SCIENTIFIC DISCUSSION

1. Introduction

Annual influenza epidemics are associated with substantial morbidity and mortality, especially in elderly and in those with underlying diseases. Although in healthy adults clinical influenza infections generally have an uncomplicated course, it may have a substantial economic impact, e.g. due to days lost from work.

The main objective of influenza vaccination in elderly is reducing the number of deaths caused by influenza infections. In younger populations, influenza associated morbidity is more important than mortality risk, especially in those at good health. In children, adults and elderly, vaccination against influenza is recommended as a single injection containing of each of the three components of the trivalent vaccine (i.e. the A/H3N2, A/H1N1 and B strain). The selection of the A and B influenza virus strains for the vaccines is based on the annual WHO and Committee for Human Medicinal Products (CHMP) strain recommendations. These viruses are the predominant strains most appropriate to the epidemiological situation in each hemisphere. Antibody development may start as early as one week following vaccination, but peak antibody responses are observed after approximately 4-6 weeks.

Since their development, inactivated influenza vaccines have been produced in the allantoic cavity of embryonated hen eggs. However, the efficiency of the method is low, requiring one or more eggs for each dose of the vaccine produced. The use of mammalian cell lines eliminates the reliance on the supply of embryonated eggs and generates more flexibility, adequate availability of substrate for virus growth and the possibility of higher virus yields. In addition, the cell culture-derived vaccines do not require extensive advance planning and can, in principle, be vital in responding to the threat of an emerging pandemic.
Novartis has developed an influenza vaccine produced in the mammalian cell line Madin Darby Canine Kidney (MDCK). Optaflu is an inactivated, detergent disrupted and purified product, which contains 15 µg per dose of haemagglutinin (HA) antigen from each of the three influenza virus strains Type A (H1N1), Type A (H3N2) and Type B filled up to volume (0.5 mL) with phosphate buffered saline (PBS) buffer. The composition of the influenza strains will be the officially recommended ones by the appropriate regulatory bodies. The final product is preservative-free, non-adjuvanted and presented in pre-filled syringes. The vaccine is intended for seasonal use.

The approved indication is:
"Prophylaxis of influenza for adults, especially in those who run an increased risk of associated complications."

Immunisation should be carried out by intramuscular (IM) injection.

This was a complete and independent/stand-alone Marketing Authorization Application, i.e. a complete dossier with administrative, quality, pre-clinical and clinical data, in accordance with Directive 2001/83/EC, as amended.

Accelerated review of the dossier was granted by the CHMP during its December 2005 meeting. However since during the first round a major objection regarding Quality had been identified, the expedited review was revoked in November 2006 and a regular timetable applied for the remainder of the procedure.

2. Quality aspects

Introduction

Novartis has developed an influenza vaccine produced in the mammalian cell line Madin Darby Canine Kidney (MDCK). Optaflu is an inactivated, detergent disrupted and purified product, which contains 15 µg per dose of haemagglutinin antigen from each of the three influenza virus strains Type A (H1N1), Type A (H3N2) and Type B buffered with PBS buffer and filled up to a final volume of 0.5 ml.

The composition of the influenza strains will be the officially recommended ones by the appropriate regulatory bodies. The final product is preservative-free, non-adjuvanted and presented in pre-filled syringes. The vaccine is intended for seasonal use.

Active Substance

The drug substance is a sterile, cell free, monovalent bulk containing purified virus surface antigens from a single influenza strain. Each monovalent bulk is prepared from influenza virus propagated in a suspension culture adapted Madin Darby Canine Kidney (MDCK) cell line.
The monovalent bulk antigen preparations are clear to slightly opalescent and contain mainly neuraminidase (NA) and haemagglutinin (HA) antigens.

Haemagglutinin content must be at least 90µg/mL at the monobulk stage. The presence of neuraminidase is confirmed on the first three monovalent bulks for each working virus seed. Host cell impurities in the monovalent bulk are limited such that each blended trivalent contains less than 20 ng/mL (10ng/0.5 mL dose) of DNA and not more than 40µg of protein other than haemagglutinin per dose per virus strain.
Monovalent bulk preparations from three distinct influenza virus strains are blended and formulated in phosphate buffered saline (PBS) to produce a trivalent bulk harvest.

- Manufacture

Description of Manufacturing Process and Process Controls

The production process of the drug substance, i.e. monovalent bulk, comprises three main steps:
1) Virus propagation in fermenter culture and virus clarification,
2) Virus purification and subunit preparation,
3) Filling monovalent bulk in storage vessel.

Cell Culture and harvest
Influenza Seed Virus: The reference virus strains provided by the WHO Reference Centres are considered the master virus seed from which working seed lots are prepared. Each reference virus strain obtained from WHO Reference Centres is propagated and passaged in MDCK cells to characterize its growth characteristics and to expand the incoming reference virus stock.

MDCK cell substrate: The MDCK cell line was initially established from the kidney of an apparently normal, adult, male cocker spaniel in 1958 by S. H. Madin and N. B. Darby at the University of California, Berkeley (USA). Novartis' MDCK cell line has been adapted to grow in suspension under serum- free, protein-free (PF) conditions. The MDCK cell line has been passaged over 500 times from its initial establishment in culture. Early stages of passage were conducted with calf serum and/or in the presence of other animal derived components. In later stages of passage the use of human and animal derived components has been eliminated at the primary level.

Cell substrate banking system: A four-tiered cell bank system represented by research seed, master, working, and end- of-production cell (EoP) banks was established. The current set of cell banks was

established after protein free adaptation. Working cell banks (WCB) derived from a single MDCK master cell bank will be used for commercial production of monovalent bulk and influenza working seed virus. The WCB represents 5 passages (~ 10 population doubling levels) from the MCB. Cells used for monovalent bulk manufacturing are stored in liquid nitrogen.

Virus propagation in fermenter culture and virus clarification

The starting material for each monovalent bulk is a single vial of the MDCK working cell bank (WCB). The cells are propagated in a chemically defined media to optimize cell growth during production. The WCB are expanded by sequential passage in spinner flasks followed by scale up in larger fermentation vessels. The limit of MDCK cell passage from culture initiation to virus infection is specified. Virus propagation in the fermenter is performed over a period of two to four days.

At the end of the infection cycle the virus suspension is centrifuged and filtered to remove residual intact cells from the culture harvest. The centrifuged, filtered bulk termed clarified virus harvest, is the end of the fermentation process. The clarified virus harvest may be stored at room temperature (16- 25 °C) in a stainless steel storage vessel for up to 24 hours.

Purification and filling

The influenza virus is purified by chromatography and ultra-/diafiltration steps, inactivated by beta-propiolactone (BPL) and disrupted by cetyltrimethylammonium bromide (CTAB) to solubilize the viral surface antigens HA and NA.

The drug substance production process concludes with a filtration of the concentrate into the final bulk vessel to obtain monovalent bulk. Each monovalent bulk is released according to established specifications for identity, safety, purity and potency. The monovalent bulk can be stored at 2- 8 °C for 18 months.

Control of Materials

The following starting materials used in the production of the monovalent bulk are of biological origin: Influenza seed virus, MDCK cell substrate, recombinant human insulin, and porcine trypsin. In general, sufficient detailed information is provided on the control and source of these starting materials.

Controls of critical steps and intermediates

In-process controls are envisaged along the cell culture and purification process. Viability, cell growth kinetics, purity and morphology are verified during expansion phase.
Control cells and virus harvests have been identified as intermediates. Control cells are taken before virus inoculation to check for extraneous agents.

Process validation and/or evaluation

Process validation and evaluation have been conducted for the upstream (fermentation) and downstream (protein purification) processes, BPL inactivation of influenza virus, BPL inactivation (hydrolysis) and removal of host cell residuals (DNA, proteins and intact cells) and column reuse and regeneration.

The inactivation studies performed provide a sufficient degree of safety with regard to influenza virus inactivation time and concentration of BPL. The inactivation process by BPL is followed by detergent splitting of the purified virions and separation of subunit antigens, which has been shown to be very effective in inactivation/elimination of enveloped and non-enveloped viruses.
The production process has been validated to reduce process-related impurities to acceptable low levels. In cases where levels are more significant (in terms of quality/safety), specifications have been set.

Manufacturing process development

Basic research on cell culture based production methods and the first manufacturing runs in small scale fermenter systems began in 1992. Formal process development was initiated in 1995.
Material for a preclinical toxicity study in rabbits and for phase I/II clinical trial use was successfully produced in 2002 using a 100 L pilot scale manufacturing process.
The upscale from a 100 L to 2500 L fermentation process was completed in 2003. Material for clinical consistency for phase III study for the EU was successfully produced under cGMP conditions in 2004 and 2005 at the 2500 L scale which is the planned commercial scale.

Characterisation

The active pharmaceutical ingredients (API) in influenza subunit vaccines are viral haemagglutinin (HA) and neuraminidase (NA) proteins. Appropriate activity and quantity of these proteins are generally conducted with international reference standards intended for conventional, egg-derived antigens.
The basic integrity of the vaccine antigens (HA & NA) has been confirmed in preclinical and clinical efficacy trials. Detailed analysis of the antigen purity (HA/total protein, SDS-PAGE), physicochemical and biological properties of the Optaflu-derived HA protein has been conducted with an emphasis on comparative analysis to egg-derived antigens from the manufacturers other marketed influenza vaccines. The results of physicochemical and biological analysis of the Optaflu HA and NA proteins support the general comparability of egg and MDCK- derived antigens (protein sequence, tertiary structure, pI, and presence of N-glycosylation). Although small physico-chemical differences between the MDCK and egg derived vaccines were observed, the results from the mice immunization studies did not indicate that these differences impact the vaccine's immunogenicity.

- Specification

Control of Drug Substance

The proposed testing protocol for the monovalent bulk complies with the Ph.Eur. monograph. Potency of the drug substance is determined by the quantification of the Haemagglutinin antigen using the single radial diffusion (SRD) assay, which also serves as identity test.
The analytical procedures (principle, equipment, standards/solution, procedure, measurement/ evaluation) are concisely described and the validation reports provided.

Haemagglutinin antigen, neuraminidase, sterility, residual infectious influenza virus, purity and total protein are determined and specified according to Ph. Eur. Polysorbate and CTAB are tested chromatographically and colorimetrically, respectively.

Batch analysis

The batch analysis results (three monovalent bulks each of three virus strains: demonstrate that the monovalent bulk production process is fairly consistent as regards to HA content and process-related impurities.

Container Closure System

The monovalent bulk is stored in polypropylene containers. Full product specifications are provided. Information is provided by the container manufacturer on product validation, including cytotoxicity, abnormal toxicity testing, leak proof, and trace metal analysis. Results from stability studies do not raise concern as regards to container-drug substance compatibility.

- Stability

All test results (up to 18 months at 2-8°C) for the 3 batches per strain of the consistency batches are well within specifications, including 6 months data under accelerated conditions at 23-27 °C. In summary all different strains investigated so far show stability up to 18 months at 2-8 °C.

Finished Product

The vaccine is an inactivated, detergent disrupted and purified product to contain 15 µg per dose of HA antigen from each of the three virus strains. The final product is PBS buffered solution, preservative-free and non-adjuvanted. It is a clear to slightly opalescent liquid for injection presented in 1 mL, type I glass (Ph. Eur.) pre-filled syringes.

Table: Composition of Optaflu

Component	**Quantity per dose = 0.5 ml**	**Reference to standards**
Active substance:		
Influenza virus surface antigens of: • A/New Caledonia/20/99 (H1N1) like strain (Reass. IVR-116) • A/California/7/2004 (H3N2) like strain (NYMC X-157) • B/Shanghai/361/2002 like strain (B/Jiangsu/10/2003) The vaccine complies with the WHO recommendation (northern hemisphere) and EU decision for the 2005/2006 season.	15 µg HA* 15 µg HA* 15 µg HA*	WHO/NIBSC
Excipients:		
PBS Water for injection (WFI) included in the buffer	Up to 0.5 ml	WFI: Ph.Eur. Buffer: International Spec.

HA* = Haemagglutinin

- Pharmaceutical Development

The formulation of the drug product is chosen on the experience of the company's egg-based flu vaccine and has not changed during development. The composition has varied in accordance with the annual strain recommendation. Minor changes have been introduced to the manufacturing process. Due to liquid losses during withdrawal, an overfill of 0.1 ml for 1 ml syringes without needle and 0.05 ml overfill for 1 ml syringes with a needle is chosen. The overfill has not changed during development.
Also the HA antigen overage has been reduced based on results of stability studies for this product at 2-8 °C and assay optimization.

The integrity of the container closure system is sufficiently proven by the stability studies for the product, which includes sterility tests. The compatibility of the drug product with the container is proven by the stability studies.

- Adventitious Agents

Nonviral Adventitious Agents (TSE aspect)

Separate TSE assessments have been conducted for the MDCK master cell bank (MCB) and the Optaflu manufacturing process.
In general, MDCK cells themselves are not likely to bear any risk of transmitting TSE as they are derived from dogs, which are excluded from the European TSE note for guidance (EMEA/410/01).
However, the cells have been in culture for a total of more than 500 passages since 1958 and have been exposed to human and ruminant derived media components over this period. However, neither human nor animal derived components, at the primary level, have been used as media supplements since 1996. The only secondary level component (material used to prepare media components) with animal origin is bovine milk (casein) of New Zealand origin that has been used to produce amino acids components in the culture media. In view of this situation a TSE risk reduction factor of 10^{31} has been determined based on the dilution of the cells in protein free media since the passaging starting in 1996.
The materials used to propagate MDCK cells since 1981 have been carefully traced and supporting documentation acquired where available. It was determined that no potential TSE risk materials have

been or are used in the virus seed materials, nor in the routine production of the subunit vaccine. The use of ruminant derived materials complies with the requirements of the current TSE note for guidance (EMEA/ 410/ 01)." TSE Certificates of Suitability have been obtained for the MDCK chemically defined media (CDM) medium component recombinant insulin.

Overall, sufficient data is provided to demonstrate compliance with the CHMP TSE NfG. The risk of transmitting TSE by Optaflu is very remote.

Viral Adventitious Agents

The company has made significant efforts to substantiate the safety of Optaflu as regards to adventitious agents potentially present. Different aspects have been taken into consideration such as the biological characteristics of the different starting materials (seed virus, cell substrate) and characteristics of the production process (cell cultivation, virus propagation, purification) including the virus inactivation/clearance performance of the production process.

The Applicant has satisfactorily supported the strategy for routine virus screening of the seed virus lots by Polymerase Chain Reaction (PCR) methodology instead of in *vivo/in vitro* testing in accordance with Ph.Eur. 2.6.16.
In addition, the Applicant committed to generate additional validation data by performing retrospectively the full extraneous agent testing according to the Ph.Eur. in comparison with the applicant's PCR procedure on seeds produced for clinical trials and other available seed material from past production campaigns, in order to validate the PCR testing approach for influenza virus seed lots by providing evidence from parallel tests with *in vitro*/*in vivo* testing that both methods render the same end results. Following a step-by-step approach according to an established time-table as agreed by the CHMP, information is submitted to support the PCR methodology and omit the conventional Ph.Eur. *in vitro* / *in vivo* testing.

Therefore, it can be concluded that the company has sufficiently assured that the Optaflu is safe as regards to adventitious agents.

- Manufacture of the Product

Manufacture / Controls of critical steps and intermediates
The manufacturing process comprises blending of the three monovalent bulks to produce trivalent bulk and filling into the final container, i.e. syringes.

The monovalent bulks of three different influenza strains are formulated according to their antigen concentration determined by single radial diffusion (SRD) assay. Resulting monovalent bulk volumes and sterile PBS are directly added to the sterile formulation steel-container under aseptic conditions.
Sterile filtration for commercial batches will be done after transportation, at the filling site prior to filling of the vaccine into the syringes. The sterile filtration is integrated in-line with the filling process (in-line sterile filtration). Each container is tested for sterility and transported to the filling and packaging facilities. During the transportation and storage the temperature is kept at 2-8 °C.

The vaccine (one dose = 0.5 ml) is filled into 1 ml, type I glass syringes. The final containers are filled by machine in a clean environment. During the filling process the filling volume is checked regularly by weighing.
The filled products are labelled and packed at room temperature.

In-process controls (IPCs) are set during blending and filling. Overall, the proposed IPCs are sufficient to monitor the manufacturing process and specifications for the final bulk are set.

Process validation and/or evaluation
Validation of a homogenous blending procedure is performed with three formulations under production conditions as part of the manufacturing of phase III clinical material. Critical process

parameters for the filling are the control of the sterility and homogeneity of the product filled into the final container. The blending and filling procedures are sufficiently validated.

- Product Specification

Specifications for the trivalent (final) bulk and drug product are provided and are in line with the Ph.Eur.
The analytical procedures (principle, equipment, standards/solution, procedure, measurement / evaluation) are concisely described but are well known.

Testing for trivalent (final) bulk: Sterility, total protein, pH and density are tested according to Ph. Eur. Osmolality is tested according USP and total DNA content is tested using the Threshold method and specified according to Ph. Eur.

Specifications for Optaflu drug product: Sterility, endotoxin, haemagglutinin antigen and identity, ATT, extractable volume and appearance are tested and specified according to Ph. Eur.

Batch analysis
Batch analysis results of three consecutively filled batches demonstrate consistent production of Optaflu drug product.

- Stability of the Product

Stability are available for the consistency batches stored at 2-8 ºC and at 23-27 º C. Preclinical, Phase I/ II and Phase III material were also put on stability testing. These data were presented as supporting data.

In general, the results support the shelf life and storage conditions as defined in the SPC (12 months at 2-8 ºC).

Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the drug substance and drug product have been presented in a satisfactory manner. The results of the tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.
At the time of CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Risk-benefit balance of the product. The applicant gave a Letter of Undertaking and committed to resolve these as follow-up measures after the opinion, within an agreed timeframe.

3. Non-clinical aspects

Introduction

Optaflu has undergone nonclinical testing designed to demonstrate immunogenicity (mice, rabbits, and ferrets), nonclinical efficacy (influenza challenge in ferrets), lack of abnormal toxicity (mice and Guinea pig), and lack of local or systemic toxicity (rabbits).

The nonclinical studies were performed using vaccine formulations that were comparable or identical to Optaflu that will be commercialized. Abnormal toxicity studies were performed on toxicology and clinical lots according to Pharmacopoeia Europa (Ph. Eur.). The ferret challenge study was performed according to Good Laboratory Practices (GLP) with a clinical lot of Optaflu. The pivotal GLP rabbit toxicology study was performed with an Optaflu lot that is comparable to clinical and consistency lots. The trivalent vaccine used in the pivotal toxicology study included seasonal influenza virus strains [A/New Caledonia/20/99 (H1N1), B/Guangdong/120/00 (B), and A/Panama/2007/99 (H3N2)] that

may differ from strains tested in the clinical development program or to be produced commercially, but strain changes in the vaccine do not constitute a 'difference' in the drug product.

Pharmacology

- Primary pharmacodynamics

The primary pharmacological effect of the vaccine is the induction of antibodies to influenza antigens and protection against infection. Therefore, the demonstration of antibody induction (immunogenicity) and efficacy (protection in an animal model of influenza virus challenge) was the focus of the non-GLP nonclinical pharmacology studies.

Mouse immunogenicity studies were performed using the intraperitoneal (IP) route to demonstrate the comparability of MDCK-derived antigens to conventional egg-derived antigens of the same type. Based on mouse immunogenicity results, MDCK-derived and conventional egg-derived antigens were comparable. In addition, the immunogenicity of the vaccine was dose-dependent. Haemagglutination-inhibiting (HI) titres were lower following administration of 1/100th of the 15µg HA per strain clinical dose versus 1/10th of the 15µg HA per strain clinical dose.

Immunogenicity was also evaluated in the pivotal GLP toxicology study in which rabbits were given 2 intramuscular administrations of Optaflu, the reference vaccine Agrippal, or placebo. HI results for serum antibodies to two of the strains (A/New Caledonia and B/Guangdong) showed no titre pre-treatment and an increase in titre after the second vaccine dose of Optaflu or Agrippal. There were background titres against strain A/Panama in control animals and pre-treatment in Optaflu and Agrippal-treated animals, which were attributed to nonspecific binding. However, a trend towards increasing amounts of A/Panama antibody titre could be seen after the second vaccine dose.

Immunogenicity and protection against influenza virus challenge has also been addressed in the ferret, which is currently considered to be the best animal model for infection with human influenza viruses. In the pivotal ferret study, animals were first primed intranasally with a heterologous virus, and then immunized twice with Optaflu, a comparator vaccine (Agrippal) or water (control). Seven days after the second immunization, animals were challenged intranasally with live virus homologous to the vaccine. Vaccine efficacy was evaluated based on body weights, body temperatures and clinical symptoms. In addition, viral shedding, leukocyte counts, and antibody titres were assessed. In this study, Optaflu was considered comparable to Agrippal because differences between the two groups were not statistically significant. Both vaccines reduced viral shedding and prevented body temperature increase as apparently the most sensitive endpoints.
During the development of the ferret challenge model, two range-finding studies were conducted to evaluate various concentrations of the challenge virus to determine an appropriate dose for use in challenge studies. Subsequently, four nonpivotal challenge studies were performed using naive (unprimed) ferrets. In these challenge studies, ferrets were vaccinated twice with vaccine formulations, and then challenged with live virus homologous to the vaccine. These animals were immunologically naive (not primed with a heterologous virus). Although the vaccine formulations were well tolerated, the immunogenicity and challenge results were highly variable. Relevant effects on endpoints such as temperature increase were only seen in one of these four studies; however, there were also low responders in the negative control group. There was no clear and consistent effect of vaccination, with the positive control vaccine or with the test vaccine. The lack of protection seen in these studies is consistent with published literature where naïve (unprimed) ferrets did not produce HA-specific antibody following immunization with concentrations of antigen shown to produce high titres of serum antibody and immunity in humans.

In the pivotal ferret study, animals *(8 animals per group)* were first primed intranasally with a heterologous virus (A/Panama/2007/99 [H3N2]), and then immunized twice with Optaflu (A/New Caledonian/20/99 [H1N1], A/New York/55/2004 [H3/N2], B/Jsiang/10/2003), a comparator vaccine (Agrippal) (based on the same antigens, also called positive control) or water (control). Two

immunizations were given intramuscularly 3 weeks apart. Seven days after the second immunization, animals were challenged intranasally with live virus homologous to the vaccine (A/New Caledonian/20/99 [H1N1]). Vaccine efficacy was evaluated based on body weights, body temperatures (see below) and clinical symptoms. In addition, viral shedding, leukocyte counts, and antibody titres were assessed. In this study, Optaflu was considered comparable to Agrippal because differences between the two groups were not statistically significant. Both vaccines reduced viral shedding and prevented body temperature increase.

- Secondary pharmacodynamics

No secondary pharmacodynamic studies were performed according to the Note for Guidance on Preclinical Pharmacological and Toxicological testing of vaccines (CPMP/465/95) and Guideline on Adjuvants in Vaccines for Human Use (EMEA/CHMP/VEG/134716/2004).

- Safety pharmacology programme

No studies investigating safety pharmacology were warranted based on the nature of the product and the existing nonclinical and clinical safety/tolerability profiles.

- Pharmacodynamic drug interactions

No studies investigating pharmacodynamic drug-drug interactions were warranted based on the nature of the product.

Pharmacokinetics

Experimental studies to demonstrate absorption, distribution, metabolism, and excretion of the active ingredients in Optaflu have not been performed. This is in line with the relevant guideline CPMP/SWP/465/95.

Toxicology

- Single dose toxicity

Single dose data in mice and Guinea pigs with Optaflu administered by the intraperitoneal route confirmed the absence of abnormal toxicity.

Single-dose toxicity and local tolerability of Optaflu was evaluated in rabbits following the administration of the first dose in the repeat-dose toxicology study. Two intramuscular injections were given 7 days apart into alternate hind limbs. Each site, therefore, received a ‘single-dose’ of Optaflu. No animals died in this study. There was no evidence of systemic toxicity after a single dose based on in-life evaluations. No detectable erythema or edema was observed at the injection sites in any animal. Histopathological findings at the injection site indicated that the vaccine was well tolerated.

- Repeat dose toxicity

The pivotal GLP study assessed the local and systemic toxicity of Optaflu in New Zealand White rabbits after two administrations and determined the reversibility of findings. Agrippal served as the reference article. Phosphate buffered saline (PBS) was the control article (placebo). The study consisted of three groups of 6 animals/sex/group. Rabbits received an intramuscular injection of 0.5 ml of either the test or reference article or placebo on Days 1 and 8. The two doses administered to rabbits in this study exceeded the intended number (one) proposed for annual interpandemic immunization.

Results showed that two intramuscular injections of the test article, Optaflu, given one week apart, were immunogenic and very well tolerated in test rabbits. There were no treatment related adverse

effects on clinical observations, dermal scoring, body weights and temperatures, food consumption, clinical pathology (haematology, coagulation, and clinical chemistry), organ weights, or macroscopic evaluations. Histopathological evaluation revealed the expected reactions (necrosis and haemorrhage) at the injection sites, which were seen in all experimental groups, attributed to the intramuscular injection, and partially to fully resolved by the end of the recovery period.

- Genotoxicity

No genotoxicity studies were conducted. This is in line with the relevant guidelines (CPMP/SWP/465/95 and CHMP/VEG/134716/2004).

- Carcinogenicity

No carcinogenicity studies were conducted, in line with the relevant guidelines.

- Reproduction Toxicity

No reproduction toxicity studies were conducted, in line with the relevant guidelines.

- Local tolerance

Local tolerability was evaluated during the repeat-dose toxicology study in rabbits. The only notable finding was mild, reversible inflammation seen at the intramuscular injection sites, which is consistent with the administration of an immunogenic vaccine by this route.

- Other toxicity studies

Tumorigenicity: Optaflu is manufactured in a novel mammalian cell substrate, therefore, an extensive panel of studies was performed to characterize the MDCK cell line. MDCK cells have been reported to exhibit varying degrees of tumorigenicity, depending upon which cells are tested and the model in which they are tested. Weakly tumorigenic MDCK cells can be converted to high tumorigenicity upon the introduction of oncogenes or genes expressing growth-related factors.

Consistent with current international guidelines for the qualification of a new cell line as a platform for vaccine production, the Applicant performed studies with intact MDCK cells, lysates of MDCK cells, and purified DNA obtained from MDCK cells. These studies were designed to characterize the tumorigenic or oncogenic potential of materials from the manufacturing process at progressive stages: intact cells, lysates prepared from these cells, and purified DNA from influenza virus infected and uninfected MDCK cells. Very sensitive species were selected to optimize the detection of possibly very rare events leading to tumorigenicity or oncogenicity: adult nude mice and infant animals (<4 days old) of three rodent species (nude mice, rats, and hamsters) with immature immune systems at the time of dosing. Each study had a 150-day duration and was designed to allow time for any potential adverse effects to emerge.

Only intact MDCK cells were tumorigenic. MDCK cell lysates and purified DNA were not oncogenic in three species of infant rodents. Although tumorigenic at low cell numbers, MDCK cells are completely removed during the manufacturing process of the vaccine. Therefore, whole cells do not pose a risk in the final product.

Impurities: There are three compounds used in the manufacturing process that are considered to warrant a toxicological assessment: cetyltrimethylammonium bromide, polysorbate 80 (Tween 80), and β-propriolactone (BPL). An extensive review of the available toxicity literature on these compounds was performed. The maximum amount of each compound theoretically contained in a vaccine dose poses no risk to the target human populations receiving the product. Although this application is not for pediatric use, no toxicity at the levels discussed would be anticipated in a pediatric population. Tolerability and toxicity of any intentional or unintentional compounds were tested in the GLP toxicology study in ~ 3kg rabbits. Animals received two clinical doses within a one-week period. Based on body weights, these doses to rabbits (~3 kg) represented approximately a 4× to

20× multiple of a single dose to a child (12 kg) or an adult (60 kg). The safety or tolerability of any intentional or unintentional residuals would, therefore, have been tested.

Ecotoxicity/environmental risk assessment

An environmental risk assessment on this product is not needed based on the final Guideline on Environmental risk assessment of non-GMO products.

Discussion on the non-clinical aspects

The documentation provided on immunogenicity and nonclinical efficacy (in ferrets) showed that Optaflu is able to induce relevant protection against an influenza challenge.

Furthermore, the toxicological documentation for Optaflu is regarded to be adequate and in line with the relevant guidelines. The rabbit study addressing repeated dose toxicity as well as local tolerance revealed no concerns regarding the safety of the vaccine.

The vaccine is produced on MDCK cells, a cell substrate up to now not used for the manufacturing of licensed vaccines. Since these cells are known to be tumorigenic, specific studies were conducted to address that issue. Tumorigenicity of MDCK cells was confirmed in the studies performed by the applicant. However, no tumorigenicity was observed for cell lysates or DNA, extracted from MDCK cells under different conditions. In conclusion, the data provided are regarded to sufficiently address the issue of oncogenicity/tumorigenicity of the MDCK cell substrate and to resolve any corresponding safety concern regarding the vaccine.

As impurities present in the vaccine, cetyltrimethylammonium bromide (CTAB), polysorbate 80 and β-propiolactone (BPL) are addressed in the preclinical part of the dossier. Based on literature data, and since these substances are not novel excipients for vaccines, the concentrations present in the final vaccine raise no safety concern.

In conclusion, the toxicological analysis is adequate for this new vaccine and reveals no concern regarding the safety of Optaflu.

4. Clinical aspects

Introduction

For the approval of egg-derived influenza vaccines, it is an accepted policy that clinical efficacy trials are considered dispensable if serological criteria as defined in the "Note for guidance on harmonisation of requirements for influenza vaccines" (CPMP/BWP/214/96) are properly met.

These CPMP criteria* are defined as follows for healthy adult and elderly populations:

	18-60 years	**>60 years**
Mean increase in GMT**	>2.5	>2
Seroconversion or significant increase	>40%	>30%
Protected post- vaccination	>70%	>60%

*Applicable to immunogenicity data based on HI-assay & SRH assay**.**
In HI tests **seroconversion** corresponds to negative prevaccination serum / postvaccination serum ≥ 40 and a significant increase to at least a fourfold increase in HI-titre. In SRH tests seroconversion corresponds to negative prevaccination serum / postvaccination serum: area ≥ 25 mm^2 and a significant increase to at least a 50% increase in area. Protected post vaccination is defined as a titre of ≥ 40 (HI-assay) or ≥ 25 mm2 (SRH-assay)

**GMT: geometric mean titre

Generally, these criteria apply to the annual strain update of egg-derived influenza vaccines and in the clinical evaluation of annually produced influenza vaccines at least one of the above three criteria should be met for each strain. However in order to demonstrate efficacy for newly developed inactivated influenza vaccines, such as this cell culture derived inactivated influenza vaccine more stringent primary clinical endpoints were defined in the CPMP guideline for cell culture inactivated influenza vaccines (CPMP/BWP/2490/00). The guideline states that all three criteria, seroprotection, seroconversion and sufficient increase in GMTs, as described as geometric mean ratio (GMR) should have been met, with postvaccination seroprotection and GMR being the most important. These parameters observed in a clinical trial of cell-derived vaccine should be non-inferior to that obtained with an equivalent egg-derived vaccine.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

Pharmacokinetics

No clinical pharmacology studies describing the pharmacokinetic properties of Optaflu were conducted in support of this application, since they do not provide useful information for establishing adequate dosing recommendations (CPMP/EWP/463/97).

Pharmacodynamics

In relation to vaccines, pharmacodynamic studies are essentially compromised of the immunogenicity studies that characterise the immune response to vaccines. The detailed characterisation of the immunological response to the proposed vaccine is the surrogate parameter for efficacy and these data are discussed below.

Clinical efficacy

- Dose response studies

Dose finding studies were not performed.

- Main studies

Five clinical studies have been conducted for the European development programme. For each influenza season, the respective influenza virus composition that was used during the clinical programme for Optaflu is shown in the table below.

Study Number	Influenza Season	Hemisphere	Influenza virus strains		
			A/H1N1	A/H3N2	B

			A/New Caledonia/20/99 IVR-116	A/Panama/2007/99 RESVIR 17	A/Wyoming/3/2003 IVR-X-147	A/New York/55/2004-X-157	B/Guangdong/120/2000	B/Shangdong/7/97	B/Jiangsu/10/2003
V58P1	2001/2002[a]	Northern	+	+			+		
V58P2	2003	Southern	+	+				+	
V58P4	2004/2005	Northern	+		+				+
V58P4E1	2005/2006	Northern	+			+			+
V58P9	2005/2006	Northern	+			+			+
V58P5	2005/2006	Northern	+			+			+

[a] Phase 1/2 study V58P1 was conducted during influenza season 2002/2003 but used the recommended influenza vaccine composition from the preceding season.

The population studied contained subjects from different countries, continents, and hemispheres and from different influenza seasons.

The European clinical development program to support licensure of Optaflu consisted of 2 exploratory studies (V58P1, V58P2) and 3 main studies (V58P4, V58P4E1, V58P9). The clinical studies were designed to evaluate the immunogenicity, safety and clinical consistency of Optaflu and that of an equivalent egg-derived control vaccine. The control egg-derived influenza vaccine (Agrippal: inactivated, subunit), licensed for use in Europe, was selected due to its similarity to Optaflu (i.e., both are inactivated subunit vaccines produced by the same manufacturer). An additional clinical Phase II study (Study V58P5) was initiated in the US evaluating the 2005/2006 seasonal influenza virus vaccine composition in subjects 18-49 years of age using a US licensed egg-derived subunit vaccine (i.e., Fluvirin, also from the same manufacturer) as comparator. The results of this study were presented as supportive data in the dossier.

All studies used the same observer blinded design, and the same methods for patient selection and randomisation. Study V58P4 was the pivotal trial, and study V58P4E1 was the one-year follow up trial, in which a subset of study V58P4 was revaccinated in a newly randomised trial.

METHODS

Study Participants

The *in- and exclusion criteria* were the same for all studies. Only subjects were *included* that fulfilled the age criterion of 18 to 60 years (adults) or 61 years and older (elderly), were mentally competent to understand the nature, scope, and consequences of the study; willing to give written informed consent, available for all the visits scheduled in the study and resident in the study area and were in good health as determined by medical history, physical examination and clinical judgment of the investigator. In short, *the exclusion criteria* encompassed unwillingness to give written informed consent; participation in another trial; suffering from an acute infectious disease; presence of any serious disease such as cancer, autoimmune disease (including RA), advanced arteriosclerotic disease or complicated diabetes mellitus, chronic obstructive pulmonary disease (COPD) requiring oxygen therapy, acute or progressive hepatic disease, acute or progressive renal disease or congestive heart failure. Furthermore exclusion criteria included planned surgery; bleeding diathesis; history of hypersensitivity or anaphylaxis to any component of the study medication, allergy to eggs or egg

products, mercury-containing compounds, or to any other vaccine component; known or suspected impairment/alteration of immune function, history of drug or alcohol abuse, laboratory-confirmed influenza disease in the past 6 months; receipt of influenza vaccine within the past 6 months; receipt of another vaccine or any investigational agent within the past 60 days, or planned within 3 weeks following the study vaccination; acute respiratory disease, infections requiring systemic antibiotic or antiviral therapy or fever of 38°C or higher within the past 3 days; pregnant/breast feeding women.

Treatments

In all studies the experimental Optaflu consisted of a 0.5 ml dose of MDCK cell-culture-derived influenza subunit vaccine contains purified viral envelope-glycoproteins hemagglutinin (15 µg) each of A/H1N1, A/H3N2 and B recommended for the influenza season in which the study was performed. Subjects assigned to the control group received a single 0.5 ml dose of egg-derived influenza subunit vaccine, containing Influenza virus antigens as recommended for the respective influenza season.

The vaccines were administered by IM injection in the deltoid muscle preferably of the non-dominant arm.

For evaluation of the antibody response blood samples were collected immediately before immunization (day 1) and three weeks after immunization (day 22).

Objectives

The *primary immunogenicity objective* of all studies was to evaluate immunogenicity of a single 0.5 mL IM injection of the cell-culture-derived and egg-derived influenza subunit vaccines, in compliance with the requirements of the European Union recommendations (CPMP/BWP/214/96). In addition, in study V58P4 *secondary immunogenicity objective* was formulated in the protocols as "To demonstrate non-inferiority of the correlates of protection (seroprotection, seroconversion and sufficient increase in GMT) of a single 0.5 mL IM injection of the cell culture-derived influenza subunit vaccine versus a single 0.5 mL IM injection of the egg-derived influenza subunit vaccine."

The studies also used the same *safety objective,* i.e. "To evaluate safety and tolerability of a single 0.5 mL IM injection of the cell-culture derived and egg-derived influenza subunit vaccines." In addition study V58P9 also defined a secondary safety objective, i.e. to compare the safety between 3 lots (A, B and C).

Outcomes/endpoints

As recommended by the CPMP/BWP/214/96 guideline, sera were assayed for anti-HA antibody against the homologous vaccine strains in clinical studies by haemagglutination inhibition (HI) as well as by single radial haemolysis (SRH) to evaluate efficacy of Optaflu. In all studies (phase I to III) the HI test was performed. In addition in studies V58P1 and V58P2 the SRH test was conducted.

All immunogenicity results were evaluated in the context of the CHMP criteria for influenza vaccines (CPMP/BWP/214/96) for adult (18-60 years) and elderly (>60 years) populations (see table above). The phase 1 and 2 studies (V58P1 and V58P2) evaluated immunogenicity according to the CPMP/BWP/214/96 requirements that at least one of the above three criteria should be met for each strain. The non-pivotal phase 3 studies (V58P9 and V58P4E1) also considered all three criteria. For the pivotal phase 3 study (V58P4), however, the primary objective was to actually meet all three CHMP criteria for all three strains in accordance with Annex CPMP/BWP/2490/00 for both adult and elderly subjects.

Sample size

Study V58P4: The planned total sample size of 1188 evaluable subjects (583 adults, 605 elderly) per group provided more than 90 % power to demonstrate non-inferiority for each of the 3 vaccine strains and each of the 3 assessment criteria (GMR, seroprotection and seroconversion) accounting for a global Type I error of 0.025 (1-sided). The following assumptions were made:

1. Lower limit for GMR ratios: 0.5, common standard deviation: 1.0
2. Non-inferiority margin for rates: -10% for the difference in incidences (test – control)
3. Assumed rates (equal rates assumed in both vaccine groups): adults - seroprotection rates: 80%, rates for seroconversion or significant increase: 60% elderly - seroprotection rates: 65%, rates for seroconversion or significant increase: 50%

Considering a 10% drop-out rate, approximately 2650 subjects (1300 adult, 1350 elderly) were to be enrolled. The overall power for demonstrating non-inferiority was less than 80% in the elderly.

Study V58P4E1: This was an extension study to study V58P4. Thus, no formal sample size calculation took place.

Study V58P9: Sample size was based on safety considerations allowing for a sufficiently large database to assess safety.

Randomisation

Except study V58P9 patients were randomised 1:1 (cell-derived / egg-derived vaccine) in blocks of 6 according to a computer generated allocation scheme provided by the Applicant. Randomisation was done separately for the predefined age strata and (in study V58P4E1) for the treatment in the parent study V58P4. In study V58P9 patients were randomised 2:2:2:1 in blocks of length 7 to one of 3 cell-derived lots or the egg-derived vaccine respectively.

RESULTS

Baseline data

Demographic characterisation was quite complete for all studies. Overall the study groups seem balanced at baseline, as far as age, gender and vaccination history was concerned. Only in study V58P1 the vaccination history in the control group was substantially higher. As this was a first exploratory analysis the finding is considered less relevant.

Numbers analysed

The number of subjects in the PP population was smaller than in the safety population, since the PP population in study V58P4E1 only included subjects in the predefined immunogenicity subset (subjects with blood samples taken for immunogenicity analyses) and excluded subjects with major protocol deviations. In the extension study V58P4E1, conducted in 2 consecutive influenza seasons (the first was the initial study V58P4), adults and elderly subjects who had received Optaflu or comparator vaccine in the previous study were randomized at a 1:1 ratio to receive either Optaflu or comparator vaccine the subsequent year, resulting in 4 treatment groups: Optaflu / Optaflu, Optaflu /comparator, comparator/ Optaflu, and comparator/comparator. Subjects continuing from study V58P4 into the extension, V58P4E1, are therefore counted twice in the total PP and safety populations.

Study	Design*	Numbers				Mean age				Vaccination history (%)			
		Immuno-genicity@		Safety		Adults (18-60)		Elderly (≥ 61 yrs)		Adults (18-60)		Elderly (≥ 61 yrs)	
		Optaflu	Con	Optaflu	Con	Optaflu	Con	Optaflu	Con	Optaflu	Con	Optaflu	Con
V58P1	OB,R,C	119	120	120	120	37.0	35.1	65.4	64.1	23	23	40	62

V58P2	OB,R,C	110	113	110	113	47.2	46.7	68.9	70.5	82	72	94	96
V58P4	OB,R,C	1322	1318	1330	1324	38.7	38.3	69.1	68.0	38	42	59	59
V58P4E1	OB,R,C	242	241	1104	1131	42.0	39.0	69.0	69.2	100	100	100	100
V58P9	OB,R,C	1017	168	1028	171	32.6	32.6	-	-	23	26	-	-
total		***2810***	***1960***	***3692***	***2859***								
V58P5	OB,R,C	307	303	309	304	33.8	34.2	-	-	19	19	-	-

@ number for immunogenicity are the per-protocol figures – in study V58P4E1 these numbers include only the subset of subjects who were randomised to the different group in study V58P4 i.e. approx. ¼ of the total exposed population.
Con= control, Agrippal for the first 5 studies, Fluvirin for study V58P5

* OB= observer blinded, R=randomised, C=controlled

The PP population also excludes an additional 35 subjects with major protocol deviations. The number of subjects excluded was balanced between the two vaccine groups and the most common reason for exclusion was failure to provide the second blood sample.

In addition, the all-randomized population is reported for the demographic and baseline characteristics, which includes all subjects, regardless of actual treatment, in order to demonstrate that the randomization resulted in balanced vaccine groups. The safety and all-randomized populations differed by only a single subject in study V58P9. There were also no relevant differences in demographic or baseline characteristics between the all-randomized and PP populations.

Outcomes and estimation

The Geometric Mean Ratio (GMR) (= ratio of the postvaccination Geometric mean titre (GMT) v.s. the prevaccination GMT), seroprotection rates pre-and postvaccination and seroconversion/ significant increase are tabled for all 5 finished trials. For results in *italic* the lower bound of the GMT was below the limit set for the CHMP criterion. For results in **bold** and ***italic*** the point estimate was below the limit set for the respective CHMP criterion.

Geometric mean ratio

GEOMETRIC MEAN RATIO postvaccination						
	H3N2		**H1N1**		**B**	
ADULTS						
	Optaflu	**Control**	**Optaflu**	**Control**	**Optaflu**	**Control**
V58P1	11 (7.5-16)	8.6(6-13)	27 (18-38)	20 (14-29)	7.2(5.4-9.5)	6.5(4.9-8.5)
V58P2	3.1(2.3-4.2)	***2.2(1.6-3.0)***	***2.3(1.6-3.3)***	4.2(2.9-6.0)	*3.1(2.4-4.0)*	3.2(2.5-4.1)
V58P4	4.9(4.4-5.3)	5.5(5.0-6.1)	7.6(6.9-8.5)	7.1(6.4-7.9)	10(9.1-11)	8.8(8.0-9.6)
V58P4E1	7.1(5.9-8.4)	3.8(3.2-4.5)	***2.0(1.8-2.3)***	***1.9(1.7-2.2)***	***2.3(2.0-2.7)***	***1.9(1.7-2.3)***
V58P9	9.5(8.8-10)	11(9.4-13.0)	13(12-14)	12(10-15)	7.1(6.6-7.6)	5.9(5.0-7.0)
ELDERLY						
	Optaflu	**Control**	**Optaflu**	**Control**	**Optaflu**	**Control**
V58P1	8.0(5.5-12)	5.2(3.6-7.6)	7.9(5.5-11)	6.4(4.5-9.1)	4.8(3.4-6.7)	4.9(3.5-7.0)
V58P2	*2.5(1.9-3.3)*	***1.7(1.3-2.2)***	***1.9(1.5-2.4)***	***1.7(1.4-2.2)***	3.1(2.3-4.0)	3.0(2.3-3.9)
V58P4	5.9(5.4-6.5)	6.5(5.9-7.2)	4.6(4.2-5.1)	4.6(4.2-5.1)	9.6(8.8-11)	7.8(7.1-8.6)
V58P4E1	8,2(6.9-9.9)	5.6(4.7-6.7)	*2.1(1.8-2.4)*	*2.1(1.8-2.4)*	2.3(2-2.6)	*2.2(1.9-2.5)*
V58P9	-	-	-	-	-	-

Seroconversion/ significant increase

SEROCONVERSION / SIGNFICANT INCREASE postvaccination (%)						
	H3N2		**H1N1**		**B**	
ADULTS						
	Optaflu	**Control**	**Optaflu**	**Control**	**Optaflu**	**Control**

V58P1	75 (62-85)	73 (60-83)	90 (79-96)	87(76-94)	68(55-80)	66(53-78)
V58P2	***30(19-44)***	***23(13-36)***	***21(12-34)***	*40(28-54)*	***36(23-50)***	***28(17-42)***
V58P4	58(54-62)	62(58-66)	63(59-67)	64(60-67)	78(75-81)	73(70-77)
V58P4E1	69(60-77)	56(47-65)	***18(11-25)***	***24(13-29)***	***24 (17-33)***	***21(14-29)***
V58P9	79(76-81)	82(78-86)	80 (77-82)	81(76-85)	71(68-74)	71(66-76)
ELDERLY						
	Optaflu	**Control**	**Optaflu**	**Control**	**Optaflu**	**Control**
V58P1	68(54-79)	52(38-65)	58(44-70)	52(38-65)	46(33-59)	53(40-67)
V58P2	***20(11-34)***	***9(3-20)***	***17(8-29)***	***13(5-24)***	*35(23-49)*	***19(22-48)***
V58P4	65(61-68)	62(59-66)	48(44-52)	47(44-51)	76(72-79)	67(64-71)
V58P4E1	79(70-86)	72(63-90)	***23(16-31)***	***22(15-31)***	***26(19-35)***	***21(11-32)***
V58P9	-	-	-	-	-	-

Seroprotection

SEROPROTECTION prevaccination (%)						
	H3N2		**H1N1**		**B**	
ADULTS						
	Optaflu	**Control**	**Optaflu**	**Control**	**Optaflu**	**Control**
V58P1	32 (20-45)	32 (21-45)	15 (7-27)	11 (5-22)	12 (5-23)	10 (4-20)
V58P2	66(52-78)	72(58-83)	54(40-67)	47(34-61)	7(2-17)	2(0-9)
V58P4	61(57-64)	60(56-64)	25(22-28)	25(22-29)	9(7-11)	10(8-13)
V58P4E1	19(13-27)	16(10-24)	66(57-74)	57(48-66)	47(38-56)	44(35-53)
V58P9	18(15-20)	18(12-25)	26(23-29)	30(23-37)	16(14-19)	17(12-24)
ELDERLY						
	Optaflu	**Control**	**Optaflu**	**Control**	**Optaflu**	**Control**
V58P1	34(22-47)	40(27-53)	15(7-27)	28(17-41)	12(5-23)	22(13-35)
V58P2	69(54-80)	73(60-84)	65(51-77)	55(41-69)	4(0-13)	7(2-17)
V58P4	64(60-67)	57(53-61)	23(20-26)	22(19-25)	13(10-15)	11(9-14)
V58P4E1	25(17-33)	26(19-35)	48(38-57)	41(32-50)	51(42-60)	44(35-54)
V58P9	-	-	-	-	-	-

SEROPROTECTION postvaccination (%)						
	H3N2		**H1N1**		**B**	
ADULTS						
	Optaflu	**Control**	**Optaflu**	**Control**	**Optaflu**	**Control**
V58P1	90 (79-96)	87 (76-94)	97(88-100)	94(84-98)	*80(68-89)*	*73(60-83)*
V58P2	91(80-97)	91(81-97)	*80(68-90)*	86(74-94)	***48(35-62)***	***40(28-54)***
V58P4	98(97-99)	98(97-99)	86(83-88)	86(83-89)	83(80-86)	82(79-85)
V58P4E1	83(75-89)	76(68-84)	92(85-96)	84(76-90)	79(71-86)	*78(70-85)*
V58P9	89(87-91)	93(88-96)	93(91-94)	93(89-97)	83(81-85)	79(72-85)
ELDERLY						
	Optaflu	**Control**	**Optaflu**	**Control**	**Optaflu**	**Control**
V58P1	80(67-89)	88(77-95)	*73(60-84)*	83(71-91)	*63(49-75)*	79(67-89)
V58P2	87(75-95)	91(80-97)	85(73-93)	79(66-88)	***44(31-59)***	***46(33-60)***
V58P4	97(96-98)	98(96-99)	76(72-79)	74(71-78)	84(81-87)	79(76-82)
V58P4E1	89(82-94)	86(79-92)	78(69-85)	70(62-78)	83(75-89)	80(71-86)
V58P9	-	-	-	-	-	-

Ancillary analyses

- Analysis performed across trials (pooled analysis)

Although studies were carried out individually, the similarity of study design across studies allowed data pooling. The applicant performed multivariate analyses of pooled data, with the objective to maximize the power to assess potential associations of specific variables with the efficacy outcome.

A total of 4287 subjects were evaluable for the immunogenicity assessment and were included in this primary analysis (HI assay with egg-derived antigen type) providing 4770 pre-vaccination and 4770 post-vaccination titres (subjects in studies V58P4 and the extension V58P4E1 had more than one observation) for each of the three viral strains (H1N1, H3N2, B). For clarity, the 4770 observations per strain are referred to as "subjects". A total of 2810/4770 (59%) subjects received the cell-derived vaccine and 1960/4770 (41%) received the egg-derived vaccine in 5 randomized clinical trials (V58P1, V58P2, V58P4, 58P4E1 and V58P9). The mean age of the subjects at enrolment was 48.8 years (range: 18-92 years of age) and 62% were adults, whilst 38% were elderly subjects. Females accounted for 58% (2744/4770) of the total study population. At the time of enrolment, 2311 subjects (48.5%) had never received an influenza vaccine, 2359 subjects (49.5%) had received at least one previous influenza vaccination, and 2% of subjects did not remember their pre-vaccination status. Subjects who had HI titre higher or equal to 40 at baseline (seroprotection): 29% (n=1360) for the H1N1 strain, 45% (n=2146) for H3N2, and 16% (n=743) for the B strain.

As could be expected, previous vaccination for influenza and baseline antibody titres strongly influenced the postvaccination immune responses. This means that , irrespective of baseline titre, a subject with vaccination history tends to respond to influenza vaccination with lower postavaccination antibody titres, but not seroprotection rates. There was a trend showing a better immune response in females and in the adults, however, the magnitude of these effects did not appear to be clinically relevant and were variable depending on the statistical method used.

- Other ancillary analyses

HI versus SRH Tests

Both the HI and SRH tests were used in the first two studies to assess the immunogenicity of Optaflu. The two tests were similar in terms of CHMP criteria, which were met for all three strains in adults and elderly and in both Optaflu and comparator vaccine groups, except for two cases:

> In Study V58P1, the adult seroprotection criterion (>70%) using the egg-derived antigen was achieved for strain A/H3N2 for both Optaflu and comparator vaccines when measured with HI, whereas with SRH it was not achieved for either vaccine group.
>
> In Study V58P2, the adult seroprotection criterion (>70%) using the egg-derived antigen was achieved for strain A/H1N1 for Optaflu when measured with HI, whereas with SRH it was not achieved.

In addition, the HI assay showed slightly lower values both at baseline and after vaccination against the B strain when compared with SRH, for both antigen sources and age groups. This is consistent with previous findings that suggest that the HI assay is less sensitive against the B strain.

Egg-derived versus Cell-derived Test Antigens

The HI findings did not change for the 3102 observations (from studies V58P1, V58P2 and V58P4) when cell-derived test antigens were used. For the H1N1 and H3N2 strains, the HI titres were higher when the cell derived rather than the egg-derived antigen was used ($p<0.0001$). For the B strain, no differences were detected ($p=0.206$).

Subpopulations - Subjects According to Previous Exposure

As shown in an extensive exploratory analysis (pooled data), both baseline antibody titres and previous influenza vaccination appeared to influenced the immune response. This influence of each of the factors is independent of the other factors, which means that both factors exert an influence on immune response regardless of the presence or absence of the other.

Interchangeability of Vaccines

In study V58P4E1, the achievement of the CHMP criteria in the adults was not influenced by whether or not Optaflu or comparator vaccines had been used previously. Thus, it can be concluded that both vaccines are interchangeable for annual vaccination campaigns.

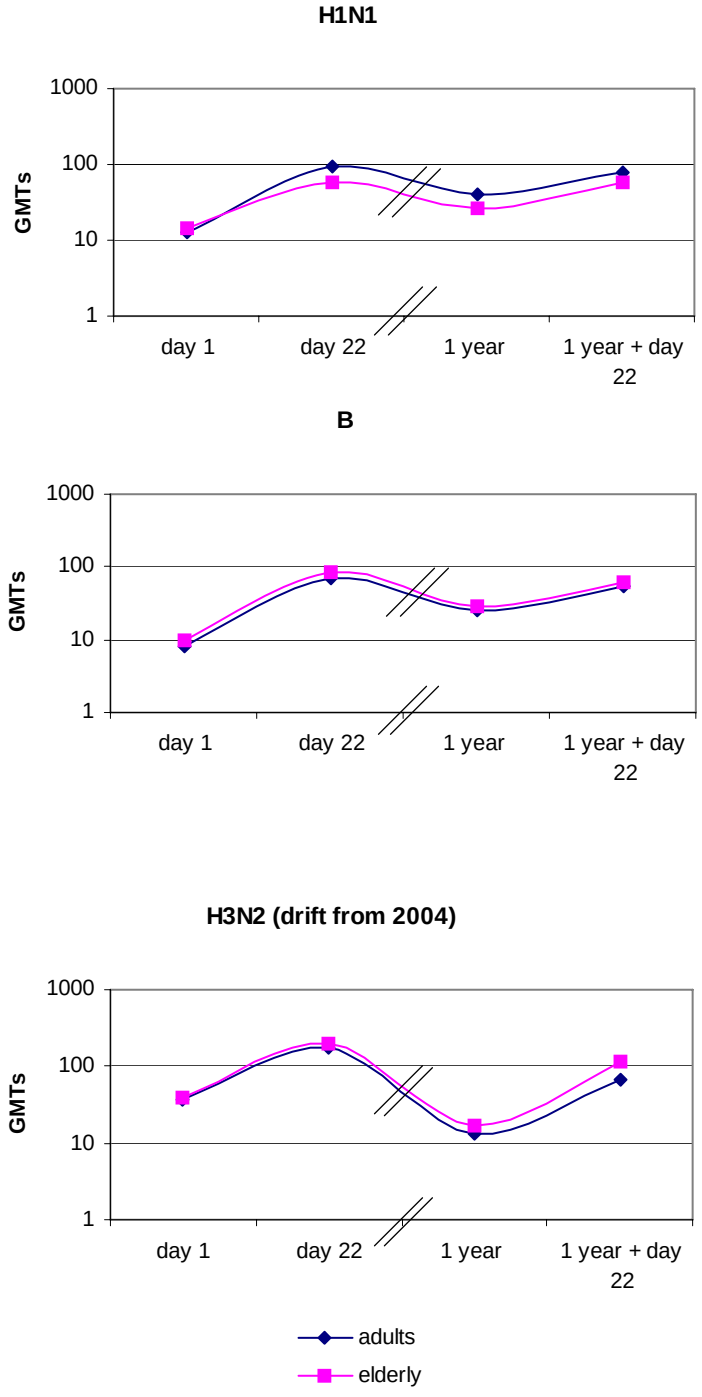
Persistence of Antibody Titres

In study V58P9, the seroprotection rates remained above the CHMP criterion of 70% until 6 months after vaccination, against the two A influenza strains. GMTs remained greater than 2.5-fold above baseline. At 6 months to the seroprotection was in agreement to CHMP criteria regardless of baseline titre status or whether the subjects had received a previous influenza vaccination.

No study was designed to evaluate the persistence of the immune response after 1 year. However, persistence could be assessed in subjects of study V58P4 who continued in the extension study V5P4E1 one year later, and whose baseline titres at day 1 therefore could reflect the persistence of antibody at 12 months (although there could be a contribution from natural exposure, the study did not allow for a differentiation between the two factors). Due to annual variation, subjects in V58P4E1 were exposed to only two of the three strains in the previous year's vaccination administered in V58P4 and therefore, persistence could only be assessed against the two identical strains in the vaccine previously administered (A/H1N1 and B).

Although after one year, the GMTs had fallen to about half their previous value against A/H1N1 and to about one third of their previous value for the B strain, seroprotection rates remained significantly higher for the identical strains for both adults and elderly subjects than one year previously (i.e., before vaccination).

Persistence of antibody titers at 1 year after vaccination in V58P4 in PP population of V58P4E1 (N=4770).



- Clinical studies in special populations

The vaccine was studied in adults and elderly in accordance with the relevant guideline. Seroresponses were assessed in the elderly population, stratified into 5 year age intervals (i.e., 60-64, 65-69, 70-74, 75-79, ≥80 years). The geometric mean ratios (GMRs) were used to investigate possible differences between the age strata. Within the confines of the small sample sizes of each of the age strata, no marked differences in the immune responses among the age and vaccine groups were observed.

The studies were not designed to evaluate other specific high-risk groups. Nonetheless, during the programme a significant number of elderly subjects developed medical conditions and therefore became representative of those who are at increased risk for complications from influenza.

In subjects with conditions associated with an increased risk of complications, e.g., cardiovascular and pulmonary conditions and diabetes mellitus, the incidence of these conditions in subjects of Optaflu and control groups was similar. Underlying conditions classified under circulatory system affected 17% and 21% of subjects in Optaflu and control group, respectively, diabetes mellitus affected 3% of subjects in each vaccine group, and conditions of the respiratory system affected 2% of subjects in each vaccine group. Due to the relatively high number of subjects with underlying disease in V58P4 compared with the other studies, this was the most appropriate study in which to evaluate at risk populations.

In V58P4, for both Optaflu and control vaccines, all CHMP criteria were passed in the subset of subjects (N = 448) with cardiovascular and pulmonary conditions as well as diabetes mellitus. Therefore, both vaccines induced acceptable immune responses also in at risk subjects with underlying medical conditions.

No other special populations, including children, were studied

- Supportive study

With the exception of a difference in the age range of adult subjects (18-49 years in V58P5 vs. 18 - 60 years in the European programme), the demographic and other baseline characteristics were consistent with those of the European programme.

These data from the statistically powered study V58P5 demonstrate all three CHMP criteria were met for both vaccine groups.

Summary of Immunogenicity of study V58P5 according to CHMP criteria

Test (antigen type)	**HI (egg-derived antigen)**				**HI (cell-derived antigen)**			
Serological Criteria to meet CPMP/BWP/214/96 requirements	**Vaccine**							
	Cell-Derived (N=307)		**Egg-Derived (N=304)**		**Cell-Derived (N=307)**		**Egg-Derived (N=304)**	
	AGE GROUP: 18-49 years (adults)							
	***A/New Caledonia/20/99* (A/H1N1)**							
Seroprotection ≥70%	96%	+	98%	+	97%	+	98%	+
GMR (day 22/day 1) ≥2.5	6.88	+	7.98	+	7.03	+	7.96	+
Seroconversion or significant increase ≥40%	61%	+	63%	+	60%	+	63%	+
	A/California/7/2004 (H3N2)							
Seroprotection ≥70%	93%	+	97%	+	96%	+	98%	+
GMR (day 22/day 1) ≥2.5	12	+	15	+	11	+	14	+
Seroconversion or	85%	+	90%	+	86%	+	90%	+

significant increase ≥40%								
	B/Shanghai/361/2002							
Seroprotection ≥70%	86%	+	87%	+	85%	+	79%	+
GMR (day 22/day 1) ≥2.5	7.09	+	6.5	+	6.83	+	5.38	+
Seroconversion or significant increase ≥40%	69%	+	70%	+	69%	+	61%	+

- Discussion on clinical efficacy

Immunogenicity was assessed using HI-assay, according to predefined CPMP criteria, both at baseline and 3 weeks post vaccination. Overall the serological responses to Optaflu were comparable to the egg- derived comparator vaccines for all parameters, i.e. GMT ratio, seroresponse (defined as significant increase or seroconversion) and postvaccination seroprotection. In addition to the HI assay, the SRH assay was applied in 2 studies (V58P1 and V58P2). In both studies, the seroprotection criterion was achieved with HI, but not with SRH
The applicant also evaluated egg-derived versus cell derived test antigens for the HI-assays. In general, it appeared that the cell -derived antigens resulted in slightly higher HI responses. However, the formal assessment according to the CPMP criteria was based upon the egg-derived test antigens.
In most studies all 3 criteria were met, except for southern hemisphere study V58P2 and extension study V58P4E1. In study V58P2, 72-95% of the vaccinees and in study V58P4E1 all vaccinees were immunised in the previous year as well. In these studies especially geometric mean ratio and seroresponse were less likely to meet the CPMP criteria. However, postvaccination seroprotection rate was always met. In addition, in these two studies where the population had a high vaccination history, it was also shown that with the use of alternative SRH assay, the criteria were met. For all 3 strains and 2 age bands, only the cell derived SRH assay consistently fulfilled the postvaccination seroprotection rate, and appeared to be the most sensitive to achieving the other parameters as well.
It was concluded that the finding was most likely an issue related to the variability of these assays.

With regard to extension study V48P4E1, not all CPMP-criteria were met for strains A/Caledonia and B/Shanghai. Since these latter 2 strains were not changed compared to the previous year, the possibility of residual immunity for these strains was considered as explanation for the inability to achieve the criteria dealing with significant titre rise and GMR. The applicant provided the baseline data, and stratified analysis for baseline titre (i.e. <10 vs ≥ 10), which supported the assumed responsiveness (as far as GMT increase is concerned) in relation to baseline titre.

In the other studies, fewer individuals had received a vaccination in the previous year, although it still ranged between 23-42% in the adults and between 40-62% in the elderly. In the meta-analysis the applicant reports that vaccination history is an independent factor for postvaccination response. However, this is based upon proportions of subjects with prevaccination seroprotection and not on prevaccination titre. Others (Beyer W. E. et al., 2004) have shown that using logistic regression models, vaccination history is only predictive for prevaccination titre, but not postvaccination titre.

Persistence of antibody titres was addressed in extension study V58P4E1. Although the titres decreased over time, the predefined seroprotection level (HI>40) was still maintained after 12 months in approximately half of the population at the time of revaccination (i.e. the prevaccination seroprotection rate of study V58P4E1).

In addition, it was noted that day 180 immunogenicity data for study V58P9 showed the lowest postvaccination seroprotection rate for B-strain, falling below 70% in both treatment arms, during an influenza epidemic season where also B strain circulated.

A specific issue with the HI titre is the between laboratory variability, which can exceed 300%. This may seriously hamper between study comparisons. However, this concern could be dismissed since all assays were performed in one laboratory only.

Overall the study population comprised of mostly healthy adults and elderly, although a proportion of individuals had underlying disease and as such was not in agreement with the inclusion criteria. The applicant provided stratified analyses and a tabulation of all conditions. The small numbers in the subanalyses precluded conclusions for these individual studies. The meta-analysis, however, also addressed co-morbidity. Analysis of this variable did not indicate a difference between the cell derived and egg derived formulation for the subgroup with comorbidity.

Especially in the elderly population the mean age of the vaccinees was relatively young. As it is known that immune responsiveness to influenza vaccine decreases with age, it was considered important that stratified analyses in different age categories in the elderly population (i.e. 60-64, 65-69, 70-74,75-79 ≥ 80 years) were provided. Non-adjusted analyses were provided for the 5-year age bands. Although for the small studies an age related decline in GMR was observed this was only obvious for A/H1N1 strain in the pivotal study V58P4. An overall analysis was not provided. Since in all age strata the CHMP criteria were met, it is however unlikely that an overall analysis would change the conclusions substantially.

Finally, in a subset of study V58P9, the clinical outcome Influenza Like illness (ILI) was addressed. As such data may be important to extend the knowledge on the efficacy of seasonal influenza vaccines and Optaflu, the applicant provided details on the 7 cases of culture confirmed influenza B infections, but a detailed analysis was not available. The infections occurred between 131 and 178 day postvaccination. Of interest was that none of the patients showed a significant increase in HI-titre against the vaccine strain postinfection, which might indicate mismatch between circulating strain and vaccine strain or co-circulation. The applicant should provide incident rate based calculations and compare the epidemic characteristics for the study area (Lithuania) with the EU, as such data may provide some insight into the expectation for the European setting based on these data (Follow-up measure).

Clinical safety

The **safety** of the vaccine has been evaluated in a total of 3693 subjects receiving at least 1 dose of the different Optaflu vaccine formulations. In the main safety study **V58P4E1** 2235 subjects were enrolled thereof 1105 received the candidate vaccine Optaflu and 1131 an egg-derived purified influenza vaccine, (Agrippal) as active control. The study population was age stratified, 1067 were adults (18-60 years of age) and 1168 belong to the elderly (>60 years of age).

- Patient exposure

In all 5 studies safety profiles were evaluated in adult subjects following 2329 doses of Optaflu and 1472 doses of the comparator vaccine. Four of these studies also evaluated safety in elderly subjects following 1363 doses of Optaflu and 1387 doses of the comparator vaccine.

Table: Numbers of Subjects and doses (Safety Population)

Studies	Adults		Elderly	
	Optaflu	Control	Optaflu	Control
Study V58P1	60	62	60	58
Study V58P2	56	57	54	56
Study V58P4	652	648	678	676
Study V58P4E1 (exposed for the first time to specific formulation / total exposed)	261/533	274/534	281/571	297/597
Study V58P9	1028	171		
Subjects exposed/ doses administered	**2057/2329**	**1212/1472**	**1073/1363**	**1087/1387**

Total completed		2312	1457	1351	1375
Withdrawal		18	15	13	11
Reason	Adverse event	0	0	3	3
	Withdrew consent	7	4	5	5
	Lost of follow up	11	11	5	3

- Adverse events

An extensive overview was given for the local and systemic reactogenicity. The data are analysed descriptively and according to so-called weighted risk ratios for any, local, systemic, or other indicator of reactogenicity, a ratio of 1 indicates no difference.

All safety data were analysed separately for adult and elderly subjects.

Table: Monitoring Periods for Safety Assessment

Study	Solicited AEs	All non solicited AEs	Non solicited AEs leading to premature withdrawal	Non solicited AEs necessitating physician visit	All SAEs*
V58P1 (Safety lab. day 1 +22)	Day 1 to 7	Day 1 to 22			Day 1 to 22
V58P2					
V58P4			Day 22 to 181		Day 22 to 181
V58P4E1				Day 22-181	
V58P9					

*SAEs: serious adverse events

Solicited AEs

There was no statistically significant difference between the two vaccines for any of the individual solicited local or systemic reactions except for pain at injection site, as was shown by the 95% confidence intervals (CIs) (a ratio of 1.0 indicates no difference). Solicited reactions classified as severe were very rare, but were balanced between the two vaccine groups in all cases (the severe indicators of reactogenicity were too infrequent in the elderly in many cases for weighted risk ratios to be calculated).

Table: Indicators of Reactogenicity

Indicator	**Weighted risk ratio (95% CI)**			
	Adults		**Elderly**	
Injection site pain	1.28	(1.11, 1.47)	1.42	(1.09, 1.84)
Ecchymosis	0.96	(0.69, 1.32)	0.91	(0.63, 1.31)
Erythema	0.93	(0.79, 1.09)	1.04	(0.82, 1.30)
Induration	0.93	(0.75, 1.16)	1.15	(0.83, 1.60)
Swelling	0.91	(0.68, 1.22)	1.02	(0.68, 1.53)
Chills	0.99	(0.70, 1.40)	1.08	(0.71, 1.64)
Malaise	1.01	(0.82, 1.23)	1.04	(0.82, 1.31)
Myalgia	0.92	(0.71, 1.19)	1.06	(0.78, 1.43)
Arthralgia	1.19	(0.84, 1.70)	1.05	(0.76, 1.44)
Headache	1.11	(0.92, 1.33)	1.01	(0.80, 1.29)

Sweating	1.08	(0.77, 1.52)	1.07	(0.79, 1.46)
Fatigue	1.02	(0.84, 1.25)	1.01	(0.81, 1.27)
Fever	0.52	(0.23, 1.17)	0.90	(0.35, 2.35)

Within each study, the percentages of adult subjects experiencing at least one solicited AE within a week of vaccination were balanced between the vaccine groups (Optaflu: 36% - 77%, control 33% - 72%). However, higher percentages of subjects from the phase 1 and phase 2 studies (V58P1, V58P2) reported solicited AEs compared with the phase 3 studies. When pooled across studies, the percentages of subjects experiencing any solicited AE only differed by 2% between vaccine groups. The weighted risk ratios suggested no difference in local reactions, systemic reactions, and other indicators of reactogenicity between the Optaflu and control vaccines.

The percentages experiencing local reactions (range, 25% - 60%) were higher than systemic reactions (range, 16% to 48%) within each study. Other indicators of reactogenicity (range, 2% to 11%) were relatively infrequent and balanced between the groups.
The most common local reactions in both vaccine groups were pain and erythema (range, 15% - 18%). The most common systemic reactions in both vaccine groups were headache, fatigue, and malaise (range, 11% - 13%).

Within each study, the percentages of elderly subjects experiencing at least one solicited local or systemic reaction within a week of vaccination were balanced between the vaccine groups (Optaflu: 26% - 63%, control 25% - 76%). However, higher percentages of subjects from the phase 1/2 and phase 2 studies (V58P1, V58P2) reported solicited reactions than the phase 3 studies. When pooled across studies the percentages experiencing any reaction between vaccine groups only differed by 1%. The weighted risk ratio with 95% CIs suggested no difference in overall percentages experiencing local reactions, systemic reactions, and other indicators of reactogenicity between Optaflu and control vaccines. The percentages experiencing local reactions (range, 16% - 62%) were higher than systemic reactions (range, 13% - 40%) when compared between vaccine groups within each study. Other indicators of reactogenicity (range, 2% to 9%) were relatively infrequent and balanced between the groups.
Pain and erythema were the local reactions experienced by the highest percentages in both vaccine groups (range, 6% - 10%). Fatigue, headache, and malaise were the systemic reactions experienced by the highest percentages in both vaccine groups (range, 9% - 10%). There was no statistically significant difference between the two vaccines for any solicited reactions except for pain for which the Optaflu groups had a weighted risk of 1.42 over the control group with 95% CIs that do not include 1. The subsequently submitted supportive US study versus a different US licensed comparator, Fluvirin, did not show the same trend. Further data are needed to exclude that pain may occur more frequently in subjects receiving Optaflu (follow up measure).

Reactogenicity after a second dose (Studies V58P4 and V58P4E1):

The extension study allowed an assessment of safety after vaccinations given in consecutive years. Adult and elderly subjects were enrolled at a 1:1 ratio into Optaflu and comparator vaccine groups of the extension study (V58P4E1) conducted in the subsequent influenza season. Each vaccine group contained equal proportions of subjects vaccinated with Optaflu and comparator vaccine the previous year. Overall frequencies of solicited AEs (especially the systemic reactions) were lower after the second dose. Results were independent of the type of influenza vaccine given previously and were similar for the two vaccine groups.

However, for Optaflu there was a slightly higher incidence of reactions in subjects receiving EGG/ Optaflu than in those receiving Optaflu / Optaflu (notably the systemic reactions malaise and myalgia for elderly, and pain for adult).

Table: Overview of reactogenecity: Percentages of subjects who received two doses, one year apart

Age	Type of	**V58P4 (N=2654)**	**V58P4E1**

group	reaction	**Optaflu** (652 adult/ 678 elderly) %	**EGG** (648 adult/ 676 elderly) %	**Optaflu / Optaflu** (N=272 adult) (N=290elderly) %	**Optaflu /EGG** (N=274 adult) (N=297elderly) %	**EGG/ Optaflu** (N=261 adult) (N=281elderly) %	**EGG/EGG** (N=260 adult) (N=300 elderly) %
Adult	Any	40	41	33	31	39	34
	Local	**32** (N=209)	**31** (N=200)	**29** (N=78)	**27** (N=73)	**32** (N=84)	**29** (N=76)
	Systemic	**22** (N=144)	**23** (N=147)	**15** (N=41)	**17** (N=46)	**18** (N=46)	**16** (N=42)
Elderly	Any	34	32	23	26	28	24
	Local	**22** (N=149)	**18** (N=121)	**16** (N=47)	**17** (N=51)	**20** (N=50)	**16** (N=47)
	Systemic	**22** (N=146)	**22** (N=147)	**13** (N=37)	**13** (N=40)	**17** (N=47)	**12** (N=37)

Non-solicited Adverse Events

Within each study, the percentages of adults experiencing at least one non-solicited AE within 3 weeks after vaccination were balanced between the vaccine groups (Optaflu: 9%-25%, comparator 7%-31%). However, higher percentages of subjects from the phase 1 and 2 studies (range, 17%-31%) reported non-solicited AEs than in the phase 3 studies (range, 7%-15%). When pooled across studies the percentages experiencing any non-solicited AE only differed by 1%. The weighted risk ratio with 95% CIs indicated no difference between Optaflu and comparator vaccines.
Most AEs were either common illnesses expected in this population or known vaccine side effects.
There were no differences in severity profiles between the vaccine groups for all non-solicited AEs when compared by study or between the pooled vaccine groups. Overall, most non-solicited AEs were mild and only 9 subjects of Optaflu group and 6 subjects of the control group experienced severe AEs.

The percentages of elderly subjects across studies experiencing at least one non-solicited AE within 3 weeks of vaccination were balanced between the vaccine groups (Optaflu: 8%-28%, control 6%-30%). However, higher percentages of subjects from the phase 1/2 and phase 2 studies (range, 15%-30%) reported non-solicited AEs than the phase 3 studies (range, 6%-13%). The percentages of non-solicited AEs between vaccine groups for the pooled population differed by only 2%. The weighted risk ratio with 95% CIs showed no difference overall between the pooled Optaflu and control vaccines. Most AEs were either common illnesses expected in this population or known vaccine side effects.
There were no differences in severity profiles between the vaccine groups for all non-solicited AEs when compared by study or between the pooled vaccine groups.

The numbers and percentages of subjects experiencing non-solicited AEs within 6 months during study V58P4 were similar to those reported within 3 weeks in both the adult and elderly subjects. This reflects the change in the criteria for assessment of safety after 3 weeks, which only required that SAEs and AEs leading to premature withdrawal from the study were monitored.

- Serious adverse event/deaths

There were three SAEs leading to death reported, during the 3- week safety follow-up. All three deaths were in study V58P4, all in elderly subjects: carbon monoxide poisoning, cerebrovascular accident and hypertension, adenocarcinoma of the lung.

For the studies V58P4E1 and V58P9, and the US study V58P5, four additional SAEs reported after the 3-week follow-up led to death in adult and elderly subjects (compression of the neck, cerebral haemorrhage on day 127, sudden cardiac death on day 33, acute myocardial infarction on day 59). All were judged to be unrelated to the vaccination.

Overall, 57 subjects experienced a total of 68 non-fatal SAEs within the 3 weeks follow up or at any time for V58P4, all were judged as unrelated to the vaccinations. The criterion for seriousness in all but one SAE (elderly subject of V58P4 [i.e., pneumonia, Optaflu group]) included hospitalization. Three of these non-fatal SAEs led to premature withdrawal from the study. The majority of non-fatal SAEs (53/68) were reported by elderly subjects.

- Laboratory findings

Clinical hematological and urinary routine analyses (hematological, including erythrocyte sedimentation rate, hematochemical, urinary analyses) were carried out on samples provided by participants in V58P1 on the day of, but prior to vaccination and 21 days after the administration of the flu vaccines. There were no consistent significant changes.

- Safety in special populations

There were no specific studies in special populations.

- Discontinuation due to adverse events

In the adult population none was due to an AE. In elderly, there was one withdrawal due to an SAE in the Optaflu group in study V58P1 as follows: a 79-year old man (the oldest subject in the study) was administered Optaflu. On day 17 he was hospitalized due to syncope, which was classified in the case report form (CRF) as severe. The subject withdrew from the study, at which time the AE persisted.

With the exception of 3 deaths, there were no other discontinuations for AEs in the elderly population in study V58P4. In extension study V58P4E1 serious AEs lead to the discontinuation of 2 elderly subjects (one in each group) (both acute myocardial infarction).

- Discussion on clinical safety

The preclinical and quality dossiers did not suggest that the differences observed between the egg-derived and cell cultured formulation has changed the antigenic characteristics. Therefore clinical evaluation of safety according to the CHMP criteria laid down in Annex (CPMP/BWP/2490/00) is acceptable. With regard to the sample size, however, it was noted that a substantial number of subjects (n=2235) were entered twice in 2 different studies with an interval of one year; hence it was considered questionable whether these individuals could be handled as independent variables in the safety assessment. In an extensive re-analysis of the reactogenicity data it was shown that exclusion of extension study V58P4E1 did not significantly change the outcome. A drawback, however, was that the total exposure in the experimental group decreased to 2588 adult and elderly vaccinees. For the purpose of reactogenicity assessment, this sample size is still well above the minimum requirement and in agreement with the requirements of the Annex. Concerning adverse events, given the experience of the egg-derived formulation, the sample size is considered sufficient for the present application, provided that the follow up on safety and reactogenicity of the new formulation is sufficiently addressed in the Risk Management Plan.

Furthermore, the observed lower frequency of local and systemic reactions in the extension study seemed related to the fact that those individuals who had less local or systemic reactions continued in the extension group. The applicant reported on a planned extension study (V58P4E2), including all subjects originally enrolled. Although the study seems interesting and informative as far as the questions concerning immunogenicity and reactogenicity following repeated annual vaccination are concerned, it does not seem adequate to solve the question with regard to selection bias.

With regard to the other prerequisites for the safety analysis, all studies were conducted as observer blinded studies, and the pivotal study V58P4 was followed up of 6 months. An extensive overview was given for the local and systemic reactogenicity. Only injection site pain was found to occur more frequently in subjects vaccinated with Optaflu. On the basis of the data provided, it cannot be excluded

that injection site pain is a result of a local reaction to the injection, which may be more frequent for Optaflu formulation compared to the control formulation Agrippal. Since it concerns an event generally graded as mild and self-limiting, it is considered appropriate that the applicant commits to active surveillance and a proper statement in the SPC.

In addition, although overall the vaccine seems to be well tolerated, and comparable to the control vaccine, it is questioned why only severe cases of systemic reactogenicity were reported in subjects who received 2 subsequent doses of Optaflu (in 2 subsequent years). One cannot fully exclude increased systemic reactogenicity to Optaflu. However, the overall safety evaluation and the statistical analysis provided, indicated that these systemic events occur at a very low frequency and will probably not be of major clinical relevance. The measures proposed seem appropriate. The applicant commits to address this issue in the Risk Management Plan.

Non-solicited (severe) adverse events were reported at a low frequency in both treatment groups and age categories. There were no marked differences between groups, and more importantly no specific safety signal. None of the severe adverse events were considered to be related to the cell cultured or egg derived vaccine. Despite the fact that the reported frequencies did not suggest a significant difference between the cell derived and egg based formulations, in adult subjects vaccinated with Optaflu in the second year, more SAEs were reported (2% vs. 1% in the controls). All adverse events will be meticulously monitored in the PSUR (Follow-up measure).

The distribution and characteristics of the severe adverse events in the elderly population also reflect the underlying health status of the population, despite the inclusion criterion of being "healthy". It highlights the need for stratified analysis of the population with and without comorbidity. Despite the fact that high risk individuals will mostly benefit from influenza vaccination, there are no safety data available for patients at particular risk for influenza complications (adults and elderly with poorly controlled underlying diseases and children). A post-marketing, prospective cohort study is planned, which will include both healthy individuals and those with underlying conditions or potential complicating conditions. The concept on how these issues (both with regard to immunogenicity and safety) will be studied is addressed in the RMP.

5. Pharmacovigilance

Detailed description of the Pharmacovigilance system

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

Risk Management Plan

The MAA submitted a risk management plan.

Populations not studied include children, pregnant or lactating women and patients with relevant co-morbidities or certain disease severity.

Vaccinations in pregnant and lactating women should be followed-up. Furthermore, the applicant should discuss in PSURs the cumulative results of the observations in pregnant and lactating women.

The applicant should provide an updated Pharmacovigilance/risk management plan within two months following a positive CHMP decision, including full details of planned, ongoing and completed studies, as for instance the second extension study, the study in children and adolescents and the safety surveillance study (including protocols and timelines for the studies to be carried out).

Table Summary of the risk management plan

Safety concern	Proposed pharmacovigilance activities	Proposed risk

		minimisation activities
Identified risks		
Severe systemic reactions associated with repeated dosing	Extension study V58P4E2, Routine Pharmacovigilance activities, PSURs	Listed as ADR in section 4.8
Increased frequency of injection site pain compared to egg-based flu vaccines	Post-marketing prospective cohort study (V58T24), additional information from other trials , PSURs, Routine Pharmacovigilance activities	Listed as ADR in section 4.8 Specific note in section 4.8
Potential risks		
Anaphylaxis	Routine Pharmacovigilance activities, PSURs, Post-marketing prospective cohort study V58T24	Listed as ADR in section 4.8
Transient thrombocytopenia	Routine Pharmacovigilance activities, PSURs, Post-marketing prospective cohort study V58T24	Listed as ADR in section 4.8
Guillain Barré syndrome	Routine Pharmacovigilance activities, Post-marketing prospective cohort study V58T24, PSURs	Listed as ADR in section 4.8
Vasculitis	Routine Pharmacovigilance activities, Post-marketing prospective cohort study V58T24, PSURs	Listed as ADR in section 4.8
Encephalomyelitis	Routine Pharmacovigilance activities, Post-marketing prospective cohort study V58T24, PSURs	Listed as ADR in section 4.8
Missing Information		
Use in children	Routine Pharmacovigilance activities PSURs, clinical trial in children and adolescents (V58P12)	Indication in section 4.1
Use in pregnant/lactating women	Routine Pharmacovigilance activities, PSURs (cumulative data)	SPC section 4.6
Concomitant administration of other vaccines	Study arm in extension study V58P4E2, Routine Pharmacovigilance activities	SPC section 4.5

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

6. Overall conclusions, risk/benefit assessment and recommendation

Quality

During the evaluation of Optaflu one major objection was identified concerning the adventitious agents risk. Satisfactory responses have been provided to resolve it. Other minor concerns have been adequately addressed, however, several commitments are made by the applicant and several follow-up measures are defined to provide further information post-approval. In conclusion, all quality issues are resolved.

At the time of CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Risk-benefit balance of the product. The applicant gave a Letter of Undertaking and committed to resolve these as follow-up measures after the opinion, within an agreed timeframe.

Non-clinical pharmacology and toxicology

Regarding the comparability between egg-derived and MDKC derived vaccines, the primary Ferret study (CBI-PCS 007) clearly shows the comparability between the egg-derived Agrippal (used as positive control) and the cell-cultured product Optaflu. No significantly different results were observed in all the parameters analysed when comparing the test and positive control products.

Efficacy

The clinical database to substantiate the immunogenicity of Optaflu includes an appropriate number of vaccinees to address the relevant questions. The consistent use of the same observer blinded randomised design, patient selection, study methods and the follow up period of 6 months allows for an overall analysis of the immunogenicity and safety data.

Overall the studies indicate similar immunogenicity to the egg-based comparator vaccine, although there are still a few remaining follow up measures (FUM). The immunogenicity and safety profile appears to be acceptable, and comparable to the egg-derived control vaccine. From the data presented no new safety issues were identified compared to the current influenza vaccines.

In a agreement with the recommendation laid down in Annex (CPMP/BWP/2490/00) to the "Note for guidance on harmonisation of requirements for influenza vaccines" (CPMP/BWP/214/96), which extends this NfG with specific requirements pertinent to the marketing authorization of influenza vaccines produced in cell culture, the MAA has shown an acceptable immunogenicity (and safety) for Optaflu.

The lack of information in children is an important issue, although at present, the vaccine is claimed for use in adults aged 18 years or older. The applicant is requested to provide details/outlines of paediatric studies and time-lines when these will be carried out.

Safety

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

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

- User consultation

A diagnostic readability test (technical readability/traceability/comprehensibility/applicability) including scoring has been performed on the English version of the Patient Information Leaflet (PIL) and from the results it can be concluded that the relevant information is accessible and understandable for the user.

Risk-benefit assessment

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

- pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns.

- no additional risk minimisation activities were required beyond those included in the product information.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered that the risk-benefit balance of Optaflu in the "Prophylaxis of influenza for adults, especially in those who run an increased risk of associated complications" was favourable and therefore recommended the granting of the marketing authorisation.