147	148	150	149
N titers at D0 and D42	147	148	150	149
SN titers (1/dil) GMT [95% CI]				
D0	10.09 [9.99;10.20]	10.22 [9.89;10.55]	10.9 [10.3;11.6]	11.8 [10.8;13.0]
D21	12.8 [11.5;14.2]	12.5 [11.3;13.9]	20.0 [16.7;24.0]	16.6 [14.2;19.5]
D42	20.7 [17.9;23.9]	15.6 [13.8;17.7]	23.4 [19.6;27.8]	17.6 [15.0;20.6]
SN ratios of titers GMTR [95% CI]				
D21/D0	1.27 [1.14;1.40]	1.23 [1.11;1.35]	1.83 [1.54;2.17]	1.41 [1.24;1.59]
D42/D21	1.61 [1.42;1.83]	1.25 [1.15;1.35]	1.17 [1.08;1.27]	1.08 [1.03;1.13]
D42/D0	2.05 [1.78;2.37]	1.53 [1.35;1.73]	2.13 [1.81;2.52]	1.51 [1.33;1.72]
Subjects with titers <20 (1/dil) (%[95% CI])				
D0	100.0 [97.6;100.0]	98.7 [95.2;99.8]	96.7 [92.4;98.9]	92.7 [87.3;96.3]
D21	86.8 [80.3;91.7]	89.9 [83.9;94.3]	69.3 [61.3;76.6]	78.7 [71.2;84.9]
D42	61.2 [52.8;69.1]	77.0 [69.4;83.5]	57.3 [49.0;65.4]	73.8 [66.0;80.7]
2-fold increase from D0 (% [95% CI])				
D21/D0	13.2 [8.3;19.7]	9.4 [5.2;15.3]	28.7 [21.6;36.6]	15.3 [10.0;22.1]
D42/D0	38.8 [30.9;47.2]	22.3 [15.9;29.9]	40.0 [32.1;48.3]	20.8 [14.6;28.2]
4-fold increase from D0 (% [95% CI])				
D21/D0	8.6 [4.7;14.3]	5.4 [2.3;10.3]	19.3 [13.3;26.6]	10.0 [5.7;16.0]
D42/D0	27.2 [20.2;35.2]	14.2 [9.0;20.9]	21.3 [15.1;28.8]	11.4 [6.8;17.6]

The applicant provided an integrated analysis of responses to the first two doses in studies GPA01 and 02 that was specific to administration of 30 µg + Ad. This analysis comprised 135 subjects aged 18-40 years, 67 aged 41-60 years and 150 aged > 60 years and immunogenicity data were available for all except nine subjects (Tables 10 and 11).

All except 51 of these subjects (who were all aged 18-40 years) were enrolled into GPA02. Thus the data for those > 60 years all come from GPA02 and have already been compared with younger subjects in the tables above. The additional analyses provide a breakdown by 18-40 *vs* 41-60 years and also allow a comparison between GPA01 and GPA02 for those aged 18-40 years only.

On comparing the HI data obtained in GPA01 and GPA02 in the age groups 18-40 years it is clear that at D21 and D42 the responses were better in the cohort studied in GPA01 (France) than in GPA02 (Belgium and UK). There is no obvious explanation for this difference between studies in terms of subjects studied.

However, in GPA01 the antigen and adjuvant were mixed extemporaneously immediately prior to injection whereas in GPA02 the vaccine was provided in a ready to use formulation, as will be commercialised. Therefore, the results from GPA02 most likely represent what may be expected with the commercial scale batches and the overall opinion on immunogenicity is mainly based on the immune responses seen in this study rather than those obtained in GPA01.

Table 9: HI data (horse erythrocytes) in pooled FAS population (Age specific stratification)

	GPA01 (18-40 yrs) (N=51)	GPA02 (18-40 yrs) (N=84)	GPA02 (41-60 yrs) (N=67)	GPA02 (>60 yrs) (N=150)	GPA02 (>=41 yrs) (N=217)
N titers at D0 and D21	50	84	67	146	213
N titers at D21 and D42	51	81	65	146	211
N titers at D0 and D42	50	81	65	147	212
Anti-HA titers (1/dil) GMT[95% CI]					
D0	4.00[4.00;4.00]	4.20[3.92;4.50]	4.06[3.94;4.19]	5.51[4.84;6.29]	5.02[4.57;5.51]
D21	14.45[8.61;24.26]	9.09[6.69;12.36]	8.04[5.86;11.04]	18.7[14.0;25.0]	14.3[11.4;18.0]
D42	48.8[28.2;84.3]	23.9[16.8;34.1]	14.9[10.5;21.3]	28.9[22.1;37.9]	23.6[19.0;29.4]
Anti-HA ratios of titers GMTR[95% CI]					
D21/D0	3.39[2.03;5.65]	2.16[1.63;2.87]	1.98[1.45;2.71]	3.36[2.64;4.29]	2.85[2.34;3.46]
D42/D21	3.38[2.19;5.19]	2.70[2.03;3.59]	1.82[1.42;2.33]	1.54[1.32;1.79]	1.62[1.42;1.85]
D42/D0	11.55[6.68;19.97]	5.68[4.05;7.98]	3.67[2.59;5.21]	5.21[4.12;6.59]	4.68[3.85;5.69]
Subjects with titers <8 (1/dil) (%) [95% CI]					
D0	100.0%[92.9;100.0]	97.6%[91.7;99.7]	98.5%[92.0;100.0]	84.7%[77.9;90.0]	88.9%[84.0;92.8]
D21	62.7%[48.1;75.9]	71.4%[60.5;80.8]	73.1%[60.9;83.2]	50.7%[42.3;59.0]	57.7%[50.8;64.5]
D42	33.3%[20.8;47.9]	39.5%[28.8;51.0]	47.7%[35.1;60.5]	34.0%[26.4;42.3]	38.2%[31.6;45.1]
Subjects with titers >=8 (1/dil) (%) [95% CI]					
D0	0.0%[0.0;7.1]	2.4%[0.3;8.3]	1.5%[0.0;8.0]	15.3%[10.0;22.1]	11.1%[7.2;16.0]
D21	37.3%[24.1;51.9]	28.6%[19.2;39.5]	26.9%[16.8;39.1]	49.3%[41.0;57.7]	42.3%[35.5;49.2]
D42	66.7%[52.1;79.2]	60.5%[49.0;71.2]	52.3%[39.5;64.9]	66.0%[57.7;73.6]	61.8%[54.9;68.4]

	GPA01 (18-40 yrs) (N=51)	GPA02 (18-40 yrs) (N=84)	GPA02 (41-60 yrs) (N=67)	GPA02 (>60 yrs) (N=150)	GPA02 (>=41 yrs) (N=217)
Subjects with titers >=32 (1/dil) (%) [95% CI]					
D0	0.0%[0.0;7.1]	2.4%[0.3;8.3]	0.0%[0.0;5.4]	6.0%[2.8;11.1]	4.1%[1.9;7.7]
D21	31.4%[19.1;45.9]	23.8%[15.2;34.3]	16.4%[8.5;27.5]	42.5%[34.3;50.9]	34.3%[27.9;41.1]
D42	66.7%[52.1;79.2]	51.9%[40.5;63.1]	38.5%[26.7;51.4]	57.1%[48.7;65.3]	51.4%[44.5;58.3]
Subjects with titers >=40 (1/dil) (%) [95% CI]					
D0	0.0%[0.0;7.1]	0.0%[0.0;4.3]	0.0%[0.0;5.4]	4.7%[1.9;9.4]	3.2%[1.3;6.5]
D21	31.4%[19.1;45.9]	23.8%[15.2;34.3]	16.4%[8.5;27.5]	37.0%[29.2;45.4]	30.5%[24.4;37.2]
D42	62.7%[48.1;75.9]	46.9%[35.7;58.3]	30.8%[19.9;43.4]	49.7%[41.3;58.0]	43.9%[37.1;50.8]
Seroconversion* or significant increase† from D0 (% [95% CI])					
D21	30.0%[17.9;44.6]	23.8%[15.2;34.3]	16.4%[8.5;27.5]	34.9%[27.2;43.3]	29.1%[23.1;35.7]
D42	66.0%[51.2;78.8]	51.9%[40.5;63.1]	38.5%[26.7;51.4]	51.7%[43.3;60.0]	47.6%[40.8;54.6]
Seroconversion* or significant increase† from D21 (% [95% CI])					
D42	39.2%[25.8;53.9]	28.4%[18.9;39.5]	18.5%[9.9;30.0]	14.4%[9.1;21.1]	15.6%[11.0;21.3]

Immune responses in subjects aged 41-60 years, who were enrolled only in GPA02, were generally lower than seen in the same study in those aged 18-40 years, especially after two doses. Responses in the group aged 41-60 were also lower than seen in those aged > 60 years in the same study. Also, in those aged 41-60 years only one of the three CHMP criteria was met at D42 (GMTR 3.67) whereas two criteria were met in the other age groups. However, the seroprotection criteria were not met in any age group in either study.

Both genders showed the abovementioned lower immune responses in subjects aged 18-40 years in study GPA02 compared to GPA01. However, within GPA02 the younger females responded better than younger males (i.e. 18-40 years) while in the 41-60 years age group males did slightly better and in the group aged > 60 years responses were similar between genders.

The SNA data (Table 10) showed a generally similar picture as for HI data by age group and by gender**.** The distribution tabulations in Appendix 15 showed that 27.7% of those aged 18-60 years reached a titre of at least 40 and 11.6% reached at least 80 compared to 24.7% and 16.7%, respectively, among those aged > 60 years. Removal of the six aged > 60 who were seropositive at baseline from the distributions did not notably affect these percentages.

Table 10: HI data (horse erythrocytes) in pooled FAS population by gender

Gender		GPA01 (18-40 yrs) (N=51)		GPA02 (18-40 yrs) (N=84)		GPA02 (41-60 yrs) (N=67)		GPA02 (>60 yrs) (N=150)		GPA02 (>=41 yrs) (N=217)		p-value **
		n	%	n	%	n	%	n	%	n	%	
Males	Anti-HA titers (1/dil) at D42(n,%)											
	<8	9	40.9	18	45.0	12	42.9	27	35.1	39	37.1	0.7949*
	>=8	13	59.1	22	55.0	16	57.1	50	64.9	66	62.9	
	>=16	13	59.1	22	55.0	14	50.0	48	62.3	62	59.0	
	>=32	13	59.1	16	40.0	13	46.4	42	54.5	55	52.4	0.1881*
	>=64	10	45.5	12	30.0	6	21.4	32	41.6	38	36.2	
	>=128	7	31.8	7	17.5	2	7.1	19	24.7	21	20.0	
	>=256	2	9.1	0	0	2	7.1	8	10.4	10	9.5	
	>=512	1	4.5	0	0	0	0	4	5.2	4	3.8	
	>=1024	0	0	0	0	0	0	3	3.9	3	2.9	
	All	22	100	40	100	28	100	77	100	105	100	
	Median titer [95% CI] ‡	45.3	[4.0;181.0]	16.0	[4.0;45.3]	17.0	[4.0;45.3]	45.3	[22.6;64.0]	32.0	[11.3;45.3]	0.2345†
	GMTs of subjects>=8 [95% CI]	121.4	[74.3;198.1]	55.5	[38.4;80.4]	50.4	[30.2;84.2]	83.9	[61.6;114.2]	74.1	[57.0;96.5]	
	Median D42/D0 [95% CI] ‡	11.3	[1.0;45.3]	4.0	[1.0;8.0]	4.2	[1.0;11.3]	5.7	[2.8;11.3]	5.7	[2.8;11.3]	0.1848†
	D42/D0 GMTR [95% CI]	7.5	[3.3;16.9]	4.1	[2.6;6.4]	4.3	[2.4;7.5]	5.2	[3.7;7.3]	4.9	[3.7;6.6]	
	N titers at D0 and D42	22		40		28		77		105		

Gender		GPA01 (18-40 yrs) (N=51)		GPA02 (18-40 yrs) (N=84)		GPA02 (41-60 yrs) (N=67)		GPA02 (>60 yrs) (N=150)		GPA02 (>=41 yrs) (N=217)		p-value **
		n	%	n	%	n	%	n	%	n	%	
Females	Anti-HA titers (1/dil) at D42(n,%)											
	<8	8	27.6	14	34.1	19	51.4	23	32.9	42	39.3	0.6102*
	>=8	21	72.4	27	65.9	18	48.6	47	67.1	65	60.7	
	>=16	21	72.4	27	65.9	18	48.6	46	65.7	64	59.8	
	>=32	21	72.4	26	63.4	12	32.4	42	60.0	54	50.5	0.4535*
	>=64	18	62.1	21	51.2	6	16.2	25	35.7	31	29.0	
	>=128	16	55.2	12	29.3	3	8.1	13	18.6	16	15.0	
	>=256	10	34.5	4	9.8	2	5.4	8	11.4	10	9.3	
	>=512	6	20.7	1	2.4	1	2.7	5	7.1	6	5.6	
	>=1024	1	3.4	1	2.4	0	0	3	4.3	3	2.8	
	All	29	100	41	100	37	100	70	100	107	100	
	Median titer [95% CI] ‡	128.0	[45.3;256.0]	64.0	[22.6;64.0]	4.0	[4.0;22.6]	32.0	[22.6;45.3]	32.0	[16.0;32.0]	0.0464†
	GMTs of subjects>=8 [95% CI]	210.0	[134.2;328.6]	97.8	[68.8;138.9]	48.9	[30.7;77.7]	74.7	[54.3;102.9]	66.4	[51.1;86.3]	
	Median D42/D0 [95% CI] ‡	32.0	[8.0;64.0]	16.0	[5.7;16.0]	1.0	[1.0;5.7]	8.0	[2.8;8.0]	5.7	[1.4;8.0]	0.0490†
	D42/D0 GMTR [95% CI]	16.2	[7.6;34.8]	7.8	[4.7;13.0]	3.3	[2.1;5.2]	5.2	[3.7;7.3]	4.4	[3.4;5.8]	
	N titers at D0 and D42	28		41		37		70		107		

SN data in pooled FAS population by gender

Gender		GPA01 (18-40 yrs) (N=51)		GPA02 (18-40 yrs) (N=84)		GPA02 (41-60 yrs) (N=67)		GPA02 (>60 yrs) (N=150)	
		n	%	n	%	n	%	n	%
Males	Median titer [95% CI] ‡	20.5	[10.0;65.2]	10.0	[10.0;16.1]	10.0	[10.0;16.4]	14.5	[10.0;25.3]
	GMTs of subjects>=20 [95% CI]	57.1	[40.6;80.4]	55.3	[41.0;74.7]	52.9	[34.5;81.2]	73.6	[51.7;104.9]
	Median D42/D0 [95% CI] ‡	2.1	[1.0;6.5]	1.0	[1.0;1.6]	1.0	[1.0;1.6]	1.0	[1.0;2.5]
	D42/D0 GMTR [95% CI]	2.5	[1.7;3.8]	1.7	[1.4;2.2]	1.6	[1.2;2.2]	2.3	[1.8;2.9]
	N titers at D0 and D42	22		41		28		79	

Gender		GPA01 (18-40 yrs) (N=51)		GPA02 (18-40 yrs) (N=84)		GPA02 (41-60 yrs) (N=67)		GPA02 (>60 yrs) (N=150)	
		n	%	n	%	n	%	n	%
Females	Median titer [95% CI] ‡	35.2	[26.1;77.1]	39.4	[22.0;55.1]	10.0	[10.0;16.7]	14.8	[10.0;28.4]
	GMTs of subjects>=20 [95% CI]	66.0	[47.5;91.6]	61.7	[48.5;78.6]	48.2	[32.6;71.2]	59.9	[44.7;80.3]
	Median D42/D0 [95% CI] ‡	3.5	[2.6;7.5]	3.9	[2.2;5.5]	1.0	[1.0;1.7]	1.2	[1.0;2.5]
	D42/D0 GMTR [95% CI]	3.8	[2.7;5.5]	3.5	[2.6;4.7]	1.6	[1.3;2.1]	2.0	[1.6;2.5]
	N titers at D0 and D42	29		41		37		71	

Antibody persistence and booster responses in GPA01 and GPA02

A major concern was raised by CHMP at D120 with regard to the poor immunogenicity of Emerflu and the failure to meet the CHMP criteria for HI seroprotection rates after the primary series with rates of only about 40% in previously seronegative adults. During the procedure additional data on antibody persistence and booster data were submitted which show the immune response after a third dose of the vaccine. Results are shown in the following tables:

Study GPA01:

The D180 data for HI performed using horse erythrocytes shown below were based on a seroprotection cut-off at ≥ 32. As would be expected, seroprotection rates and GMTs had dropped from D42 with ≤ 26% per group seroprotected. Also, SNA GMTs had dropped to within the range 12.6 to 16 and 36-44 subjects per group had no detectable neutralising antibody.

Table 11: study GPA01 – Antibody persistence at month 6

	30µg+Ad	15µg+Ad	7.5µg+Ad	All
N analyzed	50	48	48	286
Anti HA titers (1/dil)				
Geometric mean [95% CI]	9.32 [6.42;13.51]	6.21 [4.58;8.42]	5.70 [4.26;7.63]	7.21 [6.30;8.25]
Seroprotection (>=32 1/dil.) (% [95% CI])	26.0 [14.6;40.3]	8.3 [2.3;20.0]	8.3 [2.3;20.0]	16.1 [12.0;20.9]

	30µg	15µg	7.5µg	All
N analyzed	49	45	46	286
Anti HA titers (1/dil)				
Geometric mean [95% CI]	7.51 [5.32;10.58]	7.41 [5.38;10.20]	7.59 [5.18;11.13]	7.21 [6.30;8.25]
Seroprotection (>=32 1/dil.) (% [95% CI])	16.3 [7.3;29.7]	15.6 [6.5;29.5]	21.7 [10.9;36.4]	16.1[12.0;20.9]

Antibody persistence data to D365 were later provided together with the post-boost data.

In the group that received a third dose of Emerflu all three EMEA criteria were met for immune responses to the vaccine strain at D21 post-boost (bold numbers in Table 12). These HI data were supported by the SN data presented in the second table 13 below:

Table 12: H5N1/Vietnam/1194/2004 Immunogenicity Summary – HI Method on Horse Erythrocytes – GMTs, GMTRs Seriprotection and Seroconversion rates by Randomised Primary and 30 µgHA+aluminium hydroxide Booster Vaccine Group – FAS II Population [D365-D372-D386] (all subjects)

Primary series vaccination group	30µg+Ad	15µg+Ad	7.5µg+Ad	30µg	15µg	7.5µg
Booster vaccination group	**30µg+Ad**	**30µg+Ad**	**30µg+Ad**	**30µg+Ad**	**30µg+Ad**	**30µg+Ad**
n (D365-D372)	22	20	19	19	18	14
n (D372-D386)	22	20	19	19	18	14
n (D365-D386)	22	20	20	19	18	14
Anti-HA titers (1/dil) GMT [95%CI]						
D365	5.84[4.36;7.82]	5.01[3.57;7.03]	5.19[3.65;7.38]	5.45[3.73;7.99]	21.1%[6.1;45.6]	5.80[3.84;8.75]
D372	35.7[22.5;56.6]	20.0[10.7;37.4]	23.0[11.4;46.7]	11.11[6.20;19.89]	11.99[6.81;21.08]	12.49[5.76;27.11]
D386	40.5[25.4;64.6]	29.9[15.5;57.4]	44.5[24.2;81.7]	14.87[8.15;27.13]	18.3[10.4;32.1]	16.81[6.62;42.68]
Anti-HA ratios of titers GMTR [95%CI]						
D372/D365	**6.12**[3.79;9.88]	29.9[15.5;57.4]	**4.38**[2.20;8.74]	2.04[1.18;3.53]	2.12[1.28;3.51]	2.15[1.14;4.06]
D386/D372	1.134[0.972;1.323]	1.490[0.990;2.241]	2.19[1.25;3.83]	1.339[0.936;1.915]	1.53[1.07;2.17]	1.35[1.00;1.80]
D386/D365	**6.94**[4.44;10.85]	**5.96**[3.21;11.06]	**8.57**[4.75;15.46]	**2.73**[1.55;4.81]	**3.24**[1.85;5.66]	**2.90**[1.32;6.37]
Subjects with titers <8 [1/dil] (% [95% CI])						
D365	77.3%[54.6;92.2]	90.0%[68.3;98.8]	90.0%[68.3;98.8]	84.2%[60.4;96.6]	83.3%[58.6;96.4]	78.6%[49.2;95.3]
D372	13.6%[2.9;34.9]	35.0%[15.4;59.2]	36.8%[16.3;61.6]	52.6%[28.9;75.6]	44.4%[21.5;69.2]	57.1%[28.9;82.3]
D386	13.6%[2.9;34.9]	25.0%[8.7;49.1]	15.0%[3.2;37.9]	42.1%[20.3;66.5]	33.3%[13.3;59.0]	50.0%[23.0;77.0]
Subjects with titer ≥ 32 (1/dil) (%) [95%CI]						
D365	4.5%[0.1;22.8]	5.0%[0.1;24.9]	10.0%[1.2;31.7]	10.5%[1.3;33.1]	11.1%[1.4;34.7]	7.1%[0.2;33.9]
D372	**72.7%**[49.8;89.3]	45.0%[23.1;68.5]	52.6%[28.9;75.6]	21.1%[6.1;45.6]	33.3%[13.3;59.0]	42.9%[17.7;71.1]
D386	**77.3%**[54.6;92.2]	55.0%[31.5;76.9]	65.0%[40.8;84.6]	36.8%[16.3;61.6]	44.4%[21.5;69.2]	42.9%[17.7;71.1]
Seroconversion‡ or significant increase§ from D0 (%) [95%CI]						
D372	**59.1%**[36.4;79.3]	40.0%[19.1;63.9]	**42.1%**[20.3;66.5]	15.8%[3.4;39.6]	16.7%[3.6;41.4]	35.7%[12.8;64.9]
D386	**72.7%**[49.8;89.3]	**50.0%**[27.2;72.8]	**60.0%**[36.1;80.9]	21.1%[6.1;45.6]	27.8%[9.7;53.5]	35.7%[12.8;64.9]

‡ Seroconversion (defined in subjects with an undetectable baseline titer, i.e. with <8 [1/dil] pre-vaccination titer, with a post-vaccination titer ≥ 32 [1/dil])

§ Significant increase (defined in subjects with a detectable baseline titer, i.e. with ≥ 8 [1/dil] pre-vaccination titer, with at least a 4-fold increase in post-vaccination titer)

N = number of subjects in group; n = number of subjects; Ad = aluminum hydroxide adjuvant; GMT = geometric mean titer; GMTR = geometric mean titer ratio; CI = confidence interval

Mean data fulfilling the adapted EMEA criteria are shown in bold

Table 13: H5N1/Vietnam/1194/2004 Immunogenicity Summary – Seroneutralisation Method . GMTs, GMTRs, Two and Four-Fold Increase Rates by Randomized Primary and Booster Vaccine Group . FAS II Population [D365-D372-D386]

Primary series vaccination group	30µg+Ad		15µg+Ad		7.5µg+Ad		Ad+	
Booster vaccination group	30µg+Ad (N= 22)	7.5µg (N= 21)	30µg+Ad (N= 20)	7.5µg (N= 19)	30µg+Ad (N= 20)	7.5µg (N= 21)	30µg+Ad (N= 62)	7.5µg (N= 61)
Subjects with titers<20 (1/dil) [95% CI]								
D365	95.5% [77.2;99.9]	95.2% [76.2;99.9]	95.0% [75.1;99.9]	94.7% [74.0;99.9]	90.0% [68.3;98.8]	90.5% [69.6;98.8]	93.5% [84.3;98.2]	93.4% [84.1;98.2]
D372	18.2% [5.2;40.3]	57.1% [34.0;78.2]	30.0% [11.9;54.3]	52.6% [28.9;75.6]	35.0% [15.4;59.2]	47.6% [25.7;70.2]	27.4% [16.9;40.2]	52.5% [39.3;65.4]
D386	18.2% [5.2;40.3]	66.7% [43.0;85.4]	20.0% [5.7;43.7]	52.6% [28.9;75.6]	10.0% [1.2;31.7]	38.1% [18.1;61.6]	16.1% [8.0;27.7]	52.5% [39.3;65.4]
2 fold increase from D365 (%) [95% CI]								
D372	81.8% [59.7;94.8]	38.1% [18.1;61.6]	70.0% [45.7;88.1]	42.1% [20.3;66.5]	60.0% [36.1;80.9]	42.9% [21.8;66.0]	71.0% [58.1;81.8]	41.0% [28.6;54.3]
D386	81.8% [59.7;94.8]	28.6% [11.3;52.2]	80.0% [56.3;94.3]	42.1% [20.3;66.5]	85.0% [62.1;96.8]	57.1% [34.0;78.2]	82.3% [70.5;90.8]	42.6% [30.0;55.9]
4 fold increase from D365 (%) [95% CI]								
D372	72.7% [49.8;89.3]	23.8% [8.2;47.2]	55.0% [31.5;76.9]	15.8% [3.4;39.6]	50.0% [27.2;72.8]	28.6% [11.3;52.2]	59.7% [46.4;71.9]	23.0% [13.2;35.5]
D386	72.7% [49.8;89.3]	28.6% [11.3;52.2]	70.0% [45.7;88.1]	15.8% [3.4;39.6]	60.0% [36.1;80.9]	47.6% [25.7;70.2]	67.7% [54.7;79.1]	31.1% [19.9;44.3]

Study GPA02:

The seroprotection rate at 6 months was ≤10% for those aged < 60 years and about 30% for those aged > 60 years (but the latter figure includes those who were seropositive at baseline).

Table 14: Clade 1 Booster Immunogenicity Criteria by Primary Randomized Vac. - HI Horse Erythrocyte Method - Anti-HA Antibody (1/dil) Against A/Vietnam/1194/2004 (H5N1)_RG14 Strain - FAS II Pop. - [D180-D201]

	[18-60 yrs]		> 60 yrs		All	
	30µg+Ad	7.5µg	30µg+Ad	7.5µg	30µg+Ad	7.5µg
N titers at D180 and D201	38	39	41	36	79	75
PRE-BOOSTER VACCINATION(D180)						
Anti HA titers (1/dil)						
Geometric mean	6.85	5.46	13.98	8.81	9.92	6.87
95% CI	[5.14;9.13]	[4.54;6.56]	[9.34;20.92]	[5.85;13.26]	[7.66;12.84]	[5.51;8.56]
Q1 , Median , Q3	4.00 , 4.00 , 11.3	4.00 , 4.00 , 5.66	4.00 , 8.00 , 32.0	4.00 , 4.00 , 16.0	4.00 , 5.66 , 22.6	4.00 , 4.00 , 8.00
Min , Max	4.00 , 128	4.00 , 32.0	4.00 , 512	4.00 , 256	4.00 , 512	4.00 , 256
log GMT (SD)	0.84(0.38)	0.74(0.25)	1.15(0.55)	0.94(0.52)	1(0.50)	0.84(0.41)
Subjects with titers <8 (1/dil) (%) [95% CI]						
n/N	27/38	31/39	16/41	23/36	43/79	54/75
(%)	71.1%	79.5%	39.0%	63.9%	54.4%	72.0%
95 % CI	[54.1;84.6]	[63.5;90.7]	[24.2;55.5]	[46.2;79.2]	[42.8;65.7]	[60.4;81.8]
Subjects with titers >=32 (1/dil) (%) [95% CI]						
n/N	4/38	1/39	13/41	7/36	17/79	8/75
(%)	10.5%	2.6%	31.7%	19.4%	21.5%	10.7%
95 % CI	[2.9;24.8]	[0.1;13.5]	[18.1;48.1]	[8.2;36.0]	[13.1;32.2]	[4.7;19.9]
Subjects with titers >=40 (1/dil) (%) [95% CI]						
n/N	3/38	0/39	10/41	5/36	13/79	5/75
(%)	7.9%	0.0%	24.4%	13.9%	16.5%	6.7%
95 % CI	[1.7;21.4]	[0.0;9.0]	[12.4;40.3]	[4.7;29.5]	[9.1;26.5]	[2.2;14.9]

* N is the number of subjects with titers <8 (1/dil) at D180 before the second booster vaccination
† N is the number of subjects with titers >=8 (1/dil) at D180 before the second booster vaccination
‡ Seroconversion: subjects with pre-vaccination titer <8 (1/dil) and with a post-vaccination titer >=32 (1/dil)
§ Significant increase: subjects with pre-vaccination titer >=8 (1/dil) and with at least a 4-fold increase in post-vaccination titer
Note: Q1 and Q3 are the first and third quartiles

In each age group there was a very modest immune response to a dose of unadjuvanted 7.5 µg HA. No CHMP criterion was fulfilled following the dose of homologous strain 7.5µg HA vaccine in either age group (18-60 or > 60 years). It was considered that the low responses reflected poor and/or short-lived priming by the initial two doses of Emerflu.

Table 15: Clade 1 Booster Immunogenicity Criteria by Primary Randomized Vac. - HI Horse Erythrocyte Method - Anti-HA Antibody (1/dil) Against A/Vietnam/1194/2004 (H5N1)RG14 Strain - FAS II Pop. - [D180-D201]

	[18-60 yrs]		> 60 yrs		All	
	30µg+Ad	7.5µg	30µg+Ad	7.5µg	30µg+Ad	7.5µg
POST-BOOSTER VACCINATION(D201)						
Anti HA titers (1/dil)						
Geometric mean	9.96	8.67	18.8	11.53	13.8	9.94
95% CI	[7.17;13.82]	[6.32;11.89]	[12.5;28.3]	[7.56;17.59]	[10.6;18.1]	[7.69;12.85]
Q1 , Median , Q3	4.00 , 6.73 , 22.6	4.00 , 5.66 , 16.0	4.00 , 16.0 , 45.3	4.00 , 8.00 , 26.9	4.00 , 11.3 , 32.0	4.00 , 5.66 , 22.6
Min , Max	4.00 , 128	4.00 , 128	4.00 , 512	4.00 , 256	4.00 , 512	4.00 , 256
log GMT (SD)	1(0.43)	0.94(0.42)	1.27(0.56)	1.06(0.54)	1.14(0.52)	1(0.48)
Subjects with titers <8 (1/dil) (%) [95% CI]						
n/N	19/38	23/39	12/41	17/36	31/79	40/75
(%)	50.0%	59.0%	29.3%	47.2%	39.2%	53.3%
95 % CI	[33.4;66.6]	[42.1;74.4]	[16.1;45.5]	[30.4;64.5]	[28.4;50.9]	[41.4;64.9]
Subjects with titers >=32 (1/dil) (%) [95% CI]						
n/N	6/38	7/39	18/41	9/36	24/79	16/75
(%)	15.8%	17.9%	43.9%	25.0%	30.4%	21.3%
95 % CI	[6.0;31.3]	[7.5;33.5]	[28.5;60.3]	[12.1;42.2]	[20.5;41.8]	[12.7;32.3]
Subjects with titers >=40 (1/dil) (%) [95% CI]						
n/N	5/38	5/39	12/41	7/36	17/79	12/75
(%)	13.2%	12.8%	29.3%	19.4%	21.5%	16.0%
95 % CI	[4.4;28.1]	[4.3;27.4]	[16.1;45.5]	[8.2;36.0]	[13.1;32.2]	[8.6;26.3]
POST-/PRE-BOOSTER (D201/D180)						

* N is the number of subjects with titers <8 (1/dil) at D180 before the second booster vaccination
† N is the number of subjects with titers >=8 (1/dil) at D180 before the second booster vaccination
‡ Seroconversion: subjects with pre-vaccination titer <8 (1/dil) and with a post-vaccination titer >=32 (1/dil)
§ Significant increase: subjects with pre-vaccination titer >=8 (1/dil) and with at least a 4-fold increase in post-vaccination titer
Note: Q1 and Q3 are the first and third quartiles

The higher anti-HA GMTs and the higher proportion of subjects with a detectable anti-HA titre in the elderly group is probably a reflection of the higher GMTs and the higher proportion of subjects with a detectable anti-HA titre at D180 (prior to the booster vaccination) and not to a higher immune response to the booster vaccination per se in the elderly subjects. This is illustrated by the rate of seroconversion or significant increase from D180 which was low in all groups.

These HI data were supported by the SN results.

Data were also provided on responses to a dose of Emerflu containing A/Indonesia administered at 22 months after two initial doses of Emerflu containing A/Vietnam. These data are summarized in Tables 16 (HI- titres) and 17 (SN-titres).

Antibody titres could hardly be detected at M22. A single dose of Emerflu containing A/Indonesia restored antibody levels against A/Vietnam to levels comparable to those observed post-primary (anti HI 1/32 criterion corresponding to CHMP criterion for seroprotection).

The SN titres were higher compared to post primary, in particular when comparing the rate of individuals with SN-titres ≥ 1:40; Table 17).

In both assays lower response rates to booster vaccination were observed in elderly individuals.

Table 16: Immunogenicity Summary-HIH Method-Anti-HA Ab (1/dil) Against A/Indonesia/5/05-RG2 (H5N1) and A/Vietnam/1194/2004/NIBRG-14 (H5N1) – FAS

	Strain against which antibody response measured			
	A/Indonesia		A/Vietnam	
	18-60 yrs (N=60)	>60 yrs (N=57)	18-60 yrs (N=60)	>60 yrs (N=57)
n subjects with titers available at M22 and M22+7D	59	56	-	-
n subjects with titers available at M22+7D and M22+21D	59	57	-	-
n subjects with titers available at M22 and M22+21D	59	56	-	-
Anti-HA titers (1/dil) GMT (95% CI)				
M22	4.09 (3.96;4.23)	4.18 (3.83;4.56)	4.14 (4.01;4.27)	5.42 (4.40;6.67)
M22+7D	12.6 (9.56;16.5)	6.16 (5.02;7.56)	12.1 (9.35;15.8)	8.25 (6.25;10.9)
M22+21D	15.2 (11.3;20.3)	9.84 (7.06;13.7)	14.4 (10.8;19.2)	10.7 (7.71;14.9)
Individual ratios of titers GMTR (95% CI)				
M22+7D/M22	**3.07** (2.34;4.03)	1.49 (1.21;1.82)	-	-
M22+21D/M22	**3.71** (2.78;4.94)	**2.39** (1.72;3.33)	-	-
Subjects with titers ≥8 (1/dil) % (95% CI)				
M22	3.3 (0.4;11.5)	1.8 (0.0;9.6)	1.7 (0.0;8.9)	14.3 (6.4;26.2)
M22+7D	62.7 (49.1;75.0)	28.1 (17.0;41.5)	66.1 (52.6;77.9)	36.8 (24.4;50.7)
M22+21D	69.5 (56.1;80.8)	47.4 (34.0;61.0)	67.8 (54.4;79.4)	47.4 (34.0;61.0)
Subjects with titers ≥32 (1/dil) % (95% CI)				
M22	0.0 (0.0;6.0)	1.8 (0.0;9.6)	0.0 (0.0;6.0)	8.9 (3.0;19;6)
M22+7D	28.8 (17.8;42.1)	10.5 (4.0;21.5)	27.1 (16.4;40.3)	22.8 (12.7;35.8)
M22+21D	42.4 (29.6;55.9)	22.8 (12.7;35.8)	39.0 (26.5;52.6)	26.3 (15.5;39.7)
Seroconversion* or significant increase rate† from M22 (95% CI)				
M22+7D	28.8 (17.8;42.1)	8.9 (3.0;19.6)	-	-
M22+21D	**42.4** (29.6;55.9)	21.4 (11.6;34.4)	-	-

N = number of subjects in group; n = number of subjects; GMT = geometric mean titer; CI = confidence interval
* Seroconversion: subjects with pre-vaccination titer <8 (1/dil) and with a post-vaccination titer ≥32 (1/dil)
† Significant increase: subjects with pre-vaccination titer ≥8 (1/dil) and with at least a 4-fold increase in post-vaccination titer
Mean data fulfilling the adapted EMEA criteria (see Table 2.1) are shown in bold

Table 17: Immunogenicity Summary - SN Method - Neutralising Ab (1/dil) Against A/Indonesia/5/05-RG2 (H5N1) - FAS [M22, M22+7D, M22+21D]

	18-60 yrs (N=60)	>60 yrs (N=57)
n subjects with titers available at M22 and M22+7D	59	56
n subjects with titers available at M22+7D and M22+21D	59	57
n subjects with titers available at M22 and M22+21D	59	56
Neutralizing Ab titers GMT (1/dil) (95% CI)		
M22	5.54 (5.07;6.06)	5.58 (5.02;6.21)
M22+7D	41.6 (29.6;58.4)	13.9 (9.90;19.6)
M22+21D	48.9 (33.1;72.1)	23.4 (14.9;36.7)
Individual ratios of titers GMTR (95% CI)		
M22+7D/M22	7.48 (5.33;10.5)	2.49 (1.78;3.48)
M22+21D/M22+7D	1.18 (0.936;1.48)	1.68 (1.38;2.05)
M22+21D/M22	8.80 (6.04;12.8)	4.22 (2.70;6.60)
Subjects with titers ≥10 (1/dil) % (95% CI)		
M22	10.0 (3.8;20.5)	8.9 (3.0;19.6)
M22+7D	83.1 (71.0;91.6)	50.9 (37.3;64.4)
M22+21D	81.4 (69.1;90.3)	57.9 (44.1;70.9)
2-fold increase from M22 % (95% CI)		
M22+7D	83.1 (71.0;91.6)	44.6 (31.3;58.5)
M22+21D	81.4 (69.1;90.3)	55.4 (41.5;68.7)
4-fold increase from M22 % (95% CI)		
M22+7D	67.8 (54.4;79.4)	26.8 (15.8;40.3)
M22+21D	69.5 (56.1;80.8)	42.9 (29.7;56.8)
2-fold increase from M22+7D % (95% CI)		
M22+21D	22.0 (12.3;34.7)	35.1 (22.9;48.9)
4-fold increase from M22+7D % (95% CI)		
M22+21D	1.7 (0.0;9.1)	14.0 (6.3;25.8)

N = number of subjects in group; n = number of subjects; Ab=antibody; GMT = geometric mean titer; GMTR = geometric mean titer ratio; CI = confidence interval

Data on cross-reactivity study GPA01:

The ability of antibodies elicited by the primary series of Emerflu to neutralise the parental wild type Clade 1 virus strain and genetically and antigenically distant Clade 2 virus strains was investigated in a cross-neutralisation study. However, the results must be viewed with considerable caution due to the fact that these assays were performed only in highly selected subsets of sera.

Generally it seems that in the Emerflu group subjects with a neutralising antibody titre ≥20 [1/dil]) against the A/Vietnam/1194/2004/NIBRG-14 vaccine strain in the 30µgHA+aluminium hydroxide group had a neutralising antibody titre ≥20 (1/dil) against the A/Vietnam/1194/2004 wild type virus. Of the 10 tested who did not respond to the primary series 9 had a SN titre of at least 1:20 to wild type virus.

Of those tested who responded to a primary series of Emerflu sera from 90% and 42% of subjects tested cross-neutralised the wild type A/turkey/Turkey/1/2005 strain and A/turkey/Turkey/2005/NIBRG-23 strain, respectively. Cross-neutralisation of wild-type A/Indonesia/5/2005 virus was only seen in samples with high neutralising antibody titres against the Clade 1 vaccine virus (titres >100 [1/dil]).

These results cannot be extrapolated to the entire study population. In addition, the expression of SN seropositivity rates against heterologous strains after pooling of sera that were and were not seropositive for the vaccine strain was inappropriate.

Supportive studies

In study GPA04 children and adolescents were immunized with two doses of Emerflu containing A/Vietnam. These data (GPA04) demonstrated a strong immunological response, with more than 90% of subjects demonstrating a quantifiable HIH titre at Day 42, and all three EMEA criteria being fulfilled.

However, since study GPA04 was conducted in Thailand – a region endemic for highly pathogenic avian influenza virus strain H5N1 – it is doubtful whether these findings are relevant for a European population..

Study GPA11 was conducted in 100 healthy Australian adults who received two doses of Emerflu containing the A/Vietnam strain of influenza A virus subtype H5N1. This study was provided as an abbreviated report. Results are presented below under *Analysis performed across trials.*

Analysis performed across trials

Weak immunogenicity after two doses was observed across all studies regardless of the strain in the vaccine. Most notably, clinical trial lots derived from semi or full scale production (studies GPA11; GPA02 – A/Indonesia arm) were apparently even less immunogenic than lots derived from pilot production (study GPA02 – A/Vietnam arm) or the experimental formulation (GPA01).

Table 18: Immune Response Using HIH 21 Days Post-Dose 2 (Day 42) - GPA02 (A/Vietnam/1194/2004/NIBRG-14 and A/Indonesia/5/05/RG-2 strains) and GPA11 (A/Vietnam/1194/2004/NIBRG-14 strain) – Subjects Aged 18 to 60 Years

	EMEA criteria	A/Vietnam/1194/2004/NIBRG-14		A/Indonesia/ 5/05/RG-2
		GPA02 (N=150)	GPA11 (N=100)	GPA02 (N=50)
GMTR	>2.5	4.68 [3.66;5.97]	3.30 [2.66;4.11]	3.20 [2.21;4.64]
Subjects with titers ≥ 32 (1/dil) (%)	>70%	45.9% [37.6;54.3]	30.9% [21.7;41.2]	26.0% [14.6;40.3]
Seroconversion* or significant increase† from D0 (%)	>40%	45.9% [37.6;54.3]	30.9% [21.7;41.2]	26.0% [14.6;40.3]

* Seroconversion: subjects with pre-vaccination titer <8 (1/dil) and with a post-vaccination titer ≥ 32 (1/dil)

† Significant increase: subjects with pre-vaccination titer ≥ 8 (1/dil) and with at least a 4-fold increase in post-vaccination titer

GMTR = geometric mean titer ratio; N = Number of subjects in group

Data are point estimate [95% confidence interval]

Discussion on clinical immunogenicity

All the data assessed point to a conclusion that < 40% of adult or elderly individuals vaccinated with two doses of Emerflu will develop HI-titres which are considered to convey immune protection according to current CHMP criteria. Seroprotection rates may also vary depending on age and health status of vaccinees.

Whereas GPA01 showed that a third dose of vaccine containing A/Vietnam in subjects who had received pilot vaccine elicited higher seroprotection rates (less than 80%) the administration of unadjuvanted 7.5 µg HA vaccine to those primed with 30 + Al in GPA02 resulted in an increase in seroprotection rate from 10.5% to 15.8% (based on 1:32 cut-off), which is effectively no response. This was accompanied by virtually no increment in GMT.

Administration of a single dose of Emerflu A/Indonesia to subjects who had previously received (22 months before) two doses of Emerflu A/Vietnam showed that HI titres increased from pre-boost to levels observed after primary immunisation. However, only 20-40 % reached HI titres of at least 1:32 and the NA GMTs indicate very low % with 1:40.

The data presented in Thai children and adolescents indicated that responses were much better to primary vaccination with Emerflu compared to older subjects. However, it was concluded that the data were insufficient to consider any recommendation for Emerflu in subjects aged < 18 years and were of questionable relevance to the EU population. Also antibody persistence and booster data are not yet available for this age category.

As for the pandemic influenza vaccines already licensed in the EU under exceptional circumstances it is impossible to predict the protective efficacy that might be conferred by Emerflu in a pandemic situation. Nevertheless, both the HI and the NA data presented by the applicant indicate that this vaccine construct is of low immunogenicity. A third dose of a homologous strain vaccine elicited more promising responses at least in one study (and in subjects who were not primed with pre-mixed antigen and adjuvant) but this vaccine strategy is not going to be very useful in a pandemic and would consume valuable haemagglutinin manufacturing capacity.

Clinical safety

Injection site and systemic safety was evaluated during the 21 days following each injection (including the booster injection). Other reactions/events occurring between D0 and D21 after both vaccinations and all serious adverse events (SAEs) occurring between V01 and V07 (D386) were recorded.

In **study GPA01** all 300 subjects received at least one vaccination and were included in the analysis of safety.

All the 600 subjects initially vaccinated in **study GPA02** were included in the analysis of safety of which 350 were exposed to the 30µg + Ad vaccine.

During the procedure the applicant provided booster data (N=118) with one dose of Emerflu containing the A/Indonesia strain of influenza A virus subtype H5N1 from healthy adult and elderly subjects who had been immunized 22 months previously with two doses of Emerflu containing the A/Vietnam strain of influenza A virus subtype H5N1. The applicant also provided primary immunisation data (N=50) from healthy subjects between 18 and 60 years of age who received two doses of Emerflu containing the A/Indonesia strain given 21 days apart.

Adverse events to D42

GPA01

Of the 300 vaccinated 247 experienced at least one solicited reaction during the D0-D42 period. Solicited reactions were most frequently reported in the 30µg+Ad group (46; 90.2%) in which 38 subjects (74.5%) reported at least one solicited injection site reaction and the same proportion reported at least one solicited systemic reaction.

Solicited local reactions that occurred within 7 days after the first and the second doses are shown in table 19 below. The rate of injection site pain after the first dose was highest in the 30µg+Ad group.

Table 19: Occurrence of each solicited injection site reaction within 7 days after first vaccine injection – Safety Analysis Set

	30µg+Ad (N=51)			15µg+Ad (N=50)			7.5µg+Ad (N=50)			Ad+ (N=151)		
Subjects with:	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**
Injection site pain	27	52.9	[38.5;67.1]	23	46.0	[31.8;60.7]	25	50.0	[35.5;64.5]	75	49.7	[41.4;57.9]
Injection site erythema	7	13.7	[5.7;26.3]	8	16.0	[7.2;29.1]	8	16.0	[7.2;29.1]	23	15.2	[9.9;22.0]
Injection site swelling	6	11.8	[4.4;23.9]	4	8.0	[2.2;19.2]	9	18.0	[8.6;31.4]	19	12.6	[7.7;19.0]
Injection site induration	12	23.5	[12.8;37.5]	10	20.0	[10.0;33.7]	13	26.0	[14.6;40.3]	35	23.2	[16.7;30.7]
Injection site ecchymosis	0	0.0	[0.0;7.0]	3	6.0	[1.3;16.5]	3	6.0	[1.3;16.5]	6	4.0	[1.5;8.4]

	30µg (N=50)			15µg (N=50)			7.5µg (N=49)			Ad- (N=149)		
Subjects with:	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**
Injection site pain	25	50.0	[35.5;64.5]	16	32.0	[19.5;46.7]	20	40.8	[27.0;55.8]	61	40.9	[33.0;49.3]
Injection site erythema	4	8.0	[2.2;19.2]	6	12.0	[4.5;24.3]	6	12.2	[4.6;24.8]	16	10.7	[6.3;16.9]
Injection site swelling	2	4.0	[0.5;13.7]	2	4.0	[0.5;13.7]	2	4.1	[0.5;14.0]	6	4.0	[1.5;8.6]
Injection site induration	7	14.0	[5.8;26.7]	5	10.0	[3.3;21.8]	2	4.1	[0.5;14.0]	14	9.4	[5.2;15.3]
Injection site ecchymosis	4	8.0	[2.2;19.2]	1	2.0	[0.1;10.6]	2	4.1	[0.5;14.0]	7	4.7	[1.9;9.4]

After the second dose rates for solicited injection site reactions were lower than seen after the first dose. Most were mild to moderate in intensity and only two subjects presented with at least one severe solicited injection site reaction.

Within 7 days after the first dose (see table 20) at least one solicited systemic reaction was reported by 42% to 66.7% (in the 30µg+Ad group) per group. The most frequently reported were headache, myalgia and malaise, all of which had the highest rates in the 30µg+Ad group. Shivering occurred most often (up to 8%) in the 30µg+Ad and 30µg groups.

Solicited systemic reactions appeared mostly within 3 days, lasted less than 3 days in all cases, were mainly mild or moderate in intensity and resolved spontaneously except for headache that needed medication to resolve. Three subjects experienced at least one severe event of headache, which occurred on D0, D3 or D7.

Table 20: Occurrence of each solicited systemic reaction within 7 days after first vaccine injection – Safety Analysis Set

	30µg+Ad (N=51)			15µg+Ad (N=50)			7.5µg+Ad (N=50)			Ad+ (N=151)		
Subjects with:	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**
Fever	0	0.0	[0.0;7.0]	1	2.0	[0.1;10.6]	2	4.0	[0.5;13.7]	3	2.0	[0.4;5.7]
Headache	29	56.9	[42.2;70.7]	14	28.0	[16.2;42.5]	18	36.0	[22.9;50.8]	61	40.4	[32.5;48.7]
Malaise	10	19.6	[9.8;33.1]	7	14.0	[5.8;26.7]	8	16.0	[7.2;29.1]	25	16.6	[11.0;23.5]
Myalgia	16	31.4	[19.1;45.9]	11	22.0	[11.5;36.0]	13	26.0	[14.6;40.3]	40	26.5	[19.6;34.3]
Shivering	4	7.8	[2.2;18.9]	3	6.0	[1.3;16.5]	2	4.0	[0.5;13.7]	9	6.0	[2.8;11.0]

	30µg (N=50)			15µg (N=50)			7.5µg (N=49)			Ad- (N=149)		
Subjects with:	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**
Fever	0	0.0	[0.0;7.1]	2	4.0	[0.5;13.7]	3	6.1	[1.3;16.9]	5	3.4	[1.1;7.7]
Headache	20	40.0	[26.4;54.8]	15	30.0	[17.9;44.6]	23	46.9	[32.5;61.7]	58	38.9	[31.1;47.2]
Malaise	9	18.0	[8.6;31.4]	3	6.0	[1.3;16.5]	5	10.2	[3.4;22.2]	17	11.4	[6.8;17.6]
Myalgia	14	28.0	[16.2;42.5]	7	14.0	[5.8;26.7]	11	22.4	[11.8;36.6]	32	21.5	[15.2;28.9]
Shivering	4	8.0	[2.2;19.2]	1	2.0	[0.1;10.6]	2	4.1	[0.5;14.0]	7	4.7	[1.9;9.4]

The incidence of subjects with at least one solicited systemic reaction occurring within 7 days after vaccination was lower after the second injection compared to the first. The same three events were most commonly reported but with maximal rates of 30.6% for headache, 20% for myalgia and 12% for malaise. One case of severe myalgia and one of severe headache were reported.

Unsolicited events were reported as follows:

Within 7 days

Overall, 35 subjects (11.7%) presented with at least one unsolicited reaction within 7 days after any vaccination. The occurrence of unsolicited reactions was similar in all groups (12.0% to 14.0%) except for a rate of 4% in the 7.5µg+Ad group.

Six subjects reported at least one unsolicited injection site reaction (one with 30µg+Ad, two with 15µg+Ad and three with 15µg). These reactions were mild in intensity resolved within 3 days.

Also 31 (10.3%) reported at least one unsolicited systemic reaction, which were mainly general disorders (asthenia and fatigue) and gastro-intestinal disorders (diarrhoea and abdominal pain). They were mild or moderate in intensity and mostly resolved within 3 days.

After the second vaccination the number of subjects with unsolicited reactions occurring within 7 days decreased in all groups except for 30µg and 7.5µg.

From day 8 to day 21

Most of the unsolicited AEs reported by six subjects from day 8 to day 21 after any vaccination were not considered to be related to vaccination. Most frequently reported were headache, pharyngolaryngeal pain, rhinitis and abdominal pain.

GPA02

Of the 600 vaccinated initially 42.2% reported at least one solicited injection site reaction and 43.5% had at least one solicited systemic reaction over the D0-D42 period. In both dose groups the rates of solicited injection site reactions after the first vaccination were lower in the cohort aged > 60 years compared to those aged 18-60 years. Generally solicited injection site reactions started between D0 and D3 and nearly all were mild in severity.

Table 21: Solicited injection site reactions within 7 days after the first vaccination

	[18-60 yrs]						>60 yrs					
	30µg+Ad (N=151)			7.5µg (N=149)			30µg+Ad (N=150)			7.5µg (N=150)		
Subjects with at least one:	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**
Injection site pain	74/151	49.0	[40.8 ; 57.3]	40/149	26.8	[19.9 ; 34.7]	26/150	17.3	[11.6 ; 24.4]	7/150	4.7	[1.9 ; 9.4]
Injection site erythema	21/151	13.9	[8.8 ; 20.5]	16/149	10.7	[6.3 ; 16.9]	7/150	4.7	[1.9 ; 9.4]	8/150	5.3	[2.3 ; 10.2]
Injection site swelling	17/151	11.3	[6.7 ; 17.4]	6/149	4.0	[1.5 ; 8.6]	1/150	0.7	[0.0 ; 3.7]	2/150	1.3	[0.2 ; 4.7]
Injection site induration	22/151	14.6	[9.4 ; 21.2]	12/149	8.1	[4.2 ; 13.6]	5/150	3.3	[1.1 ; 7.6]	7/150	4.7	[1.9 ; 9.4]
Injection site ecchymosis	7/151	4.6	[1.9 ; 9.3]	10/149	6.7	[3.3 ; 12.0]	2/150	1.3	[0.2 ; 4.7]	7/150	4.7	[1.9 ; 9.4]

In the younger age cohort the proportions of subjects with at least one solicited injection site reaction after the second dose were lower than after the first vaccination but in the group aged > 60 years the proportions were slightly higher than after the first vaccination.

Solicited Systemic Reactions after the first dose were reported less frequently by the older age cohort.

Table 22: Solicited systemic reactions within 7 days after the first vaccination

	[18-60 yrs]						>60 yrs					
	30µg+Ad (N=151)			7.5µg (N=149)			30µg+Ad (N=150)			7.5µg (N=150)		
Subjects with at least one:	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**
Fever	3/151	2.0	[0.4 ; 5.7]	1/149	0.7	[0.0 ; 3.7]	2/150	1.3	[0.2 ; 4.7]	3/150	2.0	[0.4 ; 5.7]
Headache	44/151	29.1	[22.0 ; 37.1]	44/149	29.5	[22.3 ; 37.5]	19/150	12.7	[7.8 ; 19.1]	23/150	15.3	[10.0 ; 22.1]
Malaise	18/151	11.9	[7.2 ; 18.2]	24/149	16.1	[10.6 ; 23.0]	13/150	8.7	[4.7 ; 14.4]	16/150	10.7	[6.2 ; 16.7]
Myalgia	26/151	17.2	[11.6 ; 24.2]	24/149	16.1	[10.6 ; 23.0]	15/150	10.0	[5.7 ; 16.0]	17/150	11.3	[6.7 ; 17.5]
Shivering	11/151	7.3	[3.7 ; 12.7]	9/149	6.0	[2.8 ; 11.2]	7/150	4.7	[1.9 ; 9.4]	6/150	4.0	[1.5 ; 8.5]

In the younger age cohort there were lower rates of solicited systemic reactions after the second dose compared to the first but total rates were similar after each dose in the older cohort.

After the first vaccination dose the rates for any unsolicited reaction were 11.9% and 12.8% among younger subjects compared to 4% and 5.3% in older subjects. Almost all were systemic reactions and few were severe in intensity. After the second vaccination rates for unsolicited reactions were lower than after the first dose in the younger cohort but there was no appreciable change in older subjects. Again most were systemic and few were severe reactions.

Within 7 days of administration of Emerflu containing A/Indonesia/5/05-RG2 to previously unvaccinated subjects in study GPA02 the most frequently reported solicited injection site reaction was injection site pain, occurring in 44% and 48% of subjects following the first and second vaccinations, respectively. The incidence of the remaining solicited injection site reactions was between 2% and 18%, and was generally similar following both vaccinations. The most frequently reported solicited systemic reaction was headache, occurring in 26.0% and 22.0% of subjects following the first and second vaccination, respectively.

Table 23*:* Unsolicited Adverse Events that Occurred within 21 days after Any Vaccine Injection by System Organ Class - SafAS Population

	18-60 yrs (N= 50)	
	n/M	%
Subjects with at least one unsolicited event	27/50	54.0
Gastrointestinal disorders	8/50	16.0
General disorders and administration site conditions	4/50	8.0
Infections and infestations	12/50	24.0
Injury, poisoning and procedural complications	1/50	2.0
Musculoskeletal and connective tissue disorders	2/50	4.0
Nervous system disorders	9/50	18.0
Psychiatric disorders	2/50	4.0
Respiratory, thoracic and mediastinal disorders	3/50	6.0
Skin and subcutaneous tissue disorders	2/50	4.0

M = Number of subjects in the SafAS having at least one safety record available for the specific endpoint; N = Number of subjects analyzed according to the SafAS Population definition; n = number of subjects

The applicant's comparison between subjects aged 18-40 in GPA01 and subjects aged 18-60 in GPA02 suggested that those aged 41-60 years in the latter study likely had lower reporting rates for solicited reactions than subjects aged 18-40 years. The rates of solicited local and systemic reactions demonstrated the differences already noted within GPA02 and also showed that rates for similar or slightly higher for each solicited symptom in GPA01 compared to other subsets.

Safety of booster doses

The adverse events occurring within 21 days after booster vaccination with adjuvanted or unadjuvanted vaccine are summarised in the next table for subjects who had previously received two doses of Emerflu. Note that the immunogenicity data derived from administration of unadjuvanted HA in study GPA01 at Month 12 were not reported.

Table 24: Safety data associated with a booster dose

	GPA01						GPA02					
Age group	18 to 40 years			18 to 40 years			18 to 60 years			>60 years		
Booster vaccination group	30µg+Ad (N=22)			7.5µg (N=21)			7.5µg (N=38)			7.5µg (N=41)		
Time of booster vaccination	12 months			12 months			6 months			6 months		
	n/N	%	95%CI	n/N	%	95%CI	n/N	%	95%CI	n/N	%	95%CI
Subjects with at least one:												
Solicited reaction	18/22	81.8	[59.7 ; 94.8]	14/21	66.7	[43.0 ; 85.4]	19/38	50.0	[33.4 ; 66.6]	8/41	19.5	[8.8 ; 34.9]
- Solicited injection site reaction	16/22	72.7	[49.8 ; 89.3]	11/21	52.4	[29.8 ; 74.3]	11/38	28.9	[15.4 ; 45.9]	4/41	9.8	[2.7 ; 23.1]
- Solicited systemic reaction	11/22	50.0	[28.2 ; 71.8]	8/21	38.1	[18.1 ; 61.6]	11/38	28.9	[15.4 ; 45.9]	5/41	12.2	[4.1 ; 26.2]
Unsolicited event	8/22	36.4	[17.2 ; 59.3]	4/21	19.0	[5.4 ; 41.9]	10/38	26.3	[13.4 ; 43.1]	7/41	17.1	[7.2 ; 32.1]
- Unsolicited reaction	4/22	18.2	[5.2 ; 40.3]	1/21	4.8	[0.1 ; 23.8]	5/38	13.2	[4.4 ; 28.1]	3/41	7.3	[1.5 ; 19.9]
- Unsolicited injection site reaction	0/22	0.0	[0.0 ; 15.4]	0/21	0.0	[0.0 ; 16.1]	0/38	0.0	[0.0 ; 9.3]	0/41	0.0	[0.0 ; 8.6]
- Unsolicited systemic reaction	4/22	18.2	[5.2 ; 40.3]	1/21	4.8	[0.1 ; 23.8]	5/38	13.2	[4.4 ; 28.1]	3/41	7.3	[1.5 ; 19.9]
- SAE	0/22	0.0	[0.0 ; 15.4]	0/21	0.0	[0.0 ; 16.1]	0/38	0.0	[0.0 ; 9.3]	0/41	0.0	[0.0 ; 8.6]
Death	0/22	0.0	[0.0 ; 15.4]	0/21	0.0	[0.0 ; 16.1]	0/38	0.0	[0.0 ; 9.3]	0/41	0.0	[0.0 ; 8.6]

In GPA01 the overall incidence of AEs was higher following a booster dose of Emerflu compared to a booster with 7.5µg HA at 12 months after the first dose.

In GPA02, the incidence of AEs was higher in subjects aged 18 to 60 years compared to those aged >60 years following a booster vaccination of 7.5µg HA at 6 months after the first dose.

In GPA01, the incidence of solicited injection site reactions was generally higher in subjects who received a booster dose of Emerflu compared to a booster dose of 7.5µg HA at 12 months after the first dose. For solicited systemic reactions the distribution was similar between the two booster groups except that malaise and shivering were more common in the Emerflu booster group.

In GPA02, the incidence of solicited injection site reactions was generally higher in subjects aged 18 to 60 years compared to those aged >60 years following a booster vaccination of 7.5µg HA at 6 months after the first dose. The incidence of solicited systemic reactions was slightly lower in the elderly group.

Table 25: Injection site and systemic reactions associated with a booster dose

	GPA01						GPA02					
Age group	18 to 40 years			18 to 40 years			18 to 60 years			>60 years		
Booster vaccination group	30µg+Ad (N=22)			7.5µg (N=21)			7.5µg (N=38)			7.5µg (N=41)		
Time of booster vaccination	12 months			12 months			6 months			6 months		
	n/N	%	95%CI	n/N	%	95%CI	n/N	%	95%CI	n/N	%	95%CI
Subjects with at least one:												
Injection site pain	14/22	63.6	[40.7 ; 82.8]	10/21	47.6	[25.7 ; 70.2]	11/38	28.9	[15.4 ; 45.9]	3/41	7.3	[1.5 ; 19.9]
Injection site erythema	8/22	36.4	[17.2 ; 59.3]	2/21	9.5	[1.2 ; 30.4]	5/38	13.2	[4.4 ; 28.1]	0/41	0.0	[0.0 ; 8.6]
Injection site swelling	2/22	9.1	[1.1 ; 29.2]	2/21	9.5	[1.2 ; 30.4]	1/38	2.6	[0.1 ; 13.8]	0/41	0.0	[0.0 ; 8.6]
Injection site induration	7/22	31.8	[13.9 ; 54.9]	4/21	19.0	[5.4 ; 41.9]	1/38	2.6	[0.1 ; 13.8]	0/41	0.0	[0.0 ; 8.6]
Injection site ecchymosis	2/22	9.1	[1.1 ; 29.2]	0/21	0.0	[0.0 ; 16.1]	0/38	0.0	[0.0 ; 9.3]	1/41	2.4	[0.1 ; 12.9]
Fever	1/22	4.5	[0.1 ; 22.8]	0/21	0.0	[0.0 ; 16.1]	1/38	2.6	[0.1 ; 13.8]	1/41	2.4	[0.1 ; 12.9]
Headache	6/22	27.3	[10.7 ; 50.2]	6/21	28.6	[11.3 ; 52.2]	7/38	18.4	[7.7 ; 34.3]	4/41	9.8	[2.7 ; 23.1]
Malaise	4/22	18.2	[5.2 ; 40.3]	2/21	9.5	[1.2 ; 30.4]	3/38	7.9	[1.7 ; 21.4]	1/41	2.4	[0.1 ; 12.9]
Myalgia	5/22	22.7	[7.8 ; 45.4]	5/21	23.8	[8.2 ; 47.2]	3/38	7.9	[1.7 ; 21.4]	2/41	4.9	[0.6 ; 16.5]
Shivering	3/22	13.6	[2.9 ; 34.9]	0/21	0.0	[0.0 ; 16.1]	2/38	5.3	[0.6 ; 17.7]	0/41	0.0	[0.0 ; 8.6]

Following administration of a dose of Emerflu containing Indonesia/5/05-RG2 [H5N1]) to subjects who had initially received Emerflu containing A/Vietnam the incidence of adverse events (solicited and unsolicited, systemic and injection site) was lower for subjects aged >60 years compared to those 18 to 60 years.

Within the first 7 days incidences of all solicited injection site reactions except injection site ecchymosis were higher for subjects aged 18 to 60 years compared to those aged >60 years. The most frequently reported solicited injection site reaction in both groups was injection site pain, which occurred in 61.0% and 28.1% of subjects aged 18 to 60 years and >60 years, respectively.

The solicited systemic reactions headache, malaise and myalgia occurred within the first 7 days more often in subjects aged 18 to 60 years compared to those >60 years. The most frequently reported systemic reaction was headache, which occurred in 30.5% of subjects aged 18 to 60 years.

Table 26: Unsolicited Adverse Events that Occurred within 21 days After the Booster Vaccine Injection by System Organ Class - SafAS Population

	18-60 yrs (N= 60)		>60 yrs (N= 58)		All (N= 118)	
	n/M	%	n/M	%	n/M	%
Subjects with at least one unsolicited event	29/59	49.2	15/57	26.3	44/116	37.9
Blood and lymphatic system disorders	1/59	1.7	0/57	0.0	1/116	0.9
Eye disorders	1/59	1.7	0/57	0.0	1/116	0.9
Gastrointestinal disorders	7/59	11.9	1/57	1.8	8/116	6.9
General disorders and administration site conditions	2/59	3.4	2/57	3.5	4/116	3.4
Immune system disorders	4/59	6.8	2/57	3.5	6/116	5.2
Infections and infestations	9/59	15.3	3/57	5.3	12/116	10.3
Injury, poisoning and procedural complications	1/59	1.7	1/57	1.8	2/116	1.7
Musculoskeletal and connective tissue disorders	6/59	10.2	3/57	5.3	9/116	7.8
Nervous system disorders	12/59	20.3	2/57	3.5	14/116	12.1
Respiratory, thoracic and mediastinal disorders	3/59	5.1	1/57	1.8	4/116	3.4
Vascular disorders	0/59	0.0	2/57	3.5	2/116	1.7

M = Number of subjects in the SafAS having at least one safety record available for the specific endpoint; N = Number of subjects analyzed according to the SafAS Population definition; n = number of subjects

Serious adverse event/deaths/other significant events

In GPA01 data submitted with the initial application revealed no deaths in the D0-D180 period.

There was one non-fatal SAE, consisting of an acute asthma attack 5 months after the second dose that was not considered to be related to vaccination.

In GPA02 data submitted with the initial application revealed no deaths in the D0-D42 period.

Six subjects (two in the 30µg+Ad and three in the 7.5µg group and one elderly in the 30µg+Ad group) reported six SAEs over the D0-D42 period. None of these SAEs was considered as related to the vaccine by either the Investigator or the Sponsor.

The SAE follow-up data 6-month Follow-Up to the Primary Series (Day 42 to Day 180) (All Subjects) revealed 22 SAEs occurred during the D42-D180 period. None of these SAEs were considered to be related to the study vaccinations by the Investigator or Sponsor.

Safety Follow-Up from Day 180 to Day 455 (Centres 1, 3, 4) in Centres 1, 3 and 4, with a booster being administered at 12 months showed 13 SAEs were reported between Day 180 and Day 455. These SAEs were considered to be not related to vaccination by both the Investigator and the Sponsor.

Other safety data

GPA11 involved 99 adult subjects in Australia who received two doses of Emerflu at D0 and D21. The safety data were consistent with those from the initial phases of GPA01 and GPA02 with similar vaccine. Virtually all reactions started within 3 days of vaccination and resolved spontaneously, mostly within 3 days, with no action being taken. Importantly, the incidence of reactions after vaccination was globally no higher after the second vaccination with respect to the first. The four SAEs reported were not considered to be related to vaccination. No new safety issues were observed.

GPA04 is an ongoing pediatric clinical study in which 180 Thai children have been exposed to 7.5 µg HA or 30 µg HA with adjuvant. An additional group of 60 subjects aged 6 months to 3 years received half doses of one of these formulations. Over the D0-D42 period, the nature, intensity and duration of the adverse events reported after the second vaccination did not appear to be different with respect to those observed after the first vaccination.

No immediate unsolicited events were reported for any subject and virtually all unsolicited events reported during the follow-up period were considered to be unrelated to vaccination. The proportion of subjects (all age groups combined) experiencing solicited reactions after any vaccination with 30µg+Ad was 82.2%. A similar proportion of subjects (80.0%) experienced solicited reactions after vaccination with the 7.5µg vaccine. This increase in reactogenicity with regard to GPA01 and GPA02 is likely linked to the differences in patients' age. Supporting this finding is the observation that a higher proportion of subjects aged 6 to 35 months experienced solicited reactions in comparison with the two older age groups; this increase was mainly related to a higher occurrence of fever in younger subjects. In subjects aged 6 to 35 months, the reactogenicity profiles of half doses (15µg+Ad and 3.75µg) were not different from those observed with full doses. Only one SAE was reported over the D0-D42 period of the trial and this was considered to be unrelated to vaccination.

Table 27: Safety Summary of Adverse Events Occurring within 21 Days after Any Vaccine Injection - SafAS Population (30µgHA+aluminum hydroxide)

	30µg+Ad								
	9 to 17 years (N=30)			3 to 8 years (N=30)			6 to 35 months (N=30)		
	n/N	%	95% CI	n/N	%	95% CI	n/N	%	95% CI
Subjects with at least one:									
- Immediate unsolicited event	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)
- Immediate unsolicited reaction	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)
- Solicited reaction	21/30	70.0	(50.6; 85.3)	26/30	86.7	(69.3; 96.2)	27/30	90.0	(73.5; 97.9)
- Solicited injection site reaction	19/30	63.3	(43.9; 80.1)	24/30	80.0	(61.4; 92.3)	20/30	66.7	(47.2; 82.7)
- Solicited systemic reaction	12/30	40.0	(22.7; 59.4)	18/30	60.0	(40.6; 77.3)	24/30	80.0	(61.4; 92.3)
- Unsolicited event	7/30	23.3	(9.9; 42.3)	17/30	56.7	(37.4; 74.5)	26/30	86.7	(69.3; 96.2)
- Unsolicited reaction	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)
- Unsolicited injection site reaction	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)
- Unsolicited systemic reaction	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)
- Serious Adverse Event	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)
Death	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)	0/30	0.0	(0.0; 11.6)

N = Number of subjects analyzed according to the SafAS Population definition; n = number of subjects; Ad = aluminum hydroxide adjuvant CI = confidence interval

Discussion on clinical safety

For a vaccine intended only for intra-pandemic use it is acceptable that the limited number of subjects recruited to date does not allow the detection of rare and very rare adverse reactions.

In GPA02 the 30 µg + Ad formulation was clearly associated with a higher rate of local reactions than the 7.5 µg dose without adjuvant in persons aged 18-60 years. The former was also associated with a higher rate of pain in subjects aged > 60 years but all reporting rates were lower in the older cohort compared to younger subjects. In the younger age cohort the proportions of subjects with at least one solicited injection site reaction after the second dose were lower than after the first vaccination but in the group aged > 60 years the proportions were slightly higher than after the first vaccination.

In contrast rates for solicited systemic reactions were generally similar between the two formulations within the two age groups although lower reporting rates were again seen in the older cohort. Headache, myalgia and malaise were the most commonly reported systemic AEs. Rates for unsolicited symptoms were similar between vaccine groups and between age groups.

Data regarding the 6 months follow-up, including a list of all SAEs revealed no safety issue up to month 6 post vaccinations (GPA01 and GPA02).

The MAH provided booster (A/Indonesia Strain) data of 118 subjects who had been primary immunized 22 months ago with the A/Vietnam strain of Influenza A virus subtype H5N1 and additional primary immunization data from 50 healthy subjects who had received two doses of Emerflu containing the A/Indonesia strain (GPA02). The safety data from the 50 additional subjects who received the clade 2 30µg HA+aluminium hydroxide vaccine formulation in GPA02 was consistent with the earlier findings. The booster vaccination of the FLU H5N1 30µgHA+aluminum

hydroxide (FR) vaccine using the A/Indonesia/5/05-RG2 (H5N1) strain was well tolerated by subjects aged 18 to 60 years and by those aged >60 years (N=118). Overall, the safety profile was similar to that of the primary series.

The supportive trials GPA04 and GPA11 did not reveal any new safety problem.

2.5. Pharmacovigilance

Detailed description of the Pharmacovigilance system

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

Risk Management Plan

The MAA submitted a risk management plan, which included a risk minimisation plan

The CHMP, having considered the data submitted in the application was of the opinion that it was not appropriate to consider risk minimisation activities at this time.

2.6. Overall conclusions, risk/benefit assessment and recommendation

Quality

During the procedure major objections were identified.

The major objections precluding a recommendation of marketing authorisation pertained to the following principal quality deficiencies:

-Insufficient validation of the virus splitting and inactivation processes

-Inadequate determination of the HA content and identity in the filled product

-The potential influence of the increased adsorption of the antigen to the adjuvant over time on the safety and immunogenicity of the vaccine

Although the manufacturing process for Emerflu is based on the company's seasonal influenza vaccine, Major Objections regarding the validation of virus splitting and inactivation of the H5N1 strain have been identified and addressed. Virus splitting and inactivation are key steps in production and could have a major impact on public health, therefore additional validation data have been provided.

In addition, the lack of HA determination for final product means that batch to batch consistency is in question and the stability of the product has not been comprehensively demonstrated. The Applicant provided additional data on the validation of the SRD and HPLC method used for HA determination as well as additional stability data.

To summarise, the applicant at the time of the responses of the second list of outstanding issues, was able to satisfactorily answer the quality questions.

Non-clinical pharmacology and toxicology

The non-clinical pharmacological investigation programme for EMERFLU focuses mainly on immunogenicity and protective efficacy, which is in line with available regulatory guidances. The immunogenicity of pandemic influenza vaccine was assessed in animal studies using 4 species including mice, rabbits, monkeys and ferrets. Protection against homologous viral challenge (10^6 TCID50, intratracheally) was shown in challenge studies in monkeys and in two independent ferret models: mortality and disease model, and an infection model.

Together, the data from these two studies demonstrate protection against mortality in all ferrets that had been vaccinated with the FLU H5N1 30µgHA+aluminum hydroxide vaccine.

Based on the results from non-clinical safety studies conducted with the vaccine at the dose level of 30 µg HA adjuvanted with aluminium hydroxide (600 µg Al), no major safety concerns were raised and treatment related effects appeared to be limited to only local reactions at the site of injection.

Efficacy

Data covered immunological responses in adults and elderly persons at 21 days following two doses of Emerflu containing either the A/Vietnam (studies GPA 01, GPA 02, GPA 11) or A/Indonesia (study GPA 02) strain of Influenza A virus subtype H5N1. In addition, there were data on immune responses to a single booster dose of Emerflu containing A/Vietnam (GPA01) or supportive data from A/Indonesia (GPA 02) in subjects who had received two doses of Emerflu containing A/Vietnam 6 or 22 months previously in respective studies. Additional data on administration of two doses of Emerflu containing A/Indonesia to subjects aged 18-60 years and containing A/Vietnam to subjects aged < 18 years were also provided.

All the data assessed pointed to a conclusion that ≤ 40% of adult or elderly individuals vaccinated with two doses of Emerflu will develop HI titres of at least 1:40, which is the cut-off for assessing seroprotection according to current CHMP criteria.

Whereas a third dose of vaccine containing A/Vietnam in subjects who had received pilot batch vaccine initially elicited higher seroprotection rates (less than 80%) the administration of unadjuvanted 7.5 µg HA vaccine to those primed with 30 + Al in GPA02 resulted in no appreciable increase in seroprotection rate and virtually no increment in GMT. The additional data on administration of a third dose of Emerflu containing A/Indonesia to subjects that had previously received two doses of Emerflu containing A/Vietnam showed that the maximum seroprotection rate achieved to either strain was 42% in younger subjects, with rates <30% in older subjects.

As for the pandemic influenza vaccines already licensed in the EU under exceptional circumstances it is impossible to predict the protective efficacy that might be conferred by Emerflu in a pandemic situation. Nevertheless, both the HI and the NA data presented by the applicant indicate that this vaccine construct is of low immunogenicity.

Safety

Not unexpectedly, the adjuvanted split virus vaccine was associated with a higher incidence of local and systemic reactions compared to non-adjuvanted vaccine. However, the frequency and severity of adverse events was not thought to be unacceptable. No additional safety issues were identified during longer-term follow-up or in association with the booster doses, for which the safety profile was similar to that of the primary series. The ongoing study GPA04 in children has not revealed any new safety problem in association with administration of two doses 21 days apart.

User consultation

Not applicable

Risk-benefit assessment:

Clinical context

It is not known which strain (in terms of H and N type) will trigger the next human influenza pandemic. Emerflu is a mock-up influenza vaccine, whose scientific development is based on the guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application (CPMP/VEG/4717/03) and the guideline on submission of marketing authorisation applications for pandemic influenza vaccines through the centralised procedure (CPMP/VEG/4986/03).

Benefits

The real benefit of Emerflu could only be assessed during a pandemic and following insertion of an appropriate final pandemic strain into the vaccine. At present the potential benefit can only be evaluated based on detailed characterisation of immunological responses to vaccination.

Both the HI and the NA data presented by the applicant indicate that this vaccine construct is of low immunogenicity. This raises concern regarding the likely protective efficacy of the vaccine in a pandemic situation.

Risks

In study GPA01 in adults between 18 and 40 years of age Emerflu did not meet the minimum CHMP requirements set for pandemic influenza vaccines.

In study GPA02 and GPA 11, the vaccine used was pre-mixed as intended for commercialisation and was even less immunogenic in a comparable age group compared to the experimental formulation used in Study GPA01.

Subjects primed with two doses of Emerflu were revaccinated with 7.5 µg HA antigen without adjuvant six months later. The unadjuvanted HA dose elicited no appreciable immune response and few individual subjects showed any evidence of an anamnestic response. In addition, a third dose of commercial scale vaccine may restore but not improve upon the post-primary antibody levels.

Primary immunisation data from an additional arm of study GPA02 in which subjects received two doses of Emerflu containing the A/Indonesia strain confirmed the low immunogenicity of the two dose vaccination schedule, especially in elderly persons.

Overall there is concern that a significant proportion of subjects would not be protected against pandemic influenza following receipt of two doses of Emerflu.

Balance

The risk/benefit balance for Emerflu is negative.

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 Emerflu in the prophylaxis of influenza in an officially declared pandemic situation in individuals from 18 years of age and older, in accordance with offical guidance, was unfavourable and therefore did not recommend the granting of the marketing authorisation under exceptional circumstances.

Grounds for refusal

Whereas

- At present the potential benefit of a Pandemic Influenza Vaccine can only be evaluated based on detailed characterisation of immunological responses to vaccination. Emerflu elicited seroprotection rates after primary immunisation of previously seronegative adults (18-60 years and > 60 years) that fell short of the CHMP criterion. In adults aged < 60 years the seroprotection rate after two doses of manufacturing scale vaccine was less than 40%.

- Similar results were obtained after two doses of Emerflu containing either the Influenza A virus subtype H5N1 A/Vietnam or with the supportive set of data on the A/Indonesia strains, which demonstrated reproducibly low immunogenicity

- Contradictory results were obtained from different studies in which a dose of Emerflu containing A/Vietnam or the supportive data set on A/Indonesia or a dose of unadjuvanted haemagglutinin derived from A/Vietnam was administered to subjects who had previously received two doses of Emerflu containing A/Vietnam. These findings raised concerns regarding the ability of Emerflu to prime the immune system

- The safety and immunogenicity data presented did not support a conclusion that the benefit-risk balance for use of Emerflu during an Influenza Pandemic would be positive.

Therefore, the CHMP has recommended the refusal of the granting of the Marketing Authorisation for Emerflu.