National Institute of Pharmacy

Public Assessment Report

FLUVAL P SUSPENSION FOR INJECTION

Applicant: Omninvest Kft. (Hungary)
Vaccine Manufacturing, Researching and Trading Ltd

Date: November 2009

TABLE OF CONTENTS

ADMINISTRATIVE INFORMATION

Invented name of the medicinal product	**Fluval P suspension for injection**
INN (or common name) of the active substances	H1N1 whole virus, inactivated, adjuvated, containing antigen equivalent to: A/California/7/2009(H1N1)-like NYMC X-179/A reassorted strain: 6 µg per 0.5 ml dose Suspension of virus propagated in eggs, purified, concentrated, formaldehyde-inactivated and adsorbed on aluminium phosphate gel
Marketing Authorisation Holder	Omninvest Kft Address: H-2097 Pilisborosjenő, Fő út 7. Hungary
Indication	Prophylaxis of influenza in an officially declared pandemic situation. The vaccine provides protection against influenza virus strains with identical or similar antigen structure as that of the prototype strain used in the vaccine. Pandemic influenza vaccine should be used in accordance with official guidance.
ATC Code	J07BB02
Pharmaceutical form and strength	Suspension for injection (0.5 ml)

1. Introduction

The influenza viruses constitute a genus within the family of orthomyxoviruses that are able to infect a wide range of species. The primary site of infection and of viral replication is in the respiratory epithelium. The segmented RNA genome of influenza viruses encodes two major surface antigens – haemagglutinin (H) and neuraminidase (N). Haemagglutinin facilitates viral attachment to respiratory epithelia and neuraminidase appears to cleave the bond between the viral haemagglutinin and the host cell receptor, so facilitating the release of virions from infected cells and allowing spread to uninfected cells in the vicinity.

Both H and N antigens of influenza A and B viruses may undergo minor antigenic changes (antigenic drift) over time. Antigenic drift necessitates regular (most often annual) updating of the A and B strains that are included in inter-pandemic (seasonal) influenza vaccines. Major changes (antigenic shift) in haemagglutinins occur much less commonly and only in type A strains but are of great importance to human health. Antigenic shifts may occur *via* serial mutations or by gene re-assortment. The H1N1v strain responsible for the current pandemic is a triple reassortant containing genes variably derived from avian, swine and human adapted strains.

Influenza virus infection elicits host production of antibody against several viral components but the major antigens are the haemagglutinin and neuraminidase molecules. Antibody to haemagglutinin (anti-HA) is thought to play a major role in immunity and is effective in neutralising virus infectivity. The role of anti-neuraminidase antibody in protection is less clear and this is rarely measured except for specific experimental reasons. Serum neutralising antibody is functional but is not routinely measured because these assays are labour intensive and require use of cell cultures.

An influenza pandemic occurs when a type A influenza strain to which a higher proportion of population lacks immunity emerges.

During the years of 2005-2006, a pandemic caused by an A(H5N1) strain (the “bird flu”) was expected but it did not reach the level of a pandemic situation.

In April 2009, however, a new human influenza strain A(H1N1)v was isolated then characterised. On 11 June the World Health Organization declared Phase 6 (the most serious situation) of the influenza pandemic.

The attack rate and the seriousness of the consequences for the A(H1N1)v virus strain has been expected to be higher than for the annual (“seasonal”) strains of influenza. Because of the low levels of pre-existing immunity in the population, also the speed of the attack could be considerably high.

In order to answer this challenge, specific guidance has been developed describing fast track assessment and authorisation procedures of the pandemic flu vaccines in the European Union. It comprises, among others, submission and assessment of a core pandemic influenza vaccine dossier during the pre-pandemic period. It is based on a so called “mock-up” influenza vaccine.

The mock-up vaccine, as described in EU guidelines, should contain subtypes of influenza A to which the majority of the population is naïve. The both the immunogenicity and the safety

profile may be predicted to be similar other such subtypes of influenza A, including the later pandemic strain.

When the pandemic situation arrives, a fast track assessment of a new dossier is possible, based solely on the replacement of the virus antigens from the strain used in the manufacture of the mock-up vaccine with antigens from the identified pandemic strain. All other characteristics including the manufacturing technology remain the same. Administratively, this is not a new application but a *variation* of the marketing authorisation of the mock-up vaccine.

2. Background information on the procedure

The route of the approval was a combination of using certain data of a “mock-up” vaccine and a strain modification of an existing A(H5N1) pre-pandemic flu vaccine as marketing authorisation variation.

A temporary marketing authorisation for a pre-pandemic mock-up vaccine, Fluval H5N1 monovalent influenza vaccine (active ingredient: Influenza A/Viet Nam/1194/2004 (H5N1)-like NIBRG-14 reassortant type (inactivated)) was granted on 14 March 2006. Although this vaccine was developed as a mock-up for the expected pandemic flu vaccine, it was also suitable for the preventive treatment of H5N1 influenza infection. The temporary authorisation, with the condition that the vaccine could only be used in a pandemic situation, was granted on the basis of Article 7 of the *25th Act of 2005 on human medicines and modifications of other Acts relating the medicinal market* (a provision drawn from the Article 5(2) of the *Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human us*e). This temporary authorisation, in response to the suspected spread of 'bird flu, could be granted for one year and renewed only once for another one year.

This renewal was granted on 23 February 2007.

The national authorisation was granted 'temporarily' for the above virus had been a genetically manipulated one, thus, 'usual' marketing authorisation could only be issued by the Commission via the 'centralised marketing authorisation route' in the European Union.

Approaching the end of the one-year renewal, in order to maintain the national authorisation of the mock-up vaccine, a strain modification application was submitted in 2007 to Fluval H5N1 monovalent influenza vaccine under the new brand name of Fluval H5N1suspension injection, also for the preventive treatment of H5N1 influenza infection. The active ingredient of this vaccine was a new strain: the A/Swan/Nagybaracska/01/2006(H5N1)-like A/PR8/34/(H1N1) reassortant monovalent bulk. This application was approved on 28 September, 2007.

Fluval H5N1 suspension injection was produced in the same way as Fluval H5N1 monovalent influenza vaccine. Furthermore, the antigen content, adjuvant system and route of administration of Fluval H5N1 suspension injection were the same as for Fluval H5N1 monovalent influenza vaccine.

In order to maintain this national H5N1 vaccine marketing authorisation, its second brand application under the brand name Fluval P suspension injection was submitted to Fluval H5N1 suspension injection and approved on 24 July 2009.

To this ’second brand’ Fluval P suspension injection a pandemic strain modification application has been submitted under the same brand name, Fluval P suspension for injection (active ingredient: A/California/7/2009(H1N1)-like NYMC X-179/A reassorted strain) containing quality, nonclinical and clinical data that are new and relevant for the pandemic strain.

Dates of approvals of the influenza A(H1N1) vaccine:

- accepting the H5N1 to H1N1 strain variation and issuing marketing authorisation of the

A(H1N1) flu vaccine (indicated for adults above 18 years) 28 September 2009;

- its first variation (alternative manufacturing site for filling): 6 October 2009;
- its second variation (extension of the indication to children between 3-12 years and adolescents between 12-18 years): 15 October 2009;
- its third variation (extension of the indication to children between 12-36 months) 2 November 2009.

3. Scientific discussion

3.1 Quality assurance aspects

The site of the vaccine manufacturer, Omninvest Kft. has valid manufacturing authorisation covering vaccine manufacture, been subjected to regular inspections and found Good Manufacturing Practice (GMP) compliant. Its last NIP (National Institute of Pharmacy) GMP inspection was performed in March 2009.

The alternative manufacturing site (filling and packaging) Pharmamagist Kft. has a valid manufacturing authorisation. In spite of that, it was been inspected before accepting it and during the first filling of the Fluval P vaccine in autumn, 2009.

All relevant non-clinical (toxicological) studies were performed in test facilities, listed in the national GLP plan in the years when the tests were carried out and subjected to regular Good Laboratory Practice (GLP) inspections by the NIP.

All clinical trial sites were subjected to NIP Good Clinical Practice (GCP) inspections during clinical trials with the Fluval P suspension for inspection.

3.2 Quality aspects

Active Substance

In this application the monovalent bulk of the specified inactivated virus strain can be considered as active substance.

In the present case the virus strain, which was recommended by WHO and EMEA, is A/California/7/2009 NYMC X 179/A (H1N1). The Primary seed is originated from the Centre for Disease Control and Prevention (CDC, Atlanta, USA).

The nucleotide sequence of the Influenza A virus strain is published on the website of NCBI Influenza Virus Sequence Database/GenBank FJ 966974.1.

The manufacturing process for the monovalent bulks has been developed by the applicant's experience gained in production of its seasonal vaccines Fluval AB and the mock-up vaccine Fluval H5N1 which have been licensed in Hungary.

The steps of manufacturing process of A(H1N1) Monovalent bulk were the same as the interpandemic (seasonal) Monovalent bulk produced by Omninvest Ltd. for many years as well as the mock-up H5N1 vaccine.

The production process for monovalent bulks was adequately described. Data of PCR and SDS-PAGE methods confirmed that HA and NA genome segments as well as the specific protein components of the strain did not change during the process, therefore those in the Master seed, Working seed and Monovalent bulk were the same as those of the primary seed of A/ California/7/2009 NYMC X 179/A (H1N1).

The following technological steps were optimised and validated:

- transportation of embrionated hen's eggs,
- pre-incubation II of the embrionated hen's eggs,
- production of allantoic fluid (inoculation of diluted working seed, incubation, cooling, harvesting of allantoic fluid).
- viral inactivation procedure,
- biological purification (adsorption, elution),
- production of Monovalent bulk (dialysis, centrifugation, dilution with thiomersal and formaldehyde containing PBS buffer solution).

Control of starting materials (virus seed lots, eggs and raw materials) was acceptable. Quality specifications and test methods were presented and discussed in details. The materials were purchased from approved suppliers, who certified their products in respect of production technology, physico-chemical, chemical, microbiological and virological properties. SPF eggs and embrionated eggs from healthy flock as well as most of the other raw materials comply with Ph. Eur. requirements. The remaining non-compendial materials had appropriate in-house specifications. Moreover, relevant information concerning the viral safety of the product was also presented.

Extensive control of technological parameters during the whole manufacturing process was introduced. In the course of manufacture of batches all critical parameters were recorded in computer and were continuously observed and evaluated.

In the course of manufacturing process of Monovalent bulk the following intermediates were judged critical: Master seed, Working seed, inoculum, allantoic fluid harvested, allantoic fluid inactivated, supernatant fluid with adsorbed viruses, eluent, supernatant fluid of dialyzed product and Monovalent bulk suspension. Requirements for the in-process control (IPC) and test methods were provided.

The viral inactivation procedure was properly validated on three batches of the A/California/7/2009 NYMC X 179/A (H1N1) monovalent bulk. Samples were taken from the allantoic fluid during inactivation by formaldehyde at the beginning of the process and every second hour until hour 12. Virus titres of each sample were determined by haemagglutinin titration. The results showed a complete inactivation in about 8 hours. For this reason the duration of the procedure was set to "not less than 12 hours".

Tests for inactivation of potential adventitious viruses (both enveloped and non-enveloped) and mycoplasma in the H1N1 influenza vaccine by the formaldehyde inactivation were also performed using various model viruses and mycoplasmas.

Potential impurities and contaminants of Monovalent bulk originated from the manufacturing procedures could be as follows: microbiological contamination, ovalbumin, free formaldehyde and other pathogen viruses as well as residues of antibiotics used. The quality specification developed for Monovalent bulk introduced limits for these impurities and all batches of Monovalent bulk were tested by properly validated methods.

Residual antibiotics were tested during the final testing of the drug product.

In addition to the mentioned tests for relevant potential impurities, the specification of the monovalent bulk (the Drug Substance) included tests for appearance, sterility, for residual

infectious viruses, abnormal toxicity, HA identity and content (determination of HA titre and content by SRD), protein content, neuraminidase identity, ovalbumin and free formaldehyde content.

The test methods of sterility, effectiveness of inactivation, general safety, haemagglutinin content by SRD method and protein content corresponded to those officials in Ph. Eur. monographs.

The other testing methods were developed in-house. They are either well-known in the international analytical bibliography or based on the Ph. Eur. general method descriptions.

The A(H1N1) Monovalent bulk was filled into sterile colourless glass bottles of 1, 2 or 5 Litre volume, produced from neutral borosilicate glass having high hydrolytic resistance and closed by sterile polypropylene screw cap. Both packaging items complied with official requirements (Ph. Eur., USP and Commission Directive 2002/12/EU).

The proposed shelf life of A(H1N1)Monovalent bulk has been 12 months based on data of the mock-up and previous seasonal flu vaccines of Omninvest Ltd.

To complete and substantiate of the shelf-life testing, 3 commercial batches have been put on stability study according to the stability protocol submitted. Its aim has been to prove that the quality of the A(H1N1) Monovalent bulk stored at 5±3°C does not change considerably during storage up to 36 months. According to the Applicant's commitment, the one year stability data will already be provided to the NIP.

Medicinal Product

FLUVAL P suspension for injection containing A(H1N1) Monovalent bulk corresponding to 6 µg of haemagglutinin is a colourless, slightly opalescent suspension for intramuscular administration. A volume of 0.5 ml suspension is filled into colourless ampoules, neutral glass (high hydrolytic resistance Type I) ampoule of 1 ml with breaking-point and sealed by fusion of glass.

Active ingredient
Monovalent bulk of the specified inactivated virus strain can be considered as drug substance of FLUVAL P suspension for injection. The candidate virus nominated by the WHO was A/California/7/2009 (H1N1), classically reassorted to NYMC X 179/A. The reassortant virus originated from CDC was used for development of the new pandemic vaccine, Fluval P.

Adjuvant
Aluminium phosphate, produced during the manufacturing process from trisodium phosphate 12 H_2O and aluminium chloride hexahydrate under validated conditions. It is hydrophilic gel, which helps to reach the optimal efficacy of influenza vaccines.

Other excipients
Potassium dihydrogen phosphate
Disodium phosphate dehydrate
Sodium chloride
Potassium chloride

Thiomersal
Water for injection

The excipients used in the composition of pandemic FLUVAL P suspension for injection were the same as used in composition of inter-pandemic (seasonal) Fluval AB vaccine regularly as well as in the H5N1 mock-up vaccine. They are well-known, generally used in vaccines, regarded safe for human use in the applied concentration, and had important function to ensure and maintain the quality, stability and efficacy of the product. They are official in European Pharmacopoeia except the trisodium phosphate dodecahydrate, which is qualified by an in-house specification.

Thiomersal is an effective antimicrobial preservative, which has still been used in several vaccines.

The vaccine adjuvanted this way, administered intramuscularly forms depot, from which the haemagglutinin dissolves gradually and uniformly giving higher immunogenic efficacy.

The manufacturing process of pandemic FLUVAL P suspension for injection was the same as applied for producing the seasonal Fluval AB vaccines and the pre-pandemic H5N1 vaccine. The product was prepared according to the rules of *controlled aseptic technology*. The technological steps chosen were well-known and usual in the manufacture of a medicinal product of this type. Manufacturing steps included formation of the monovalent virus pool with suitable titre; preparation of the aluminium-phosphate gel containing the suitable amount of the antimicrobial preservative, preparation of the adjuvanted final bulk and the filling procedure.

Prospective validation was accomplished with the first three commercial batches.

All parameters and (IPC) results were collected during the whole manufacturing process from master seed to final lot.

Equipments and process parameters have already been used for many years in the manufacture of the mock-up vaccine and the seasonal Fluval AB vaccines and the appropriate IPC and quality control (QC) results confirmed their suitability as all the three final lots (including 1 – 7 sub-batches) met all requirements of the specification.

The Final bulk was tested for sterility, bacterial endotoxin, thiomersal content, pH, abnormal toxicity and efficacy. Requirements for the final bulk were in accordance with the relevant monograph of European Pharmacopoeia and recommendations of the World Health Organization. The following test were included in the specification: appearance, volume, pH, total protein, ovalbumin content, free formaldehyde content, haemagglutinin content, abnormal toxicity, haemagglutinin adsorption to aluminium gel, aluminium content, thiomersal content, antibiotic content, bacteriological endotoxin and sterility.

The test methods for sterility, bacterial endotoxin, general safety, pH value, haemagglutinin content by SRD method and free formaldehyde content corresponded to those officials in Ph. Eur. monographs. The remaining test methods (haemagglutinin identification, immunological effectiveness, aluminium and thiomersal content, haemagglutinin binding to aluminium gel and antibiotics content by HPLC method) were developed in-house. They were well-known and usual in the international analytical bibliography or based on the Ph. Eur. general method descriptions.

The ovalbumin content, the HA content (SRD) and protein content were determined only in the Monovalent bulk and were then calculated to the final lot.

All analytical methods including bacterial endotoxins by LAL test as well as the aluminium and thiomersal contents were validated or verified according to the relevant ICH guidelines.

FLUVAL P suspension for injection was filled into glass ampoules, the usual container for single-dose injections. The ampoule chosen is colourless glass ampoule of 1 ml volume with breaking-point, sealed by fusion of glass. The ampoules were produced from neutral glass of high hydrolytic resistance Type I. The quality specifications and routine test methods met the relevant official specifications (Ph. Eur., USP, DIN and ISO). The analytical results and quality certificates of the ampoules were provided in the file

The proposed shelf life of the product was 12 months when stored at 2°C – 8°C protected from light. It can not be frozen.

As the shelf life of the product was proposed on the basis of the stability studies performed with the mock-up FLUVAL H5N1 suspension for injection and the seasonal FLUVAL AB vaccines, it was accepted as a temporary value. In order to verify it, a stability protocol was submitted. According to the Applicant's commitment three commercial batches would be put on stability study and after its completion the stability report provided to the NIP. Interim analysis after one-year storage would also be submitted.

3.3 Non-clinical aspects

All the three vaccines indicated below (for their relationship see *Chapter.2 Background information on the procedure*) underwent similar non-clinical testing, as follows:

- Fluval H5N1 monovalent influenza vaccine (Influenza A/Viet Nam/1194/2004 (H5N1)-like NIBRG-14 reassortant type (inactivated)), the mock-up vaccine,
- Fluval H5N1 suspension injection (Influenza A/Swan/Nagybaracska/01/2006(H5N1)-like A/PR8/34/(H1N1)), the strain variation of the mock-up vaccine and
- Fluval P suspension injection (active ingredient: A/California/7/2009(H1N1)-like NYMC X-179/A reassorted strain).

Pharmacology

Primary pharmacodynamics

The following studies were performed:

- dose finding (guinea pigs and mice),
- single dose immunogenicity (guinea pigs),
- repeated dose immunogenicity (guinea pigs),
- the influence of aluminium phosphate gel on the efficiency (guinea pigs).

The pharmacological tests were designed and performed in line with the "Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines (CPMP/SWP/465/95) and the "Guideline on Dossier Structure and Content for Pandemic Influenza Vaccine Marketing Authorization Application" (CPMP/VEG/4717/03).

No animal experiments for effectiveness in protection against a challenge from the pathogenic organism of the vaccine (e.g. in ferrets) were carried out because of the identity of the manufacturing steps with seasonal influenza A vaccines. Their effectiveness was intended to be proved by human clinical studies.

The presented results demonstrated that the vaccine was able to achieve specific antibody titre elevation in the injected animals. The nonclinical sections of the Summary of Product Characteristics (SPC) were also acceptable.

Secondary pharmacodynamics

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

Safety pharmacology and *Pharmacodynamic drug interactions*

No such studies were performed. Grounds for non-providing these and other new data were justified adequately.

Pharmacokinetics

Experimental studies to demonstrate absorption, distribution, metabolism and excretion of the active ingredients of the above vaccines were not performed, in accordance with the relevant guidelines CPMP/SWP/465/95 and CPMP/VEG/4717/03.

Toxicology

The following toxicological studies were performed:
- single-dose toxicity
- local tolerance testing
- local tolerance testing – repeat-dose.

The tests were carried out according to the GLP requirements. The site of investigation (Bela Johan National Epidemiologic Center) had the appropriate GLP certificate.

Single-dose toxicity

Fluval H5N1 monovalent influenza vaccine: it was administered im. to 48 guinea-pigs (12 animals per sample) in the dose range of 3.5; 6.5; 12; 20 µgHA/0.5 ml and ip. to 48 guinea pigs (12 animals per sample) also in the above dosage range. Physiological saline was administered to 6 controls. Even the highest dose was free of toxic signs during the observation period (7 days for im. and 21 days for ip. administration). No local or systemic signs of toxicity were observed. No lethal outcome was reported after 20 µgHA/0.5 ml im. and after the ten times higher dose 20 µgHA/5 ml Fluval H5N1 monovalent vaccine ip. administration. No clinically important signs were observed during the observation period and the body weight increased in a normal manner like that of the control group.

Fluval H5N1 suspension injection: the study has been carried out according to CPMP/VEG/4717/03 principles. Six batches of the vaccine were administered intramuscularly to 14 guinea-pigs (2 per sample) or 20 mice (5 per sample) in the dose range of 3.5, 6, 15 µgHA/0.5 ml. Even the highest dose was free of toxic signs during the observation period. No local or systemic sign of toxicity was observed. No lethal outcome was observed after 15 µgHA/0.5 ml intraperitoneal administration. No clinically important signs were observed during the observation period and the body weight increased in a normal manner like that of the control group.

Fluval P suspension injection:

a) the vaccine was administered ip. to 12 guinea-pigs in the dose of 10 or 17 µgHA/0.5 ml (6 animals in each group). These doses were free of toxic signs during the observation period (7 days for ip. administration). No local or systemic signs of toxicity were reported. No lethal outcome was observed after 10 or 17 µgHA/0.5 ml ip. administration. No clinically important signs were observed during the observation period and the body weight increased in a normal manner like that of the control group. In the experiments mice and guinea pigs were used.
b) co-injection with FLUVAL AB suspension for injection (the „seasonal flu vaccine): Fluval P vaccine and Fluval AB seasonal vaccine were simultaneously co-administered ip. to 18 guinea-pigs (6 animals per sample) in the dose of 17 µgHA/0.5 ml (Fluval P) and 15 µg/virus strain/0.5 ml (Fluval AB). These doses and posology were free of toxic signs during the observation period (7 days for ip. administration). No local or systemic signs of toxicity were described. No lethal outcome was observed after 17 µgHA/0.5 ml (Fluval P) + 15 µg/virus strain/0.5 ml (Fluval AB) ip. administration. No clinically important signs were observed during the observation period and the body weight increased in a normal manner like that of the control group. In the experiments mice and guinea-pigs were used.

Local tolerance testing

Fluval H5N1 monovalent influenza vaccine: 48 mice (12 animals per sample) were injected intramuscularly with Fluval H5N1 monovalent vaccine in a dose range of 3.5; 6.5; 12; 20 µgHA/0.5 ml. Physiological saline was administered to 6 controls. The results of the local tolerance tests show that Fluval H5N1 monovalent vaccine in the investigated dose range (3.5; 6.5; 12; 20 µgHA/0.5 ml) was well tolerated by the animals following single-dose intramuscular vaccination. No local alterations were observed on the surface of the skin of the animals during the observation period. No animal died during the period of observation. No clinical toxic symptoms were observed. The weight of the experimental animals did not decrease in the observation period.

Fluval H5N1 suspension injection: 16 guinea pigs (8 per sample) were injected intramuscularly with the vaccine in a dose range of 6; 15 µgHA/0.5 ml. Six animals were used as control. The results of the local tolerance tests show that the vaccine in the investigated dose range was well tolerated by the animals following single-dose intramuscular vaccination. No local alterations were observed on the surface of the skin of the animals during the observation period. No animal died during the observation period. No clinical toxic symptoms/signs were observed. The weight of animals did not decrease in the observation period.

Fluval P suspension injection:

a) Guinea-pigs were injected im. with Fluval P suspension for injection in a dose range of 10 or 17 µgHA/0.5 ml. The results of the local tolerance tests show that Fluval P suspension for injection in the investigated doses (10; 17 µgHA/0.5 ml) was well tolerated by the animals following single-dose intramuscular vaccination. No local alterations were observed on the surface of the skin of the animals during the observation period. No animal died during the period of observation. No clinical toxic symptoms were observed. The weight of the experimental animals did not decrease in the observation period;
b) co-injection with FLUVAL AB suspension for injection (the „seasonal flu vaccine): the Fluval P suspension for injection and Fluval AB suspension for injection was simultaneously co-administered im. to guinea-pigs in the dose of 17 µgHA/0.5 ml (Fluval P) and 15 µg/virus strain/0,5 ml (Fluval AB). The results of the local tolerance tests show that Fluval P suspension for injection and Fluval AB suspension for injection co-administration in the investigated doses (17 µgHA/0.5 ml Fluval P, 15 µgHA/virus strain 0,5 ml Fluval AB) was well tolerated by the animals following intramuscular vaccination. No local alterations were observed on the surface of the skin of the animals during the observation period. No animal died during the period of observation. No clinical toxic symptoms were observed. The weight of the experimental animals did not decrease in the observation period.

Local tolerance testing repeated dose

Fluval H5N1 monovalent influenza vaccine (co-injection with FLUVAL AB suspension for injection, the „seasonal flu vaccine): the Fluval H5N1 monovalent and Fluval AB trivalent vaccines were administered im. to guinea-pigs (10 animals in each group) twice. Physiological saline was administered to 5 controls. The first dose was given at the 0 day and the second on the 21st day of experiment. The dose for an administration session was 12 µg HA of H5N1 monovalent vaccine and 3x15 µg HA of Fluval AB trivalent vaccine. No local and no systemic signs of toxicity were described. No lethal outcome was reported during the observation period.

Fluval H5N1 suspension injection: the study has been carried out according to CPMP/VEG/4717/03 principles. Two batches of the vaccine were administered intramuscularly to 16 guinea-pigs twice. Six animals served as control. The first dose was given at the 0 day and the second on the 21st day of the study. The dose for an administration session was 6 µg HA and 15 µg HA/0.5 ml. No local or systemic signs of toxicity were observed. No lethal outcome was recorded during the observation period.

Genotoxicity

The vaccines were not tested. No indication of genotoxicity was evident.

Carcinogenicity

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

Reproductive and Developmental Toxicity

Not performed. Grounds for not providing these data were justified.

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.

Overall conclusion of the non-clinical results

The presented results demonstrated that the vaccines were safe in animals (guinea pigs and mice).

Since the mock up (Fluval H5N1 monovalent influenza vaccine) and final pandemic vaccine (Fluval P suspension for injection) are similar except than in strain-antigen content and the non-clinical characteristics are also similarly favourable, there are no objections to approval of Fluval P suspension injection from a non-clinical point of view.

3.3 Clinical aspects

Introduction

The H5N1 avian influenza strain was considered as a possible candidate to cause the next influenza pandemic. There fore, its dossier was used as a mock-up vaccine dossier. However, the marketing authorisation holder decided to perform those clinical trials that are, as a rule, necessary for strain modification variation, moreover, to test the vaccines at least a limited number of children.

Pharmacokinetics

Not applicable according to the note for guidance on clinical evaluation of new vaccines (CPMP/EWP/463/97) and the Guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application (CPMP/VEG/4717/03)..

Pharmacodynamics

For vaccines, the pharmacodynamic assessment means that of the immune responses (see Clinical efficacy below)

Clinical efficacy and safety

Like their non-clinical testing, all the three vaccines indicated below (for their relationship see *Chapter.2 Background information on the procedure*) underwent some clinical trials, as follows:

- Fluval H5N1 monovalent influenza vaccine (Influenza A/Viet Nam/1194/2004 (H5N1)-like NIBRG-14 reassortant type (inactivated)), the mock-up vaccine,
- Fluval H5N1 suspension injection (Influenza A/Swan/Nagybaracska/01/2006(H5N1)-like A/PR8/34/(H1N1)), the strain variation of the mock-up vaccine and
- Fluval P suspension injection (active ingredient: A/California/7/2009(H1N1)-like NYMC X-179/A reassorted strain).

Individual clinical trials

Fluval H5N1 monovalent influenza vaccine

- *Dose ranging study on the safety and immunogenicity of 3.5, 6 and 12 µgHA/0.5 ml Fluval H5N1 monovalent mock-up vaccine in two age groups of healthy adults*

The clinical part of the study was performed according to the study protocol. No amendment was submitted. The data analysis was conducted according to the analysis plan written in the protocol. Administration of the Fluval H5N1 influenza vaccine was well tolerated by the volunteers. As far as efficacy (immunogenicity) was concerned, in Group 1 (2x3.5 µg HA/dose) all three CPMP criteria were fulfilled 21-28 days after the final (second) vaccination. In Group 2 (1x3.5 µg HA/dose) one out of the three CPMP criteria was fulfilled in the age group 18-60 years, and two in the age group over 60 years. (If one criterion is fulfilled out of the three, the vaccine complies with the immunogenicity requirements.) In Group 3 (1x6 µg HA/dose) and Group 4 (1x12 µg HA/dose) all criteria were fulfilled in both age groups. HI and MN antibody titres were lower at day 80-90 after the final vaccination, but the results met CPMP criteria on GMT increase in all groups and age groups. In conclusion, the adequate dose seems to be 6 µg, as immune responses seen were similar in patients receiving 2x3.5, 1x6 and 1x12 µg. Thus, doses exceeding 6 µg or two injections seemed to be unnecessary.

Summary charts on efficacy results 21-28 days after final vaccination:

Age group 18-60 years					
Immunogenicity criteria	Criteria	Results 21-28 days after final vaccination			
		Group 1	Group 2	Group 3	Group 4
Seroconversion	> 40 %	72.4 (+)	37.7	71.7 (+)	70.5 (+)
Increase in GMT	> 2.5	19.8 (+)	11.1 (+)	21.1 (+)	22.8 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	72.4 (+)	37.7	71.7 (+)	70.5 (+)
Age group >60 years					
Immunogenicity criteria	Criteria	Results 21-28 days after final vaccination			
		Group 1	Group 2	Group 3	Group 4
Seroconversion	> 30 %	60.7 (+)	35.2 (+)	64.8 (+)	66.7 (+)
Increase in GMT	> 2.0	14.5 (+)	10.1 (+)	14.8 (+)	15.2 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 60 %	60.7 (+)	35.2	64.8 (+)	66.7 (+)

(+) Met CPMP criteria

Safety evaluation was based on monitoring of adverse events (AEs) and clinically significant changes in physical status and vital signs.

Safety parameters were:
- local reactions: pain at injection site, erythema, swelling, induration, ecchymosis;
- systemic reactions: fever, headache, malaise, myalgia, shivering;
- clinically significant changes in physical status and vital signs: skin, mucous membranes, BP, heart rate, lungs, abdomen, liver, extremities, neurology.

The vaccine proved to be safe; no clinically significant changes in the physical condition or the vital signs of the volunteers were observed. Side effects were rare and mild, no vaccine-

related Serious Adverse Event was observed.

- *Safety and immunogenicity of NIBRG-14 H5N1 01-2005 mock up vaccine (identical to Fluval H5N1 monovalent influenza vaccine) in adults*

The clinical part of the study was performed according to the study protocol. No amendment was submitted. The data analysis was conducted according to the analysis plan written in the protocol. As far as the primary objective was concerned (to assess the efficacy of the vaccine in humans at Day 21-28 after immunization) volunteers in both age groups met efficacy criteria on seroconversion and increase in GMT. The third possible efficacy criterion, i.e. the post-vaccination titres of >1:40 failed to reach 70% and 60%, respectively, in the age groups. As far as secondary objectives were concerned (to assess the duration of protective immunogenicity in humans at day 80-100 and at day 170-180 after immunization) volunteers in age group 18-60 years met efficacy criteria on increase in GMT and seroconversion at day 80-100, met that on increase in GMT and practically met that on seroconversion at day 170-180. Volunteers in age group over 60 years met all the three efficacy criteria both at day 80-100 and day 170-180, but results in this age group were not considered representative due to limited number of subjects.

Overall summary chart on immunogenicity criteria and results in the total study population:

Total study population				
Immunogenicity criteria	Criteria	Results		
		Day 21-28	Day 80-100	Day 170-180
No. of participants	N.A.	146	101	91
Seroconversion	> 40 %	63.7 % (+)	67.3 % (+)	41.8 % (+)
Increase in GMT	> 2.5	5.58 (+)	5.87 (+)	3.85 (+)
Seropositivity (post-vaccination titres $\geq$ 1:40)	> 70 %	63.7 % (-)	67.3 % (-)	41.8 % (-)

(+) Met CHMP standards.

Administration of the vaccine was well tolerated by all volunteers. The vaccine proved to be safe; no clinically significant changes in the physical condition or the vital signs of the volunteers were observed. No Severe Adverse Event was observed, no subject showed systemic adverse events.

- *Safety and immunogenicity of Fluval H5N1 monovalent vaccine in children and adolescents*

The clinical part of the study was performed according to the study protocol. No amendment was submitted. The data analysis was conducted according to the analysis plan written in the protocol. Regarding efficacy, due to the low number of cases, no statistical significance was calculated. However, adolescents displayed higher HI and MN titres than children.

Mean HI titres before and 21-28 days after vaccination

Population	Before vaccination		After vaccination		Increase in GMT
	GMT	95% CI	GMT	95% CI	
Total	2	2.0 – 2.0	33.9	15.8 – 72.6	17.0
Children	2	2.0 – 2.0	18.0	3.8 - 85.0	9.0
Adolescents	2	2.0 – 2.0	64.0	64.0 – 64.0	32.0

Number of seroconversion and individuals reaching titres ≥40 (and, as exploratory assessment, ≥32) on Day 21-28

Population	Seroconversion		Titre ≥40		Titre ≥32	
	Nr.	%	Nr.	%	Nr.	%
Total	9	75	9	75	9	75
Children	3	50	3	50	3	50
Adolescents	6	100	6	100	6	100

Mean MN titres before and 21-28 days after vaccination

Population	Before vaccination		After vaccination		Increase in GMT
	GMT	95% CI	GMT	95% CI	
Total	2	2.0 – 2.0	9.0	3.8 – 21.2	4.5
Children	2	2.0 – 2.0	4.0	1.8 – 8.9	2.0
Adolescents	2	2.0 – 2.0	20.2	4.8 – 84.3	10.1

Mean HI titres before and 80-90 days after vaccination

Population	Before vaccination		After vaccination		Increase in GMT
	GMT	95% CI	GMT	95% CI	
Total	2	2.0 – 2.0	18.0	12.4 – 25.9	9.0
Children	2	2.0 - 2.0	12.7	7.0 – 23.0	6.3
Adolescents	2	2.0 - 2.0	25.4	17.4 – 37.0	12.7

Number of seroconversion and individuals reaching titres ≥40 (and, as exploratory assessment, ≥32) on Day 80-90

Population	Seroconversion		Titre ≥40		Titre ≥32	
	Nr.	%	Nr.	%	Nr.	%
Total	5	42	0	0	5	42
Children	1	17	0	0	1	17
Adolescents	4	67	0	0	4	67

Mean MN titres before and 80-90 days after vaccination

Population	Before vaccination		After vaccination		Increase in GMT
	GMT	95% CI	GMT	95% CI	
Total	2	0.9 – 3.1	4.2	2.3 – 7.8	2.1
Children	2	0.4 – 3.6	2.5	1.4 – 4.6	1.3
Adolescents	2	0.4 – 3.6	7.1	2.4 – 20.8	3.6

Administration of the vaccine was well tolerated by the children and adolescents. The vaccine proved to be safe; no clinically significant changes in the physical condition or the vital signs of the participants were observed. No Severe Adverse Event was observed, no subject showed systemic adverse events.

Fluval H5N1 suspension injection

- *Tolerability and indicative immunogenicity study of 6 µg HA Fluval Avian monovalent flu vaccine in healthy adults*

Summary:
The clinical part of the study has been performed according to the study protocol. No amendment was submitted. The data analysis was conducted according to the analysis plan written in the protocol. Administration of Fluval Avian monovalent influenza vaccine (identical to the Fluval H5N1 suspension injection) was very well tolerated by all volunteers. The vaccine proved to be safe; no clinically significant changes in the physical condition or the vital signs of the volunteers were observed. No Severe Adverse Event was observed, no subject showed systemic adverse reactions. As far as immunogenicity was concerned 21-28 days after immunization, the study population as total met all three CHMP efficacy criteria.

Details:
The primary objective of this open, uncontrolled, one centre trial was to assess tolerability, and, as secondary objective, immunogenicity of FLUVAL AVIAN monovalent flu vaccine (= Fluval H5N1 suspension injection) in healthy adults.
The secondary objectives included
- the assessment of immunogenicity of the investigational medicinal product in humans by serology testing of blood taken at Day 21-28 after immunization;
- the assessment of the long term immunogenicity of the investigational medicinal product in humans by serology testing of blood taken at Day 170-180 after vaccination.

Enrolment of up to twenty (20) healthy volunteers of age over 18 years meeting the predetermined inclusion and exclusion criteria was permitted in the study to ensure a study population of at least fifteen (n = 15) evaluable cases. After screening, seventeen (n = 17) healthy volunteers were involved in the study and were vaccinated. The data of all seventeen (n = 17) individuals were available and evaluated at Day 21-28. The data of sixteen (n = 16) individuals were available and evaluated at Day 170-180.

After physical examination and blood sampling, 0.5 ml of the vaccine was injected at one side into the deltoid muscle with a deep intramuscular injection. Vaccinated individuals were

observed for 6-12 hours after the injection. Blood samples from the cubital vein to test for specific antibodies against H5N1 flu virus by serology testing were taken at Day 0 (prior vaccination), and at Day 21-28 and Day 170-180 after vaccination.

Tolerability evaluation was based on monitoring of adverse reactions and clinically significant changes in physical status and vital signs. Tolerability parameters included:
- local reactions: pain at injection site, induration, redness, swelling, warmth;
- systemic reactions: headache, malaise, myalgia, shivering, fever;
- clinically significant changes in physical status and vital signs: skin, mucous membranes, BP, heart rate, lungs, abdomen, liver, extremities, neurology.

Frequency, mean time of appearance and duration of all adverse reactions were calculated by simple descriptive statistics.

Tolerability was analyzed in all ITT patients vaccinated.

Serum antibody titres were measured by the following tests:
a) Haemagglutination inhibition (HI): 1U HA = virus quantity / 0.05 ml causing agglutination of 0.05 ml 0.5% chicken erythrocyte suspension. Virus haemagglutination inhibitions were assayed in Takátsy microtitre plates.
b) Virus neutralization assay (MN): titre inhibiting virus infection of 2×10^3 PFU. This neutralization test for influenza viruses is based on the inhibition of cytopathogenic effect (CPE)-formation in Madin-Darby Canine Kidney (MDCK) cell cultures. In this assay it is tested if serum neutralizing antibodies to influenza virus HA inhibit the infections of MDCK cells with 100 x TCID50% / 50 µl virus. Neuraminidase inhibition (NI) and cell-mediated immunity by Granzyme B assay were also assessed as secondary immunological variables.

The primary efficacy variables were the change of HI and MN titres in time. The secondary efficacy variables were the change of NI and Granzyme B protein. HI, MN and NI antibody titres, as well as quantity of Granzyme B protein (biomol units/mg) were determined at baseline, at Day 21-28, and Day 170-180.
HI titres were used to calculate seroconversion rates, increase in GMT, and seroprotection rates. Since at present there is no defined seroprotective titre level (and/or requirement) related to MN and NI, assessment of immunogenicity related to MN and NI included description of changes in MN and NI titres and increase in GMT.

In respect of cellular immunity response, increase of Granzyme B protein was assessed.

Immunogenicity related to HI was assessed according to the criteria of the EMEA concerning potential pandemic flu vaccine (EMEA/CHMP/VWP/263499/2006). According to that an effective vaccine should meet all three criteria as defined in guideline CPMP/BWP/214/96:
(i) seroconversion, i.e.: a ≥4-fold titre increase reaching a titre of ≥32;
(ii) mean geometric increase (GMT), i.e.: increase in geometric mean of postvaccination serum anti-HA antibody titres to that of prevaccination serum anti-HA antibody titres, and
(iii) seroprotection, i.e.: achievement of an HI titer ≥1:40. (In the course of antibody titrations a serial dilution of 1:4, 1:8, 1:16, 1:32, 1:64 ... was used. Since the calculation for percentage of population reaching postvaccination titre ≥40 used values of 64 or higher, the results show a downward bias. Therefore an explanatory assessment for the calcula-

tion for percentage of population reaching postvaccination titre ≥32 was also performed.)
For the purposes of calculation, any HI result <8 (=undetectable) was expressed as 4.
According to the CPMP/BWP/214/96 guideline the following requirements have to be met in subjects to <60 years old:
(i) seroconversion rate should be > 40%,
(ii) increase in GMT should be > 2.5-fold, and
(iii) seroprotection rate should be > 70%;
and the following requirements have to be met in subjects to >60 years old:
(i) seroconversion rate should be > 30%,
(ii) increase in GMT should be > 2.0-fold, and
(iii) seroprotection rate should be > 60%.

With respect to the limited number of participants, and, especially, to that of participants over 60 years, the participants were grouped into only one age group. Therefore in this study the more rigorous set of requirements (i.e. that concerns participants < 60 years old) has been applied without respect to the age of the subjects. *Statistical analysis* on immunogenicity results was carried out using the data of all participants completing the study (PP population).

Safety results:
Administration of the vaccine at 6 μg HA/dose was well tolerated by all participants of the study. The influenza vaccine proved to be safe; no clinically significant changes in the physical condition or vital signs of the volunteers were observed. No Severe Adverse Event was observed. No subject showed systemic adverse reaction. Only one (1) subject out of the vaccinated 17 ones (5.9 %) had local reaction: this patient felt pain at the injection site after vaccination. This AE was classified as mild. The relationship of this AE to the study drug was evaluated as probable. No medical intervention was neccessary, the subject recovered in 24 hours without sequel. The AE has not endangered the volunteer's safety. No other local reaction (induration, redness, swelling, warmth) was observed.

Immunogenicity results:
About half of the participants displayed measurable levels of H5N1 HI or MN antibodies before immunization, what can be explained by former vaccination with FLUVAL H5N1 influenza vaccine containing A/Viet Nam/1194/2004(H5N1)-like NIBRG-14 reassortant virus strain. (The intervals between such former H5N1 vaccination and the present one were ranging from 9 months up to 2 years.) The geometric mean HI titres at Day 0 (before immunization) were 1:12.88 in the whole population. HI titres of ≥1:40 were detected in case of 3, those of ≥1:32 were detected in case of 5 subjects (18.75% and 31.25% respectively). The geometric mean of MN titres at Day 0 (before immunization) was 1:7.03 in the whole population.
The geometric mean of HI titres 21-28 days after immunization was 1:53.82 in the whole population (a 4.18-fold increase in GMT).

Seroconversion 21-28 days after immunization occurred in 62.5% of the total population.
HI titres of ≥1:40 were obtained in 75.0%, those of ≥1:32 were obtained in 87.5% of the subjects.
The geometric mean of HI titres 170-180 days after immunization was 1:10.83 in the whole population (a 0.84-fold increase in GMT).

Seroconversion 170-180 days after immunization occurred in 6.3% of the total population.
HI titres of ≥1:40 were obtained in 0.0%, those of ≥1:32 were obtained in 25.0% of the sub-

jects.
The geometric mean of MN titres 21-28 days after immunization was 1:53.82 in the whole population (a 7.66-fold increase in GMT). Significant (i.e. at least fourfold) increase in MN titres 21-28 days after immunization occurred in 62.5% of the total population.

The geometric mean of MN titres 170-180 days after immunization was 1:11.31 in the whole population (a 1.61-fold increase in GMT). Significant (i.e. at least fourfold) increase in MN titres 170-180 days after immunization occurred in 25.0% of the total population.

As far as HI is concerned 21-28 days after immunization, volunteers met, according to the requirements set out in 4.16.2., *all three* efficacy criteria (seroconversion rate, increase in GMT, seroprotection rate).
Further to this, Day 0 immunogenicity results show that former Fluval H5N1 vaccinations containing A/Viet Nam/1194/2004(H5N1)-like NIBRG-14 reassortant virus generated cross-immunity against A/Swan/Nagybaracska/01/2006 (H5N1)-like virus.

Overall summary chart on immunogenicity criteria and results in the total PP trial population 21-28 days after immunization

Total PP study population			
Immunogenicity	Criteria	Results (Day 21-28)	
		HI	MN
No. of participants (PP)	N.A.	16	16
Seroconversion	> 40 %	62.5 % (+)	62.5 %
Increase in GMT	> 2.5	4.18 (+)	7.66
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	75.0 % (+)	N/A

(+) Met CHMP standards

Fluval P suspension injection

- *Tolerability and immunogenicity study of Fluval P monovalent influenza vaccine (identical to Fluval P suspension injection) in adults and elderly people – an interim report*

The aim of this randomized, single-blind, uncontrolled, interventional, prevention study was to determine the tolerability and immunogenicity of FLUVAL P suspension injection in adults and elderly people, with the objective to verify efficacy and tolerability of the study drug.

The primary objective included
- the assessment of tolerability/safety (incidence of adverse events) of the study drug,
- the assessment of efficacy (immunogenicity) of the study drug by serology testing of blood samples taken at Day 21-28 after immunization in groups and age groups.

The secondary objectives included
- the assessment of the long-term safety of the study drug 50-60 days after immunization,

- the determination of tolerability of simultaneous administration of Fluval P monovalent pandemic influenza vaccine (=Fluval P suspension injection) and Fluval AB trivalent seasonal influenza vaccine in case of adults and elderly people,
- the assessment of efficacy of the study drug by optional epidemiological follow-up of the participants until the end of the influenza season,
- the assessment of immunogenicity of the study drug by optional cross-reactive immunity tests performed with non-homologous influenza A and B virus strains.

A total of 355 subjects (203 adult subjects aged 18-60 years of age and 152 elderly subjects aged over 60 years of age) meeting the predetermined inclusion and exclusion criteria were enrolled in the study. The subjects were randomly assigned in a 1:1 ratio to one of the following vaccine groups:

Group 1:

103 adults (of 18-60 years of age), and 75 elderly volunteers (over 60 years of age) from both sexes were enrolled. Treatment: Vaccination with Fluval P at 6 µg HA/0.5 ml active ingredient content and aluminium phosphate gel adjuvant (dose: 0.5 ml /total 6 µg HA/ in both age groups, single dose). The Fluval P vaccine was injected into the deltoid muscle of the left arm.

Group 2:

100 adults (of 18-60 years of age) and 77 elderly volunteers (over 60 years of age) from both sexes. *Treatment:* Vaccination with Fluval P at 6 µg HA/0.5 ml active ingredient content and aluminium phosphate gel adjuvant (dose: 0.5 ml /total 6 µg HA/ in both age groups, single dose) AND with Fluval AB trivalent influenza vaccine with 15 µg HA/0.5ml/strain active ingredient content and aluminium phosphate gel adjuvant (dose: 0.5 ml /total 3x15 µg HA/ in both age groups, single dose).

178 subjects in Group 1 received Fluval P influenza vaccine once, and 177 subjects in Group 2 received Fluval P AND Fluval AB influenza vaccines simultaneously once.

During the study no further study medication or concomitant medication as cause of adverse drug reaction was administered so far.

The Fluval P vaccine was injected into the deltoid muscle of the left arm, the Fluval AB vaccine was injected into the deltoid muscle of the right arm.

Out of the 355 vaccinated subjects 352 attended the visit at Day 21-28 after vaccination. Three subjects failed to attend the control visit. Subject VE-111 stayed abroad during the concerned period, while subjects PV-130 and PV-132 could not manage travelling to the study site. All the three subjects contacted their investigators, and reported no medical complaints. These three subjects were included in the tolerability analysis, but were excluded from the immunogenicity one.

Statistical analysis: safety and tolerability have been analysed in all ITT patients vaccinated. Immunogenicity has been analysed in all subjects completing the control visit at Day 21-28 after vaccination. In the course of tolerability/safety assessment frequency, severity, mean time of appearance and duration of all local and systemic AEs have been calculated in all groups by simple descriptive statistics according to CPMP/BWP/214/96: "Note for Guidance on Harmonization of Requirements for Influenza Vaccines", 12 March 1997, Para. 2.4., 2.6., and 3.2.

The *first* interim tolerability/safety assessment was performed after follow-up calls at Day 7 following the vaccination. The *second* interim tolerability/safety assessment has been performed after visits at Day 21-28 following the vaccination. *Final* assessment of tolerability and safety will be performed after visits at Day 50-60 following the vaccination.

Serum antibody titres were measured by haemagglutination inhibition (HI) test at baseline and at Day 21-28 after vaccination. Haemagglutination inhibition (HI): 1U HA = virus quantity/0.05 ml causing agglutination of 0.05 ml 0.5% erythrocyte suspension. Virus haemagglutination inhibition was assayed in Takátsy micro titer plates. A 1:2 serial dilution of sera was performed in the micro wells. 0.025 ml (8 U) antigen was added and after vigorous shaking, plates were allowed to stay at room temperature for 30 min. Subsequently, 0.05 ml 0.5% chicken red blood cells were added, and after 20 min. incubation at room temperature, plates were analyzed. Antibody titrations were done in duplicate; pre- and post-vaccination sera were titrated simultaneously. The titre assigned to each sample was the geometric mean of two independent determinations. HI titres were used to calculate seroconversion rates, seroprotection rates, and increase in geometric mean titres (GMTs).

In the course of immunological assessment fulfilment of the following efficacy variables are considered in groups and age groups according to CPMP/BWP/214/96: "Note for Guidance on Harmonization of Requirements for Influenza Vaccines", 12 March 1997, Para. 3.1.:
(i) number of seroconversions or significant (i.e. $\geq$4-fold) increase in HI antibody titre;
(ii) mean geometric increase; and
(iii) the proportion of subjects achieving an HI titre $\geq$40.

An interim tolerability and immunogenicity assessments at Day 21-28 after vaccination including at least 50 volunteers per group and age group provide sufficient data to meet the requirements as set out in guidelines concerning licensure of influenza vaccines in case of modification of the strain composition of influenza vaccines.

The investigators monitored the occurrence of adverse events during the course of the study. The investigators instructed the patients prior to the initiation of the administration of study drug to record and report any physical changes or new symptoms they notice during the course of the study.

Adverse events and serious adverse events were monitored by telephone follow-up on Days 1, 2, 3 and 7 after vaccination. During the visit at Day 21-28 the investigators cross-checked and reconfirmed the AEs reported by the telephone follow-up, and recorded new symptoms the subjects noticed in the meantime. The adverse events were then classified by the investigators on the basis of the following guidelines:

Grade 1: mild: Transient or mild discomfort; no limitation in activity; no medical intervention/therapy required.

Grade 2: moderate: Mild to moderate limitation in activity; some assistance may be needed; no or minimal medical intervention/therapy required.

Grade 3: severe: Marked limitation in activity; some assistance usually required; medical intervention/therapy required, hospitalization possible.

Grade (life threatening): extreme limitation in activity; significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable.

An adverse event was considered a serious adverse event (SAE) if it:

a) was fatal;
b) was life-threatening (i.e., results in an immediate risk of death);
c) was persistently or significantly disabling/incapacitating (i.e., severe or permanent disruption of one's ability to carry out normal life functions or daily activities);
d) resulted in, or prolonged hospitalization;
e) is a congenital anomaly/birth defect;
f) is an event which, in the opinion of the Investigator/Medical Monitor, may jeopardize the patient or may require medical or surgical intervention to prevent one of the outcomes listed in this section.

The efficacy data were assessed in all groups and age groups.

Immunogenicity related to HI was assessed according to guideline CPMP/BWP/214/96:
- In case of adult subject aged 18 to 60 years:
  (i) number of seroconversions or significant (i.e. ≥4-fold) increase in HI antibody titre should be >40 %;
  (ii) mean geometric increase should be >2.5; and
  (iii) the proportion of subjects achieving an HI titre ≥40 should be >70 %.
- In case of adult subject aged over 60:
  (i) number of seroconversions or significant (i.e. ≥4-fold) increase in HI antibody titre should be >30 %;
  (ii) mean geometric increase should be >2.0; and
  (iii) the proportion of subjects achieving an HI titre ≥40 should be >60 %.

Safety results:
A total of 73 possibly or probably related Adverse Events were reported by 49 subjects (13.8%). These adverse events were all mild and similar as observed in connection with common flu vaccinations.
Since the telephone follow-up at Day 7 after vaccination no new possibly or probably related Adverse Event was reported.

Thirty-seven (37) not related Adverse Events were reported by 34 subjects (9.58%).

In *Group 1* twenty-nine (29) possibly or probably related Adverse Events were reported by 18 subjects (10.11%). All of these adverse reactions were mild.
In *Group 2* number of possibly or probably related Adverse Events (both local and systemic ones) were higher, but remained still low and well tolerable: Forty-four possibly or probably related Adverse Events were reported by 31 subjects (17.5%). All of these adverse reactions were mild.

The vaccine related adverse events appeared mostly on the day after vaccination, and disappeared mostly by the second or third day after vaccination. By Day 7 after vaccination practically all vaccine related adverse events disappeared without sequel. Most frequent vaccine related adverse event in both groups was mild pain at injection site (eight cases /4.49%/ in Group 1, and 18 cases /10.17%/ in Group 2.). Most frequent systemic reaction was fatigue for 1-2 days after vaccination (three cases /1.69%/ in Group 1, and 5 cases /2.82%/ in Group 2.). In Group 2 there were no significant difference between local reactions on Fluval P influenza vaccine and Fluval AB influenza vaccine. Out of the 18 cases of pain at injection site 3 cases were related to Fluval P vaccine only, 3 cases were related to Fluval AB vaccine only, and 12 cases were related to both vaccines. There was no death or Serious Adverse

Events during this study so far.

Efficacy results:

Primary objective of the study was to assess the efficacy of the study drugs in humans by serology testing of blood taken at Day 21-28 after immunization. In this respect changes in HI titres were considered as primary efficacy parameter. The mean (95% CI) GMT titres and changes from baseline are shown in the table below.

Geometric mean of HI titres (with 95% CI lower and upper limit) before and after immunization

Group 1 *(vaccination with Fluval P vaccine)*

Antigen	Population	Day 0	Day 21-28	Change in GMT (Day 21-28)
H1N1 swl.	18-60 years	5.0 (5.0 - 5.0)	45.3 (38.3 - 53.5)	9.1 (7.7 – 10.7)
	>60 years	5.0 (5.0 - 5.0)	31.5 (26.0 – 38.1)	6.3 (5.2 – 7.6)
	Males	5.0 (5.0 - 5.0)	40.3 (33.7 - 48.3)	8.1 (6.7 – 9.7)
	Females	5.0 (5.0 - 5.0)	37.4 (31.2 - 44.9)	7.5 (6.2 – 9.0)
H1N1	18-60 years	16.6 (14.2 - 19.4)	19.2 (17.1 – 21.6)	1.2 (1.1 – 1.2)
	>60 years	18.6 (16.2 - 21.4)	21.5 (19.9 - 23.4)	1.2 (1.1 – 1.3)
	Males	16.5 (14.1 - 19.2)	19.5 (17.6 – 21.7)	1.2 (1.1 – 1.3)
	Females	18.3 (15.8 - 21.3)	20.8 (18.6 – 23.1)	1.1 (1.1 – 1.2)
H3N2	18-60 years	18.6 (16.1 - 21.4)	20.6 (18.5 – 22.9)	1.1 (1.1 – 1.2)
	>60 years	17.7 (15.4 - 20.4)	21.0 (19.0 - 23.1)	1.2 (1.1 – 1.3
	Males	16.1 (13.7 - 18.8)	19.0 (17.0 – 21.3)	1.2 (1.1 – 1.3)
	Females	20.3 (17.9 – 23.0)	22.3 (20.3 - 24.7)	1.1 (1.0 – 1.2)
B	18-60 years	25.4 (23.1 - 28.0)	25.6 (23.3 - 28.1)	1.0 (1.0 – 1.0)
	>60 years	21.1 (18.8 - 23.8)	22.1 (20.1 - 24.4)	1.1 (1.0 – 1.1)
	Males	21.2 (18.9 - 23.9)	22.1 (20.1 - 24.4)	1.0 (1.0 – 1.1)
	Females	25.7 (23.3 - 28.3)	25.9 (23.6 - 28.4)	1.0 (1.0 – 1.0)

Geometric mean of HI titres to A/H1N1/09 swl. antigen significantly increased 21-28 days after immunization in Group 1 in both sexes and age groups (change in GMT titres ranges between 6.3 and 9.1). Geometric mean of HI titres to seasonal H1N1, H3N2, and B antigens

practically remained unchanged in *Group 1*.

The percentage of seropositive (= post-vaccination titres of ≥1:40) individuals and seroconversion are shown in the table below.

*Seropositivity (%) and seroconversion (%) (with 95% CI lower limit and upper limit) after immunization Group **1** (vaccination with Fluval P vaccine)*

Antigen	Population	Seropositivity (%) (Day 21-28)	Seroconversion (%) (Day 21-28)
H1N1 swl.	18-60 years	74.3 (64.6 - 82.4)	74.3 (64.6 - 82.4)
	>60 years	61.3 (49.4 - 72.4)	61.3 (49.4 - 72.4)
	Males	69.5 (58.4 - 79.2)	69.5 (58.4 - 79.2)
	Females	68.1 (57.7 - 77.3)	68.1 (57.7 - 77.3)
H1N1	18-60 years	25.7 (17.6 - 35.4)	0.0
	>60 years	17.3 (9.6 - 27.8)	0.0
	Males	18.3 (10.6 - 28.4)	0.0
	Females	25.5 (17.1 - 35.6)	0.0
H3N2	18-60 years	26.7 (18.4 - 36.5)	0.0
	>60 years	17.3 (9.6 - 27.8)	0.0
	Males	17.1 (9.7 - 27.0)	0.0
	Females	27.7 (18.9 - 37.9)	0.0
B	18-60 years	40.6 (30.9 - 50.8)	0.0
	>60 years	24.0 (14.9 - 35.3)	0.0
	Males	25.6 (16.6 - 36.4)	0.0
	Females	40.4 (30.4 - 51.1)	0.0

The percentage of seropositive (= post-vaccination titres of ≥1:40) individuals in Group 1 to A/H1N1/09 swl. antigen was over 70% in age group of 18-60 years and over 60% in age group above 60 years. The percentage of seropositive individuals to seasonal H1N1, H3N2 and B antigens was far below 70% and 60% in age groups respectively.

The rate of seroconversion to A/H1N1/09 swl. antigen was far above 40% in the age group of 18-60 years and far above 30% in age group of >60 years. The rate of seroconversion to seasonal H1N1, H3N2 and B antigens in Group 1 remained 0.0%.

*Geometric mean of HI titres (with 95% CI lower and upper limit) before and after immunization – **Group 2** (vaccination with Fluval P vaccine AND Fluval AB trivalent seasonal vaccine simultaneously)*

Antigen	Population	Day 0	Day 21-28	Change in GMT (Day 21-28)
H1N1 swl.	18-60 years	5.0 (5.0 - 5.0)	38.1 (32.2 - 45.1)	7.6 (6.4 – 9.0)
	>60 years	5.0 (5.0 - 5.0)	40.0 (33.3 - 48.0)	8.0 (6.7 – 9.6)
	Males	5.0 (5.0 - 5.0)	36.8 (30.4 - 44.4)	7.4 (6.1 – 8.9)
	Females	5.0 (5.0 - 5.0)	40.9 (34.8 - 48.1)	8.2 (7.0 – 9.6)
H1N1	18-60 years	13.6 (11.7 - 15.8)	50.1 (43.9 - 57.1)	3.7 (3.2 – 4.3)
	>60 years	15.0 (12.8 - 17.5)	40.7 (35.1 - 47.3)	2.7 (2.3 – 3.2)
	Males	14.8 (12.6 - 17.3)	46.6 (40.4 - 53.7)	3.2 (2.7 – 3.7)
	Females	13.7 (11.8 - 15.9)	45.0 (39.1 -51.8)	3.3 (2.8 – 3.9)
H3N2	18-60 years	14.0 (12.1 - 16.2)	53.7 (46.4 - 62.1)	3.8 (3.3 – 4.5)
	>60 years	15.1 (12.8 - 17.8)	43.0 (37.1 - 49.8)	2.8 (2.4 – 3.4)
	Males	14.5 (12.2 - 17.2)	47.4 (40.9 - 54.9)	3.3 (2.8 – 3.8)
	Females	14.5 (12.6 - 16.6)	49.9 (42.9 - 58.0)	3.5 (2.9 – 4.1)
B	18-60 years	12.2 (10.4 - 14.3)	46.7 (38.3 - 55.4)	3.8 (3.2 – 4.5)
	>60 years	14.1 (11.9 - 16.6)	44.2 (38.4 - 50.8)	3.1 (2.7 – 3.7)
	Males	12.4 (10.4 - 14.7)	42.8 (36.2 - 50.6)	3.5 (2.8 – 4.1)
	Females	13.5 (11.6 - 15.9)	48.1 (41.2 - 56.2)	3.6 (3.0 – 4.2)

Geometric mean of HI titres to A/H1N1/09 swl. antigen significantly increased 21-28 days after immunization in both sexes and age groups (change in GMT titres ranges between 7.4 and 8.2). Geometric mean of HI titres to seasonal H1N1, H3N2, and B antigens also increased significantly in both sexes and age groups.

The percentage of seropositive (= post-vaccination titres of ≥1:40) individuals and seroconversion are shown in the table below.

*Seropositivity (%) and seroconversion (%) (with 95% CI lower limit and upper limit) after immunization – **Group 2** (vaccination with Fluval P vaccine AND Fluval AB trivalent seasonal vaccine simultaneously)*

Antigen	Population	Seropositivity (%) (Day 21-28)	Seroconversion (%) (Day 21-28)
H1N1 swl.	18-60 years	76.8 (67.2 - 84.7)	76.8 (67.2 - 84.7)
	>60 years	81.8 (71.4 - 89.7)	81.8 (71.4 - 89.7)
	Males	73.2 (62.2 - 82.4)	73.2 (62.2 - 82.4)
	Females	84.0 (75.1 - 90.8)	84.0 (75.1 - 90.8)
H1N1	18-60 years	76.8 (67.2 - 84.7)	67.7 (57.5 - 76.7)
	>60 years	68.8 (57.3 - 78.9)	40.3 (29.2 - 52.1)
	Males	75.6 (64.9 - 84.4)	54.9 (43.5 - 65.9)
	Females	71.3 (61.0 - 80.1)	56.4 (45.8 - 66.6)
H3N2	18-60 years	78.8 (69.4 - 86.4)	70.7 (60.7 - 79.4)
	>60 years	70.1 (58.6 - 80.0)	49.4 (37.8 - 61.0)
	Males	78.1 (67.5 - 86.4)	57.3 (45.9 - 68.2)
	Females	72.3 (62.2 - 81.1)	64.9 (54.4 - 74.5)
B	18-60 years	75.8 (66.1 - 83.8)	59.6 (49.3 - 69.3)
	>60 years	75.3 (64.2 - 84.4)	53.3 (41.5 - 64.7)
	Males	74.4 (63.6 - 83.4)	53.7 (42.3 - 64.8)
	Females	76.6 (66.7 - 84.7)	59.6 (49.0 - 69.6)

The percentage of seropositive (=post-vaccination titres of ≥1:40) individuals to A/H1N1/09 swl. antigen was over 70% in age group of 18-60 years and over 60% in age group above 60 years. The percentage of seropositive individuals in Group 2 to seasonal H1N1, H3N2 and B

antigens was also over 70% and 60% in age groups respectively.

The rate of seroconversion to A/H1N1/09 swl. antigen was far above 40% in the age group of 18-60 years and far above 30% in age group of >60 years. The rate of seroconversion to seasonal H1N1, H3N2 and B antigens in Group 2 was also over 40% and 30% in age groups respectively.

Immunogenicity data in Group 1 at Day 21-28 (Antigen: A/H1N1/09 swl)

Immunogenicity criteria	Age group			
	18 - 60 years		> 60 years	
	Criteria	Result	Criteria	Result
Seroconversion	> 40 %	74.3 (+)	> 30 %	61.3 (+)
Increase in GMT	> 2.5	9.1 (+)	> 2.0	6.3 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	74.3 (+)	> 60 %	61.3 (+)

Immunogenicity data in Group 1 at Day 21-28 (Antigen: A/H1N1):

Immunogenicity criteria	Age group			
	18 - 60 years		> 60 years	
	Criteria	Result	Criteria	Result
Seroconversion	> 40 %	0.0	> 30 %	0.0
Increase in GMT	> 2.5	1.2	> 2.0	1.2
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	25.7	> 60 %	17.3

Immunogenicity data in Group 1 at Day 21-28 (Antigen: A/H3N2):

Immunogenicity criteria	Age group			
	18 - 60 years		> 60 years	
	Criteria	Result	Criteria	Result
Seroconversion	> 40 %	0.0	> 30 %	0.0
Increase in GMT	> 2.5	1.1	> 2.0	1.2
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	26.7	> 60 %	17.3

Immunogenicity data in Group 1 at Day 21-28 (Antigen: B):

Immunogenicity criteria	Age group			
	18 - 60 years		> 60 years	
	Criteria	Result	Criteria	Result
Seroconversion	> 40 %	0.0	> 30 %	0.0
Increase in GMT	> 2.5	1.0	> 2.0	1.1
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	40.6	> 60 %	24.0

(+) Met CPMP criteria

Immunogenicity data in Group 2 at Day 21-28 (Antigen: A/H1N1/09 swl):

Immunogenicity criteria	Age group			
	18 - 60 years		> 60 years	
	Criteria	Result	Criteria	Result
Seroconversion	> 40 %	76.8 (+)	> 30 %	81.8 (+)
Increase in GMT	> 2.5	7.6 (+)	> 2.0	8.0 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	76.8 (+)	> 60 %	81.8 (+)

Immunogenicity data in Group 2 at Day 21-28 (Antigen: A/H1N1):

Immunogenicity criteria	Age group			
	18 - 60 years		> 60 years	
	Criteria	Result	Criteria	Result
Seroconversion	> 40 %	67.7 (+)	> 30 %	40.3 (+)
Increase in GMT	> 2.5	3.7 (+)	> 2.0	2.7 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	76.8 (+)	> 60 %	68.8 (+)

Immunogenicity data in Group 2 at Day 21-28 (Antigen: A/H3N2):

Immunogenicity criteria	Age group			
	18 - 60 years		> 60 years	
	Criteria	Result	Criteria	Result
Seroconversion	> 40 %	70.7 (+)	> 30 %	49.4 (+)
Increase in GMT	> 2.5	3.8 (+)	> 2.0	2.8 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	78.8 (+)	> 60 %	70.1 (+)

Immunogenicity data in Group 2 at Day 21-28 (Antigen: B):

Immunogenicity criteria	Age group			
	18 - 60 years		> 60 years	
	Criteria	Result	Criteria	Result
Seroconversion	> 40 %	59.6 (+)	> 30 %	53.3 (+)
Increase in GMT	> 2.5	3.8 (+)	> 2.0	3.1 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	75.8 (+)	> 60 %	75.3 (+)

(+) Met CPMP criteria

In Group 1 administration of Fluval P pandemic influenza vaccine with 6 µg HA/dos active ingredient content induced strong immune response against A/H1N1/09 swl. antigen to meet all three CPMP criteria 21-28 days after immunization in both age groups. Administration of Fluval P influenza vaccine in Group 1 has not induced significant immune response against seasonal A/*H1N1, A/H3N2 and B antigens.*

In Group 2 simultaneous administration of Fluval P pandemic influenza vaccine and Fluval AB seasonal trivalent influenza vaccine induced strong immune response against A/H1N1/09 swl. antigen as well as against seasonal A/H1N1, A/H3N2 and B antigens to meet all three CPMP criteria 21-28 days after immunization in both age groups.

- *Tolerability and Immunogenicity Study of Fluval P Monovalent Influenza Vaccine in Children and Adolescents – an interim report*

The aim of this randomized, single-blind, controlled, interventional, prevention study was to determine as primary objective the tolerability and safety of Fluval P monovalent influenza vaccine (6 µg HA/0.5 ml active ingredient content and aluminium phosphate gel adjuvant) in children and adolescents and assess, as secondary objective, the efficacy (immunogenicity) of the vaccine by serology testing.

Study population: 58 volunteers were enrolled in this study (28 children of 3-12 years of age and 30 adolescents of 12-18 years of age). The subjects enrolled in this study were randomly assigned in a 1:1:1 ratio to one of the vaccine groups below.

Group 1: 10 children (of 3-12 years of age), and 10 adolescents (of 12-18 years of age) from both sexes were enrolled and vaccinated. Treatment: Vaccination with Fluval P monovalent influenza vaccine with 6 µg HA/0.5 ml active ingredient content and aluminium phosphate gel adjuvant. Dose: 0.25 ml (total 3 µg HA) in age group 3-12 years, and 0.5 ml (total 6 µg HA) in age group 12-18 years, single dose.

Group 2: 9 children (of 3-12 years of age), and 10 adolescents (of 12-18 years of age) from both sexes were enrolled and vaccinated. Treatment: Fluval AB trivalent influenza vaccine with 15 µg HA/0.5ml/strain active ingredient content and aluminium phosphate gel adjuvant. Dose: 0.25 ml (total 3x7.5 µg HA) in age group 3-12 years, and 0.5 ml (total 3x15 µg HA) in age group 12-18 years, single dose.

Group 3: 9 children (of 3-12 years of age), and 10 adolescents (of 12-18 years of age) from both sexes were enrolled and vaccinated. Treatment: Fluval AB Novo trivalent in-

fluenza vaccine with 6 µg HA/0.5ml/strain active ingredient content and aluminium phosphate gel adjuvant. Dose: 0.25 ml (total 3x3 µg HA) in age group 3-12 years, and 0.5 ml (total 3x6 µg HA) in age group 12-18 years, single dose.

Actual number of subjects enrolled in the study

Vaccine Age group	Group 1: Fluval P	Group 2: Fluval AB	Group 3: Fluval AB Novo	Total
3-12 years	10	9	9	28
12-18 years	10	10	10	30
Total	20	19	19	58

58 subjects were vaccinated once: 20 subjects in Group 1 received Fluval P influenza vaccine once, 19 subjects in Group 2 received Fluval AB influenza vaccine once, and 19 subjects in Group 3 received Fluval AB Novo influenza vaccine once. During the study no further study medication was administered.

Safety evaluation

In the course of safety/tolerability assessment frequency, severity, mean time of appearance and duration of all local and systemic adverse events (AEs) were calculated in all groups by simple descriptive statistics according to CPMP/BWP/214/96: "Note for Guidance on Harmonization of Requirements for Influenza Vaccines", 12 March 1997, Points 2.4., 2.6., and 3.2. According to Points 2.4, 2.6 and 3.2 of CPMP/BWP/214/96, adverse reactions for 3 days following vaccination, either local (induration, erythema, ecchymosis, pain) or general (fever, shivering, malaise, other side-effects) and any other adverse reactions lasting 2 days beyond vaccination should be recorded and assessed.

Efficacy evaluation

Secondary objective of the study was to assess the efficacy of the study drugs in the subjects by serology testing of blood taken at Day 21-28 after immunization. In this respect changes in HI titres were considered as primary efficacy parameter. HI titres were used to calculate seroconversion rates, seroprotection rates, and increase in geometric mean titres. HI tests on blood samples were performed at National Institute of Epidemiology, Department for Respiratory Viruses.

Antibody titrations were done in duplicate. Pre- and post-vaccination sera were titrated simultaneously. In case of an invalid result the HI test on the given sample should have been repeated. There was no invalid result during the tests.

No subject displayed measurable level of HI antibodies against the pandemic A/H1N1/09 virus at the baseline visit at Day 0. However they showed elevated level of HI antibodies against the seasonal A/H1N1 and A/H3N2 viruses, and lower level against the seasonal B virus. (It may be noted that the B-strain in the seasonal vaccines was changed this spring by the recommendations of the WHO and EMEA on seasonal flu vaccine composition.)

Immunogenicity related to HI was assessed according to the criteria defined in guideline CPMP/BWP/214/96:

(i) seroconversion, i.e.: a ≥4-fold titre increase reaching a titre of ≥40;
(ii) mean geometric increase (GMT), i.e.: increase in geometric mean of postvaccination serum anti-HA antibody titres to that of prevaccination serum anti-HA antibody titres, and
(iii) seroprotection, i.e.: achievement of an HI titre ≥1:40.

For the purposes of calculation, HI results <10 (=undetectable) were expressed as 5.

In absence of adopted requirements for children and adolescents those specified for adults were considered.

According to CPMP/BWP/214/96 the following requirements have to be met in subjects of <60 years of age:

(i)	seroconversion rate should be	> 40%,
(ii)	increase in GMT should be	> 2.5-fold, and
(iii)	seroprotection rate should be	> 70%.

Safety results

A total of 34 possibly or probably related AEs were reported by 20 subjects (34.5%). These adverse events were mild (30) and moderate (4), and mostly similar as observed in connection with common flu vaccinations. Forty-seven (47) not related Adverse Events were reported by 36 subjects (62.1%).

In Group 1 (vaccination with Fluval P pandemic vaccine) twelve (12) possibly or probably related Adverse Events were reported by 8 subjects (40.0%). All of these adverse reactions were mild.

In Group 2 (vaccination with Fluval AB seasonal vaccine) eleven (11) possibly or probably related Adverse Events were reported by 8 subjects (42.1%). Eight adverse reactions were mild, three ones moderate (mild headache between days 2 and 7 after vaccination in case of subject VE07-323, and low fever of 37.5^{o}C and headache between days 7 and 10 after vaccination in case of subject VE07-805).

In Group 3 (vaccination with Fluval AB Novo seasonal vaccine) eleven (11) possibly or probably related Adverse Events were reported by 4 subjects (21.1%). Ten adverse reactions were mild, one was moderate (fever of 39.0^{o}C on day 3 after vaccination).

The vaccine related adverse events appeared mostly 1-2 days after vaccination, and disappeared mostly in one or two days. Latest by the last day of the telephone follow-up (Day 10) all study drug related adverse events disappeared without sequel. Most frequent vaccine related adverse event in all groups was mild pain at injection site (four cases /20%/ in Group 1, 5 cases /26.3%/ in Group 2, and 1 case /10%/ in Group 3). Most frequent systemic reaction was headache (one case /5%/ in Group 1, 3 cases /15.8%/ in Group 2, and 3 cases /15.8%/ in Group 3).

Efficacy results

In Group 1 administration of Fluval P pandemic influenza vaccine with 6 µg HA/0.5ml active ingredient content induced strong immune response against A/H1N1/09 swl. antigen to meet all three CPMP criteria 21-28 days after immunization in both age groups. Administration of Fluval P influenza vaccine in Group 1 has not induced significant immune response against seasonal A/H1N1, A/H3N2 and B antigens.

Immunogenicity data in Group 1 at Day 21-28 (Antigen: A/H1N1/09 swl):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	85.0 (+)	80.0 (+)	90.0 (+)
Increase in GMT	> 2.5	8.6 (+)	6.1 (+)	12.1 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	85.0 (+)	80.0 (+)	90.0 (+)

Immunogenicity data in Group 1 at Day 21-28 (Antigen: A/H1N1):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	0.0	0.0	0.0
Increase in GMT	> 2.5	1.3	1.2	1.3
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	35.0	30.0	40.0

Immunogenicity data in Group 1 at Day 21-28 (Antigen: A/H3N2):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	10.0	0.0	20.0
Increase in GMT	> 2.5	1.4	1.2	1.6
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	55.0	60.0	50.0

Immunogenicity data in Group 1 at Day 21-28 (Antigen: B):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	5.0	10.0	0.0
Increase in GMT	> 2.5	1.1	1.2	1.0
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	5.0	10.0	0.0

(+) Met CPMP criteria for adults between 18-60 years of age

In Group 2 administration of Fluval AB seasonal influenza vaccine with 15 µg HA/strain/0.5ml active ingredient content induced strong immune response against seasonal A/H1N1, A/H3N2 and B antigens to meet all three CPMP criteria 21-28 days after immunization in both age groups in case of all strains *with the exception* of strain B and age group 3-12 years where the seropositivity rate was just 66.7% instead of the required >70%. However, seroconversion rate and GMT ratio was above 40% and 2.5 respectively, meeting thus

CPMP criteria on seasonal flu vaccines in case of this strain and age group as well. Administration of Fluval AB seasonal influenza vaccine in Group 2 has not induced significant immune response against A/H1N1/09 swl. antigen.

Immunogenicity data in Group 2 at Day 21-28 (Antigen: A/H1N1/09 swl):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	0.0	0.0	0.0
Increase in GMT	> 2.5	1.1	1.1	1.1
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	0.0	0.0	0.0

Immunogenicity data in Group 2 at Day 21-28 (Antigen: A/H1N1):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	79.0 (+)	77.8 (+)	80.0 (+)
Increase in GMT	> 2.5	6.0 (+)	5.4 (+)	6.5 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	100.0 (+)	100.0 (+)	100.0 (+)

Immunogenicity data in Group 2 at Day 21-28 (Antigen: A/H3N2):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	68.4 (+)	66.7 (+)	70.0 (+)
Increase in GMT	> 2.5	5.6 (+)	6.9 (+)	4.6 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	89.5 (+)	88.9 (+)	90.0 (+)

Immunogenicity data in Group 2 at Day 21-28 (Antigen: B):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	73.7 (+)	66.7 (+)	80.0 (+)
Increase in GMT	> 2.5	6.7 (+)	7.4 (+)	6.1 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	79.0 (+)	66.7	90.0 (+)

(+) Met CPMP criteria for adults between 18-60 years of age

In Group 3 administration of Fluval AB Novo seasonal influenza vaccine with 6 µg HA/strain/0.5ml active ingredient content induced strong immune response against seasonal A/H1N1, A/H3N2 and B antigens to meet all three CPMP criteria 21-28 days after immunization in both age groups in case of all strains *with the exception* of strain B and age group 3-

12 years where the seropositivity rate was just 66.7% instead of the required >70%. However, seroconversion rate and GMT ratio was above 40% and 2.5 respectively, meeting thus CPMP criteria on seasonal flu vaccines in case of this strain and age group as well. Administration of Fluval AB Novo seasonal influenza vaccine in Group 3have not induced significant immune response against A/H1N1/09 swl. antigen.

Immunogenicity data in Group 3 at Day 21-28 (Antigen: A/H1N1/09 swl):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	0.0	0.0	0.0
Increase in GMT	> 2.5	1.1	1.0	1.2
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	0.0	0.0	0.0

Immunogenicity data in Group 3 at Day 21-28 (Antigen: A/H1N1):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	79.0 (+)	77.8 (+)	80.0 (+)
Increase in GMT	> 2.5	7.7 (+)	8.0 (+)	7.5 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	94.7 (+)	100.0 (+)	90.0 (+)

Immunogenicity data in Group 3 at Day 21-28 (Antigen: A/H3N2):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	73.7 (+)	66.7 (+)	80.0 (+)
Increase in GMT	> 2.5	6.0 (+)	4.0 (+)	8.6 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	84.2 (+)	77.8 (+)	90.0 (+)

Immunogenicity data in Group 3 at Day 21-28 (Antigen: B):

Immunogenicity criteria	Criteria	Age group		
		All	3-12 years	12-18 years
Seroconversion	> 40 %	73.7 (+)	66.7 (+)	80.0 (+)
Increase in GMT	> 2.5	5.6 (+)	5.9 (+)	5.3 (+)
Seropositivity (post-vaccination titres ≥ 1:40)	> 70 %	73.7 (+)	66.7	80.0 (+)

(+) Met CPMP criteria for adults between 18-60 years of age

- *Tolerability and Safety Study of FLUVAL P Monovalent Influenza Vaccine in Children – an interim report*

The aim of this open, uncontrolled, interventional, prevention trial was to determine the tolerability and safety of FLUVAL P monovalent influenza vaccine in small children.

The primary objective was to assess tolerability/safety (incidence of adverse events) of the investigational medicinal product after Day 28 following the vaccination.

The secondary objective included the assessment of long-term safety of Fluval P monovalent influenza vaccine (6 µg HA/0.5 ml active ingredient content and aluminium phosphate gel adjuvant) 180-210 days after immunization. Furthermore, the assessment of the efficacy of the study drug is conducted by optional epidemiological follow-up of the participants until the end of the influenza season.

Trial population: 10 children aged 11-31 months of age (note: based on CPMP/BWP/214/96 the 6 – 36 months age group is regarded as a single entity) from both sexes (6 males and 4 females) were enrolled in one treatment group.

Treatment: Vaccination with Fluval P monovalent influenza vaccine with 6 µg HA/0.5 ml active ingredient content and aluminium phosphate gel adjuvant. Dose: 0.25 ml (total 3 µg HA), single dose.

Methods of data analysis: in the course of tolerability/safety assessment frequency, severity, mean time of appearance and duration of all local and systemic AEs were calculated by simple descriptive statistics. Final assessment of tolerability and safety will be performed after follow-up at Day 180-210 following the vaccination.

Methods of safety evaluation: in the course of safety/tolerability assessment frequency, severity, mean time of appearance and duration of all local and systemic adverse events (AEs) were calculated by simple descriptive statistics according to CPMP/BWP/214/96: "Note for Guidance on Harmonization of Requirements for Influenza Vaccines", 12 March 1997, Points 2.4., 2.6., and 3.2. According to Points 2.4, 2.6 and 3.2 of CPMP/BWP/214/96, AEs for 3 days following vaccination, either local (induration, erythema, ecchymosis, pain) or general (fever, shivering, malaise, other side-effects) and any other adverse reactions lasting 2 days beyond vaccination should be recorded and assessed.

Results:
A total of 3 possibly or probably related AEs were reported in case of 2 subjects (20%). These adverse events were all irritation and were classified as mild. Three (3) unrelated AEs were reported in case of 3 subjects (30%). The possibly or probably vaccine related adverse events appeared 2 or 7 days after vaccination, and disappeared in one day. Latest by the 10th day of the telephone follow-up all study drug related adverse events disappeared without sqeual.

Summary of the clinical assessment

Administration of all the three studied vaccines,

- Fluval H5N1 monovalent influenza vaccine (Influenza A/Viet Nam/1194/2004 (H5N1)-like NIBRG-14 reassortant type (inactivated)) (mock-up vaccine),

- Fluval H5N1 suspension injection (Influenza A/Swan/Nagybaracska/01/2006(H5N1)-like A/PR8/34/(H1N1)) and
- Fluval P suspension injection (active ingredient: A/California/7/2009(H1N1)-like NYMC X-179/A reassorted strain)

proved to be safe and were well tolerated by all participants of the studies (see the details in Chapter 3.2 – Clinical study reports).

The immunogenicity of Fluval P suspension injection (the pandemic vaccine) met all the three CPMP criteria on days 21 – 28 after immunisation in volunteers aged 18 – 60 and above 60 years(adults and elderly) when it was administered alone or together with Fluval AB seasonal trivalent influenza vaccine. Simultaneous administration of Fluval P and Fluval AB vaccines induced strong immune response against A/H1N1/09 swl. antigen, as well as seasonal H1N1, H3N2 and B antigens.

No clinically significant changes in the physical condition or vital signs of the volunteers were observed. No vaccine-related moderate or any Serious Adverse Event was observed. Therefore, the potential benefit in case of a pandemic outweighs the potential risks.

In children between 3 – 12 years and adolescents between 12 – 18 years the administration of Fluval P pandemic influenza vaccine induced strong immune response against pandemic A/H1N1/09 swl. antigen, and Fluval AB and Fluval AB Novo seasonal influenza vaccines induced strong immune response against seasonal A/H1N1, A/H3N2 and B antigens, but they could not induce at the same time significant cross-protection immunity against the seasonal A/H1N1, A/H3N2 and B antigens or the pandemic A/H1N1/09 swl. antigen, respectively. No clinically significant changes in the physical condition or vital signs of the volunteers were observed. No possibly or probably Fluval P vaccine related moderate and no Serious Adverse Event was observed. Fluval P vaccine related side effects were mild. No medical intervention was necessary. No serious adverse events were observed in any of the groups. On the basis of the study, administration of Fluval P pandemic vaccine in the age group of children between 3 – 12 and adolescents between 12 – 18 years is safe and— as far as the small number of subjects permits this statement – effective.

In children between 12 months – 36 months the administration of 0.25 ml of Fluval P influenza vaccine with 6 µg HA/0.5ml proved to be safe and were well tolerated by the participants (10 children between 11 months – 31 months). No clinically significant changes in the physical condition or vital signs of the volunteers were observed. Fluval P vaccine related side effects were all mild (transient irritation). No medical intervention was necessary. No possibly or probably vaccine related moderate and no Serious Adverse Event was observed.
On the basis of the study administration of Fluval P pandemic vaccine in the age group of children between 12 months – 36 months is safe.

Naturally, the very rarely occurring adverse effects, like Guillain-Barré syndrome, could not be evaluated.

The vaccines, as is the rule in such clinical trials, have not been studied in pregnant women.

The vaccine may be authorised provided an appropriate risk management is appended and the adverse events will be actively monitored and reported.

4. Pharmacovigilance system

4.1 Description of the Pharmacovigilance System

Routine Pharmacovigilance

This RMP describes the routine pharmacovigilance practice applied by at Omninvest. All pharmacovigilance activities are covered by Standard Operation Procedures. The company has a SOP to describe modified pharmacovigilance activities which reflects the recommendations of the CHMP (EMEA/359381/2009).

The Pharmacovigilance system as described by the applicant fulfilled the requirements and provided adequate evidence that the applicant possessed the services of a qualified person responsible for pharmacovigilance (QPPV) and had the necessary means for the notification of any adverse reaction.

Additional pharmacovigilance activities and action plans

Simplified periodic safety update reports

Since it is a vaccine for pandemic situation a submission of simplified PSUR is obligatory on a monthly basis. Beside these simplified PSURs submission of regular PSURs as described in Regulation 52/2005 (XI.18) are also required.

In order to effectively monitor of the safety profile of the candidate vaccine during an officially-declared H1N1 pandemic, Omninvest will prepare monthly simplified PSURs, accompanied by a summary of vaccine distribution, as described in the CHMP recommendations for pharmacovigilance plans of H1N1 pandemic influenza vaccines (EMEA/359381/2009).

Other activities

Special attention will be paid to rare serious adverse events, such as Guillain-Barré syndrome. By investigation such cases identification of potential risk factors will be performed.

Safety monitoring of immunocompromised patients and pregnant women will be part of follow-up.

Effectiveness of the Fluval P vaccine will be studied with collaboration of National Centre for Epidemiology through its “sentinel physicians” network. With serum samples originated from patients participated in clinical trials cross-reactivity testing of drifted A/H1N1 influenza virus will be performed if such virus variants will arise.

4.2 Risk Management Plan

The Risk Management Plan (RMP) for Fluval P vaccine was submitted according to the RMP guideline (EMEA/CHMP/96268/2005) and CHMP Recommendations for the Pharmacovigilance

4.2.1 Limitations of the human safety database

- The populations not studied in the pre-authorisation phase comprised

- pregnant or lactating women,
- patients who are under immunosuppressive therapy or were in the preceding 36 months,
- patients with chronic infection,
- active neoplasm,
- patients with known allergy to eggs or other components of vaccine,
- patients with Guillain-Barré syndrome in their health history,
- patients with immunodeficiency.
- children aged 0-6 months.

- Potential of overdose: according to Summary of Product Characteristics of Fluval P vaccine children below 12 years of age should be administered 0.25 ml of vaccine however this size is not available. Based on experience with previous pandemic vaccine Fluval H5N1 (product containing 12 µg active substance**)** no higher rate of adverse effects was observed.

- Important missing information:
    - Children aged 6 - 12 months
    - Pregnant or lactating women
    - Patients who are under immunosuppressive therapy
    - Patients with chronic infection,
    - Patients with active neoplasm,
    - Patients with known allergy to eggs or other components of vaccine,
    - Patients with Guillain-Barré syndrome in their heath history,
    - Patients with immunodeficiency.

4.2.2 Identified and unidentified (potential) risks and important missing safety information

Identified risks:

- local reactions:
    - pain at injection site,
    - erythema,
    - swelling,
    - induration.
- systemic reactions:
    - fever,
    - headache,
    - malaise,
    - myalgia,
    - arthalgia,
    - shivering,
    - fatigue,
    - sweltering.

Potential risks:

- Guillain-Barré syndrome,
- anaphylaxis,
- neuritis,
- convulsion,
- encephalitis,
- vasculitis,
- demyelination,
- Bell's palsy.

4.2.3 Post-authorization Safety Study

A prospective non-interventional cohort study has been required as a condition of the marketing authorisation and, consequently, will be performed in the population defined below. The primary objective will be to investigate the incidence of adverse events in the different age groups with an active surveillance method. Secondary objective will be collection all information on any AESIs and unexpected severe adverse events and the epidemiological effectiveness of vaccine in the vaccinated population.

Age	Number of subjects
12-36 months	100
3-12 years	300
12-18 years	300
18-44 years	4500
45-60 years	500
over 60 years	500
Total	6200

Medical information will be provided for all subjects at time of entry in order to allow collection and analysis of data. Medical information collected is as follows:

- chronic obstructive pulmonary disease in elderly patients,
- immunocompromised,
- cardiovascular disorder,
- diabetes mellitus,
- chronic neurological diseases,
- pregnancy information at baseline and during the study.

4.2.4 Additional safety data collection

Safety data collection is planned on pregnant women in one centre. From the beginning of vaccination period up to 6 months all women admitted to this centre in order to give birth or to be treated after spontaneous abortion will be asked on vaccination (vaccine(s), date of vaccination, adverse events, and important safety information on foetus / newborn).

Two studies involving subjects less than 18 years of age are ongoing. They will be extended to further 6 months follow-up. Other study in minor is under discussion. Concerning safety data from elderly an observational safety trial is planned in few centres for a period of time of 6 months.

Enhanced passive surveillance: Omninvest establishes a web-based system in order to make easier adverse event reports for healthcare professionals and consumers, respectively.

4.2.5 Evaluation of the need for risk minimisation activities and risk minimisation plan

Fluval P pandemic influenza is administered by healthcare specialists, so the risk of medication error is assessed as minimal.

According to EMEA/359381/2009 CHMP Recommendation revision 1.1 Omninvest pays special attention to Adverse Events for Special Interest.

Routine risk minimisation activities: Omninvest has an availability of 7/24 (persons dealing with reports on every day from 0 to 24 hours). All employees have been trained on receiving and handling safety information/ADRs. The company summarises safety information on a monthly basis in a periodic safety update report. Moreover, two positions have been established within pharmacovigilance department of Omninvest. A physician and an assistant will join the company soon.

*Additional risk minimalisation activities***:**

- *Dear Healthcare Professional Letters (DHPL):* Omninvest compiles three sorts of DHPLs: one for physicians who vaccinate in order to call their attention to the importance of ADR reporting. The second one has been addressed to neurologists with the request of paying attention to AESIs. Finally pharmacists will also be sent a letter emphasising their role in collecting ADRs from all consumers. DHPLs have been distributed by National Public Health and Medical Officer Service.
- *Safety Board (SB):* Omninvest established a Safety Board in order to assess ADRs and detect signals continuously during the pandemic period. Safety Board consists of members from National Centre for Epidemiology, National Institute of Pharmacy, Omninvest and independent physician experts. The Safety Board has a personal meeting or teleconference once a week. It overviews ICSRs received on the previous week, detects signals and decides on further activities if is necessary. SB can invite experts. Minutes will be compiled after each meeting.
- *Communication*: contact person at Omninvest is the pharmacovigilance qualified person (QPPV) for communication.
- *Product complaints*: if justified, product recall and even stop of vaccination is planned.

5. Decision on the marketing authorisation

Based on the review of the data on quality, safety and efficacy, the National Institute of Pharmacy (NIP) considered that the application for pandemic variation of Fluval P suspension for injection, to be used for the prevention of new A(H1N1) influenza in the pandemic situation, could be approved.

Conditions for the marketing authorisation

Legal Status: prescription-only medicinal product.

Follow-up measures: see the Risk Management Plan.

Specific obligations: see the Risk Management Plan.