SCIENTIFIC DISCUSSION

1. Introduction

An influenza pandemic is a global outbreak of influenza disease that occurs when a new type A influenza strain emerges in the human population, causes serious illness, and then spreads easily from person to person worldwide. Pandemics are different from seasonal outbreaks of influenza, as the latter are caused by subtypes of influenza viruses that are already present among people, whereas pandemic outbreaks are caused by new subtypes or by subtypes that have not circulated among people for a long time. Consequently, and in contrast to seasonal influenza, virtually all people are immunologically naïve for such a pandemic strain.

EMEA/CHMP have established a fast track assessment procedure for pandemic influenza vaccines, as described in the *Guideline on Submission of Marketing Authorisation Applications for Pandemic Influenza Vaccines through the Centralised Procedure* (CPMP/VEG/4986/03). The procedure involves the submission and evaluation of a core pandemic dossier during the interpandemic period, followed by a fast track assessment of the data for the recommended pandemic strain as a variation to the MAA. The dossier requirements for the core dossier are laid down in the *Guideline on Dossier Structure and Content for Pandemic Influenza Vaccine Marketing Authorisations Application* (CPMP/VEG/4717/03).

GlaxoSmithKline Biologicals has submitted a Marketing Authorisation Application (core pandemic dossier) for Daronrix in line with the above mentioned guidelines. Daronrix contains the mock-up strain H5N1 (NIBRG-14) derived by reverse genetics from the avian influenza virus A/Viet Nam/1194/2004. The vaccine contains a mixture of aluminium hydroxide and aluminium phosphate as adjuvant. Manufacturing, non-clinical and clinical information has also been gained with other mock-up vaccines containing the A/Hong Kong/1073/99 H9N2 and the A/Singapore/1/57 H2N2 influenza strains.

From an epidemiological point of view it is very unlikely that influenza strain A/Vietnam /1194/2004 would be the next pandemic strain, since the virus will either undergo further antigenic drift or the pandemic will be caused by another subtype of influenza vaccines (antigenic shift). Antigenic shift and drift are natural phenomenona related to all influenza viruses. For example, additional mutations will be required to enable the virus to transmit effectively from human to human. It is highly unlikely, therefore, that Daronrix containing the antigens from the strain derived from A/Vietnam /1194/2004 will provide adequate protection when using during a pandemic. In line with the developed core dossier concept, a variation would therefore have to be submitted to introduce the WHO/EU recommended strain, prepared from the influenza virus causing the pandemic, prior to use of Daronrix in a pandemic. This will assure that the pandemic vaccine will induce a satisfactory immune response to the influenza virus causing the pandemic. Daronrix has also not been developed for prophylactic use during the prepandemic period.

2. Part II: Chemical, pharmaceutical and biological aspects

Composition

GlaxoSmithKline applies for a Pandemic Influenza Vaccine which is mainly built up on the knowledge, equipment and manufacturing experience gained with the already licensed inter-pandemic (seasonal) split antigen vaccine Fluarix.

Daronrix is a suspension for injection, presented in multidose vials and multidose ampoules. Monodose pre-filled syringes and monodose ampoules are possible additional presentations.

The Mock-up vaccine application is based on the whole virion inactivated H5N1 Reverse Genetics Strain NIBRG 14, adjuvanted with aluminium. NIBRG 14 is derived from the highly pathogenic avian influenza strain A/Vietnam/1194/2004.

A 0.5 ml dose of the vaccines contains 15 µg H5N1 antigen. The total amount of aluminium (Adjuvant) per dose is 0.5 mg (0.45 mg as aluminium phosphate and 0.05 mg as aluminium hydroxide). Thiomersal is added as a preservative. The other ingredients used as buffer or to ensure isotonicity are sodium chloride, disodium phosphate dodecahydrate, potassium dihydrogen phosphate, potassium chloride, magnesium chloride hexahydrate.

Active substance

- ***Manufacture***

The manufacturing process of the monovalent bulks for the pandemic influenza vaccine can be divided in three main parts:

1. Propagation of the working seed virus in fertilized hen's eggs, harvesting and pooling of infected allantoic fluids to obtain the "crude monovalent whole virus bulk".
2. Purification of the crude monovalent whole virus bulk by an adsorption step and a sucrose gradient (isopycnic ultracentrifugation) leading to the "purified monovalent whole virus bulk".
3. The purified monovalent whole virus bulk is then diluted and sterile filtered. Then it is inactivated by incubation with formaldehyde, in order to obtain the "monovalent inactivated whole virus bulk", or "Monovalent Bulk".

The first and second part of the manufacturing process, propagation and purification, is similar to the process of the licensed interpandemic influenza vaccine from GSK (Fluarix), with as only difference a simplification in the fraction collection at the end of the ultracentrifugation. . The third part, inactivation of the monovalent bulk, differs from the Fluarix process since the pandemic vaccine is a whole virus vaccine without splitting process.

1. Production of the Crude Monovalent Whole Virus Bulk

The production of the vaccine is based on a seed lot system. A master seed (MS) and a working seed (WS) are prepared for the recommended pandemic virus strain. The total number of passages between the original virus and the WS does not exceed 15 and the final vaccine represents one passage from the Working Seed Lot. Each WS is tested for Sterility and Mycoplasma as well as for NA and HA identity, to confirm identity to the original prototype strain. During a pandemic, the applicant proposes to start with the production based on negative results for mycoplasma obtained by PCR. The release of the final container will then only be performed upon completion of all tests for absence of mycoplasma. The passages for the production of the master and working seeds are conducted in Specific Pathogen Free (SPF). For the production of monobulk material embryonated hens' eggs are obtained from healthy flocks corresponding to specifications laid down by the company.

The inoculum is prepared on the day of inoculation by diluting the working virus seed lot with phosphate buffer containing gentamicin sulfate and hydrocortisone. The eggs are inoculated with virus inoculum and incubated. At the end of incubation the eggs are killed by cooling. The allantoic fluid is harvested by egg harvesting machines and collected in thermo-regulated stainless steel tanks. At this stage the product is called "Crude Monovalent Whole Virus Bulk", which is immediately transferred to the clarification step.

2. Production of Purified Monovalent Whole Virus Bulk

The first step of purification is the clarification of the Crude Monovalent Whole Virus Bulk by moderate centrifugation to remove big particles (e.g. parts of egg shells). The second step permits to further clarify the allantoic fluid by an adsorption step, followed by a resuspension.

The resuspended influenza sediment is filtered through a 6-µm filter membrane to remove potential remaining pellets. The influenza virus is further purified (removal of proteins and phospholipids) and concentrated by isopycnic ultracentrifugation in a linear sucrose gradient. The gradient is formed

using two sucrose solutions containing thiomersal in order to control the process bioburden, as the centrifugation is performed at room temperature. Three different fractions are recovered by measuring the sucrose concentration via a refractometer. Depending on the biophysical characteristics of the selected pandemic strain, the ranges recovered for the 3 fractions may be modified. The upper limit of Fraction 2 is selected to balance between a high purity coefficient HA/protein and a maximum recovery of whole virus. The lower limit of Fraction 2 is selected on the basis of the HA content found in the low sucrose gradient range. Fraction 2 is stored at 2-8°C until the next manufacturing step.

3. Production of Monovalent Inactivated Whole Virus Bulk

Fraction 2 is diluted with phosphate buffered saline and filtered gradually ending with a sterile grade membrane of 0.22 µm. A sonication of the virus material is performed to facilitate the filtration.

After filtration an inactivation of the virus with formaldehyde is performed. The resulting material is distributed in 10-litre glass bottles (Type I) and called the "monovalent inactivated sterile whole virus bulk" or "monovalent bulk". Formulation of monovalent bulks takes place at Sächsisches Serumwerk Dresden (SSW) or alternatively at GlaxoSmithKline Biologicals (GSK Bio) in Rixensart. The storage temperature is preserved during transfer.

In addition to the control of the monovalent inactivated whole virus bulk according to the release specifications, a variety of in-process tests is performed at the main manufacturing steps. Internal consistency limits will be established from historical data generated on a minimum of 15 batches produced at full industrial scale.

Process validation was performed on 3 batches of monovalent bulk (H5N1) manufactured at full scale. Following parameters were evaluated to demonstrate process consistency: preparation of virus inoculation, inoculation and incubation of embryonated eggs, harvesting step, clarification by separation, adsorption and filtration, ultracentrifugation, Fraction 2 after dilution and filtration, final sterile filtration and inactivation. The removal of ovalbumin and phospholipids, neuraminidase identity, process yield, residual gentamicin sulphate and hydrocortisone were evaluated. Inactivation of A/Viet Nam/1194/2004 (H5N1) NIBRG-14 during the manufacturing of whole inactivated virus monobulks was validated on 3 batches (at commercial scale). From the data presented it can be concluded that the formaldehyde inactivation guarantees complete inactivation of A/Viet Nam/1194/2004 (H5N1) NIBRG-14. Validation of mycoplasma inactivation by formaldehyde was performed using lots of H9N2 vaccine manufactured at pilot scale.

- ***Specification***

Release specifications for the monovalent inactivated, whole virus bulk were set taking into account relevant pharmacopoeia and guideline texts.

All analytical methods applied to the pandemic influenza monovalent bulk are also applied to the licensed interpandemic vaccine Fluarix, and were fully validated for Fluarix. For some methods specific validations for the pandemic monovalent bulk have been conducted in order to demonstrate that the performance of the analytical methods is not adversely impacted by the composition of the pandemic vaccine matrix. The concerned methods are sterility, thiomersal content, endotoxin content (LAL), sucrose content and formaldehyde content, and results are provided. The validation reports for the assays for the determination of the haemagglutinin content, ovalbumin content and protein content are provided: the company committed to set specification limits for these 3 assays on basis of data generated on a minimum of 15 batches produced at industrial scale.

- ***Stability of the monovalent bulk***

H9N2 and H5N1 monovalent bulks were included in the stability programme. Monovalent H9N2 bulks were stored at 2-8 °C for up to 12 months. The data on HA content measured by SRD clearly show a dramatic reduction of 70% and more. To further analyse the stability of the product the applicant developed a mouse potency test and defined that, for a vaccine to meet the stability criteria, the antibody response of the vaccine in stability should not be significantly different from the antibody

response elicited by the vaccine at release. In practice, this criterion implies that, upon stability, the titres measured for the vaccine dilutions that are within the linear part of the response curve (mainly vaccine dilutions of 1:5 and 1:25) must not differ by more than one dilution step (1 $\log_2$) with the titres obtained at release. No loss of immunity was seen with the mouse potency assay over a storage period of 12 months. Stability results for 4 H5N1 bulks showed that the decrease in HA content measured by SRD is less marked than with H9N2: about 75 % of the initial HA content remained after 6 months storage at 2-8°C..

A shelf life for the monovalent bulk of 3 months at 2-8 °C is supported by the stability data and was accepted at the time of initial authorisation.

Other ingredients

All excipients are European Pharmacopoeia Grade. Aluminium phosphate (adjuvant) is not described in the European Pharmacopoeia and is controlled following a GSK monograph.

Product development and finished product

- ***Pharmaceutical Development***

The development of GSK Biologicals' pandemic influenza vaccine builds on the experience with the licensed interpandemic split antigen influenza vaccine (Fluarix). The approach for pandemic influenza vaccines is to increase vaccine supply resources by using whole virus instead of split and adjuvanting the vaccine with aluminium.

In an early development phase, the reactogenicity profiles of monovalent aluminium adjuvanted whole virus vaccines were investigated using lots derived for the H3N2 strain. To evaluate immunogenicity in unprimed populations, monovalent "mock-up pandemic vaccines" (with the reference viruses A/Singapore/1/57 (H2N2) and A/Hong Kong/1073/99 (H9N2)) were tested in two bioequivalence studies. The H9N2 strain offered the opportunity to study the vaccination with an avian virus that was transmissible to humans, but had not been circulating in humans so far.

The present core pandemic dossier describes the H5N1 mock-up vaccine. Supportive information and results obtained with the clinical lots derived from the other three strains (H9N2, H3N2 and H2N2) are also provided.

- ***Manufacture of the Product***

The manufacturing process of the pandemic influenza final vaccine consists of three steps:

1. Formulation of the final bulk by mixing the monovalent bulk with the adjuvant and excipients:
   - Preparation of the aluminium adjuvant
   - Adsorption of the influenza monovalent bulk.

The aluminium adjuvant is produced shortly before the formulation. The autoclaved aluminium adjuvant is stored at 2 – 8 °C awaiting final formulation with the influenza monovalent bulk. The validation of the shelf life of 6 months at room temperature is ongoing and will be submitted as soon as they become available. For the adsorption process, the calculated amount of formulation buffer is first transferred to the formulation vessel, followed by the calculated volume of Thiomersal stock solution, aluminium adjuvant and monovalent bulk. The final bulk is stirred for 15 minutes to allow adsorption of the antigen.

The formulated final bulks will be kept in the formulation vessel until filling into final containers at SSW, or alternatively filled into high-density polyethylene (HDPE) containers and shipped to Rixensart or Wavre for filling into final containers. For filling at SSW, the storage duration in the formulation vessel is set to maximum 7 days at 2-8°C prior to the filling start. The HDPE containers are wrapped with two polyethylene plastic bags each, sealed and stored at 2-8°C prior to shipping to GSK Bio Rixensart or Wavre for filling and packaging. Currently, the final bulk is held in the HDPE containers for up to 30 days prior to filling in final containers.
The final bulk will be tested for sterility, Thiomersal content and free formaldehyde. The company will test the completeness of adsorption of the HA to the aluminium (in accordance of the PhEur

monograph on vaccines for human use) of the first 15 full-scale batches as characterisation testing. If the degree of adsorption is stable, than this testing will be discontinued.

2. Filling of the vaccine into final containers
3. Labeling and packaging.

- ***Product Specification***

The specifications have been set in accordance to existing pharmacopoeias and guidance. In terms of the heamagglutinin (HA) content, the applicant explored alternatives to confirm the identity and potency of the final vaccine. The use of a sandwich Enzyme Linked Immunosorbent Assay (ELISA) following desorption was investigated. The proposal of the applicant to use the ELISA test to assess the antigen content in the final vaccine, as well as the identity is acceptable and should be used to complement the mouse potency test.

Stability of the product

A mouse potency test has been developed to evaluate the antigen stability because the Single Radial Diffusion (SRD) method cannot be applied to adsorbed formulations. The stability of the H5N1 final containers was evaluated by testing 4 batches of adsorbed vaccines and 4 batches of unadsorbed vaccine in this mouse potency assay. The HI titres measured for the adsorbed and unadsorbed vaccine lots remain stable for up to at least 6 months. Taking into account the reduced shelf life of the monovalent bulk (3 months) and supportive data obtained with H9N2 final lots, the proposed shelf-life of 12 months at 2 – 8 °C was accepted at the time of initial authorisation.

Nevertheless, since stability of the vaccine is also strain dependent the stability program of the H5N1 final lots will be completed as committed and any out of specification results should be communicated immediately.

Issues related to manufacture and quality control under pandemic conditions

Due to the constrains in a pandemic situation the applicant provided additional information with regard to the optimisation of the manufacturing process, alternative testing for mycoplasma, the supply of SPF and production eggs and measures already in place to comply with BSL2+ containment.

The handling of the production parameters that may be strain-dependent are already optimised due to the time constrains of the yearly production. To further improve the virus adsorption to the adjuvant significant modifications in the manufacturing process would be needed, thus the company will therefore continue to apply the formulation process described in the MAA, which is acceptable.

For a faster testing of the seed material for mycoplasma the applicant proposes to start with the production based on negative results obtained by PCR. The release of the final container will then only be performed upon completion of all tests for absence of mycoplasma.

With regard to the availability of eggs the applicant stated that from January 2006 onwards a full year capacity of total production amount of eggs is available from two different qualified suppliers (pandemic back up in case of pandemic threat).

3. Part III: Toxico-pharmacological aspects

The manufacturing process of the whole virion antigen of Daronrix is similar to the approved manufacturing process of the seasonal inactivated split vaccine Fluarix (the splitting and purification steps are specific for the Fluarix). Taking this into consideration, the applicant submitted a reduced non-clinical package for Daronrix. This is in accordance with the guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application, CPMP/VEG/4717/03.

Pharmacodynamics

Two primary pharmacodynamic studies were ongoing at the time of authorisation, and the applicant committed to provide the final reports post authorisation (follow-up measures). The humoral and cellular immuneresponse to whole cell adjuvanted influenza vaccine is investigated in naïve C57Bl/6 mice. In a second study the immunogenicity of whole cell adjuvanted influenza vaccine and protection against homologous challenge is evaluated in naïve ferrets. Both studies are using the H5N1 A/Vietnam/1194/2004 vaccine.

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

Pharmacokinetics

Experimental studies to demonstrate absorption, distribution, metabolism, and excretion of the active ingredients in Daronrix have not been performed. This is in line with the relevant guidelines CPMP/SWP/465/95 and CPMP/VEG/4717/03.

Toxicology

- *Single dose toxicity / repeat dose toxicity (with toxicokinetics)*

Daronrix is a whole virion inactivated influenza vaccine. The manufacturing process of the antigen is similar to the approved manufacturing process of the seasonal inactivated split vaccine Fluarix (except for the deoxycholate splitting step which is specific for the Fluarix) thus the no single dose toxicity/ repeat dose toxicity studies are required according CPMP/VEG/4717/03.

- *Genotoxicity, carcinogenicity and reproduction toxicity*

No genotoxicity, carcinogenicity and reproduction toxicity studies were conducted. This is in line with the relevant guidelines CPMP/SWP/465/95 and CPMP/VEG/4717/03.

- *Local tolerance*

The applicant has conducted a local tolerance studies with influenza candidate vaccines H5N1 and H5N1/A1 in male and female rabbits after two intramuscular injections at 27 µg/dose. The test vaccines were compared to the reference vaccine (Fluarix) and saline, which acted as the control.

Erythema at the injection site was observed in one two female rabbits, one in the H5N1 treated group and one in the H5N1/A1 treated group.

Fasciitis was observed in all vaccine treated groups, indicating an inflammatory response. There was no difference in severity between the different vaccines.

Perivascular cuffing as observed in the vaccine treated animals is consistent with an inflammatory reaction induced locally by a vaccine formulation. There was no difference in severity or incidence between the vaccines.

The muscle necrosis recorded only for the H5N1/A1 vaccine suggests of a more irritant response, when compared with the other test and reference vaccines.

The microscopic findings of granulomatous/needle track myositis were comparable across all groups, indicating a local inflammatory reaction caused by the administration method rather than the candidate vaccines (H5N1 and H5N1/A1) or reference vaccine (Fluarix).

In conclusion, 27µg/500µl-dose of H5N1/A1 induces an increased local effect (muscle necrosis) as compared to H5N1 and Fluarix.

- *Other toxicity studies*

Animal studies with inactivated adjuvanted virus vaccines (e.g. RSV, measles virus) have shown evidence of vaccine-enhanced disease (treatment with vaccine and subsequently challenged with live virus). Aluminium adjuvanted inactivated virus vaccines may induce too strong a Th2 response, which, upon challenge with live virus, results in vigorous T-cell recall producing immune-enhanced disease. There is therefore a theoretical concern that alum adjuvanted whole virus vaccines, when given to naïve populations (e.g. infants, young children) might predispose them to (even) more serious influenza disease during a pandemic (immune enhancement).

The applicant will address this issue in the ferret challenge model to evaluate the protective efficacy of candidate flu vaccines. An outline of the study to investigate the possible disease enhancement with Daronrix was submitted. If the results of these studies are inconclusive a study in a cotton rat model is envisaged. The applicant commits to submit the non-clinical reports (immunogenicity study in naïve mice and challenge studies in naïve ferrets) when they become available and submits a variation to amend the product information, e.g. if the outcome of animal studies would raise any concerns for the use of this vaccine in children.

- *Ecotoxicity/environmental risk assessment*

After injection, the active substance is taken up by immunocompetent cells and metabolised. The aluminium adjuvant and some other excipients may be excreted in the environment, but in concentrations that do not merit concern.

Overall, it can be concluded that neither the inactivated whole influenza virus nor the excipients will enter in the environment in quantities that merit ecological concern.

4. Part IV: Clinical aspects

Clinical trials on protective efficacy for the mock-up vaccine cannot be performed. Therefore a detailed characterisation of the immunological response to the mock-up vaccines is required. The vaccine virus strains chosen for these studies should allow simulating a situation where the target population for vaccination is immunologically naïve.

The criteria for these studies are laid down the Guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application, CPMP/VEG/4717/03. With no other criteria to suggest at present, mock-up vaccine should be able to elicit sufficient immunological response to meet all three of the current standards set for existing vaccines in adults or older adults laid down in CPMP/BWP/214/96:

In adults aged 18-60 years:

- Number of seroconversions or significant increase in antihaemagglutin antibody titre > 40%
- Mean geometric increase > 2.5;
- Proportion of subjects achieving an Haemagglutin inhibition (HI) titre $\geq$ 40 or SRH titre $\geq$ 25 mm^2 > 70%.

In adults > 60 years:

- Number of seroconversions or significant increase in antihaemagglutin antibody titre > 30%
- Mean geometric increase > 2.0;
- Proportion of subjects achieving an HI titre $\geq$ 40 or SRH titre $\geq$ 25 mm^2 > 60%.

In addition neutralising antibodies should be present. The development program for Daronrix is based on this guideline.

Early investigations on the reactogenicity profile of a monovalent aluminium adjuvanted whole virus vaccine were performed using lots derived from the H3N2 strain included in the licensed interpandemic vaccine during the season of 1998-1999, i.e. A/Sydney/5/97. Following demonstration of clinically acceptable rates of local and general symptoms in this study, further studies were performed with A/HongKong/1073/99 (H9N2) and H2N2.

H9N2 represents an avian virus, which is transmissible to humans, but had not been circulating in the human population so far. H2N2 circulated from 1957 until 1968. Thus, individuals born after 1968 were also regarded to be immunologically naïve.

Since the avian influenza strain H5N1 strain considered as a possible candidate to cause the next influenza pandemic, the applicant decided to base the mock-up dossier on studies performed (immunogenicity and safety) with A/Vietnam/1194/2004 (H5N1) strain containing vaccine

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

Clinical pharmacology

Pharmacodynamics

In relation to vaccines, pharmacodynamic studies are essentially included in the immunogenicity studies that characterise the immune response to vaccines. The detailed characterisation of the immunological response to the mock-up vaccines is the surrogate parameter for efficacy (CPMP/VEG/4717/03) and these data are discussed below.

Pharmacokinetics

Pharmacokinetic studies were not performed in accordance with the Guideline on clinical evaluation of new vaccines (CHMP/VWP/164653/2005) and the Guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application (CPMP/VEG/4717/03).

Clinical efficacy

The reactogenicity profiles of the monovalent aluminium adjuvanted whole virus formulations (A/H3N2) containing decreased antigen doses were evaluated in feasibility study Flu-037 in healthy subjects aged between 18-60 years (see safety).

Following demonstration of clinically acceptable rates of local and general symptoms in the latter study, study Flu-038 was performed in immunologically naïve (18-30 years old, born after 1968) and primed cohorts (>30 years old, born before 1968) with different doses of a monovalent whole virus vaccine (H2N2 strain) to evaluate immunogenicity.

In study Flu-041, the immunogenicity of different doses of a monovalent whole virus vaccine (avian strain H9N2) was assessed in an unprimed cohort of 18-60 year old subjects. The study also included two monovalent vaccine groups using A/H2N2, the strain used in study Flu-038, for comparative purpose.

Both studies (Flu-038 and Flu-041) were conducted with non-circulating influenza strains to establish the proof of concept for a pandemic influenza vaccine and indicated the need for a two-dose schedule.

Study Flu-045, evaluated the cell-mediated and humoral immune response of a monovalent aluminium-adjuvanted whole virus formulation (A/H9N2) according to two different schedules in a 18-30 year old cohort considered as immunologically naïve to the H9N2 strain. The study aimed to determine the most optimal vaccination regimen in case of influenza pandemic.

Study Flu-059 evaluated the reactogenicity and immunogenicity of different formulations of a monovalent (H9N2) whole virus vaccine (using different antigen doses, with or without aluminium as adjuvant) in subjects above 60 years of age.

The pivotal study H5N1-001 was conducted in healthy adults aged 18 to 60 years with a monovalent adjuvanted whole virion vaccine containing the mock-up strain H5N1 (A/Vietnam/1194/2004).

Dose-response studies and main clinical studies

- **Flu-038**

Study Flu-038 was an open, randomised, comparative, multi-centre trial with four groups to evaluate the immunogenicity and safety/reactogenicity of a monovalent H2N2 whole virus vaccine as compared to a monovalent H2N2 split virus vaccine.

Four hundred (400) healthy adults and patients with well-controlled underlying diseases (cardiovascular, respiratory or metabolic disorder), who were 18-30 years old (unprimed cohort) or above 30 years old (primed cohort) were enrolled in the study. Subjects were randomised to receive vaccination with a monovalent aluminium-adjuvanted whole virus formulation (A/H2N2) of different antigen concentrations (1.9, 3.8 and 7.5 µg HA per dose) or a split virus vaccine (15 µg HA per dose) without aluminium adjuvant according to a 2-dose schedule (day 0, day 21).

Results: For the unprimed cohort (18-30 years old), seroconversion to haemagglutination inhibition (HI) antibodies was seen in 17.6%-29.2% of vaccine recipients ten days after the first dose, and seroprotective levels of HI titres (≥ 1:40) were obtained in 19.1% to 31.3% of subjects in the four study groups. Twenty-one days after the first dose, 40.0%-58.3% of the 18-30 year old subjects showed seroconversion and 40.0%-62.5% had seroprotective HI titres. No significant dose response effect was seen after the first dose based on any of the serological criteria.

After the second dose, GMTs, seroconversion rates, seroprotection rates and seroconversion factors tended to be higher with increasing amounts of HA per dose, although the group immunized with 3.8 µg HA demonstrated consistently higher immunological parameters than the group immunized with 7.5 µg HA. The highest percentage of individuals with seroprotective titres was observed in the groups vaccinated with either 3.8 µg HA or 15 µg HA.

In the primed cohort of 31-60 years old, seroconversion and seroprotection rates ranged between 8.8%-83.3% and 76.5%-100% respectively in the four groups ten days after the first dose, and between 11.8%-86.1%% and 76.5%-100% respectively at 21 days.

After the second dose, all subjects except 3 from the 1.9 µg HA vaccine group had seroprotective HI titres. In the primed cohort of >60 years old, all subjects were seroprotected after the second dose with seroconversion rates ranging from 38.5% to 81.8%.

- **Flu-041**

Study Flu-041 was an open, randomised, comparative, multi-centre trial with six groups to evaluate the immunogenicity and safety/reactogenicity of a monovalent H9N2 whole virus vaccine.

Three-hundred (300) healthy adults and patients with well-controlled underlying diseases, who were 18-30 years old and above 30 years old (unprimed cohort) were enrolled. Subjects were randomised to receive vaccination with a monovalent aluminium-adjuvanted whole virus formulation (A/H9N2) containing 1.9, 3.8 or 7.5 µg HA per dose or a non-adjuvanted whole virus vaccine (15 µg HA per dose) according to a 2-dose schedule (day 0, day 21).

Two other groups received a monovalent aluminium-adjuvanted whole virus formulation (A/H2N2) containing 1.9 µg HA per dose or a monovalent non-adjuvanted split virus vaccine (15 µg HA per dose).

Results: In the H9N2 adjuvanted vaccine groups, seroconversion and seroprotection rates ranged between 16.7%-28% and 20.8%-28% respectively 10 days after the first dose in 18-30 years old, and between 33.3%-40% and 37.5%-40% respectively at 21 days.

In the comparator group (H9N2 plain vaccine, 15 µg HA), seroconversion and seroprotection rates were 25.0% and 33.3% respectively 10 days after the first dose, and 50.0% and 58.3% respectively at 21 days.

Following administration of a second dose at day 21, the GMTs, seroconversion factors, seroconversion rates and seroprotection rates increased in all H9N2 vaccine groups, fulfilling all CHMP requirements for annual registration procedures of influenza vaccines. Similar trends in the immune response were observed following administration of the H2N2 adjuvanted whole virus vaccine (1.9 µg HA).

In subjects above 30 years old who received H9N2 formulations, all serological parameters were decreased as compared to those seen in the 18-30 year old age group.

- **Study H5N1-001**

Study H5N1-001 is a partially-blind randomised multicentre study in adults aged between 18 and 60 years designed to evaluate the reactogenicity and immunogenicity of one and two doses of pandemic monovalent (H5N1) influenza vaccines (whole virus formulation) administered at different doses (3.8 µg, 7.5 µg, 15 µg and 27 µg HA) adjuvanted or not with Aluminium salts.

- **Study Participants**

The study subjects were healthy adults aged between 18 and 60 years. Female subjects were either of non-childbearing potential, i.e., either surgically sterilised or one year post-menopausal. For women of childbearing potential, abstinence or using adequate contraceptive was required from 30 days prior to first vaccination, until two month after completion of the vaccination series.

The ATP cohort for analysis of immunogenicity included all evaluable subjects for whom data concerning immunogenicity endpoint measures are available. This included subjects for whom assay results are available for antibodies against at least one study vaccine antigen component after vaccination.

- **Treatments**

Subjects were randomised to receive vaccination monovalent whole virus influenza vaccines H5N1 (A/Vietnam/1194/2004) adjuvanted or not with Aluminium salts at different doses (3.8 µg, 7.5 µg, 15 µg and 27 µg HA) adjuvanted or not with Aluminium salts. The subjects were vaccinated at day 0 and day 21.

- **Objectives**

The primary objectives were to evaluate the safety and reactogenicity and the humoral immune response (in term of anti-haemagglutinin antibody) of one and two doses of pandemic monovalent (H5N1) whole influenza vaccines containing different antigen doses adjuvanted or not with Al.

The secondary objectives were to evaluate the humoral immune response (in term of neutralising antibody) and cell-mediated immune response (CMI) of one and two doses of pandemic monovalent (H5N1) whole influenza vaccines containing different antigen doses, adjuvanted or not with Al.

Outcomes/endpoints

The co-primary endpoints were defined as follows:

- Geometric mean titer (GMT) of serum anti-HA antibodies with 95% CI at days 0, day 21 and days 42.
- Seroconversion rate with 95% CI at day 21 day and day 42 defined as the proportion of subjects with either a pre-vaccination anti-HA titer < 1:10 and a post-vaccination titre ≥ 1:40, or a pre-vaccination titer ≥ 1:10 and a minimum four-fold increase in post-vaccination titer.
- Seroprotection rate with 95% CI at day 0, 21 and day 42 defined as the proportion of subjects with a serum anti-HA titer ≥ 1:40.
- Conversion factor at day 21 and day 42 defined as the fold increase in serum anti-HA GMT on day 21 or 42 compared to day 0.

Sample size

The target sample size was 400 enrolled subjects (50 subjects in each group) in order to reach 360 evaluable subjects (45 subjects in each group).

The co-primary GMT endpoint was used to estimate the sample size. Using a conservative approach, the alpha error has been corrected (divided by 6) to allow up to six independent comparisons between vaccine groups to assess the adjuvantation effect (with or without Al) and haemagglutinin-dose effect (3.8, 7.5, 15 and 27 µg of HA).

A sample size of 45 evaluable subjects per group had 96% power to detect a 3-fold increase in the H5N1 antibody response between two groups, assuming the common deviation is 0.5 (in log unit) and using a two-group- t-test with a 0.008 two-sided significance level.

- **Results**

Baseline data

The demographic profile of the different vaccine groups of subjects was comparable with respect to mean age, gender and racial distribution. In the total vaccinated cohort, the mean age at the time of informed consent was 35.4 years with a standard deviation of 13.25 years. Female subjects (58.5%) were more represented than male subjects and the population was predominantly white/caucasian (99.8%).

Numbers analysed

The Total Vaccinated cohort included all vaccinated subjects for whom data are available. For the Total analysis of immunogenicity, this included vaccinated subjects for whom data concerning immunogenicity endpoint measures are available. The Total Vaccinated cohort analysis was performed per treatment actually administered.

For each treatment, at each time-point when a serological result was available the following data were tabulated:

- Geometric mean antibody titers (GMTs) of anti-HA antibodies with 95% CIs.
- Seroconversion factor of anti-HA antibodies with 95% CIs.
- Seroconversion rate of anti-HA antibodies with 95% CIs.
- Seroprotection rate of anti-HA antibodies with 95% CIs.

Outcomes and estimation

Hemagglutination inhibition responses against vaccine strain H5N1 A/Vietnam/1194/2004 of the monovalent pandemic influenza A vaccine (H5N1) in adults from study H5N1-001 (ATP immunogenicity cohort)

Study (Age of vaccination)	Timepoint	Strain	HA (µg per dose)	Al	N	GMT			SCF			SCR			SPR		
						Value	95% CI		GMR	95% CI		%	95% CI		%	95% CI	
							LL	UL		LL	UL		LL	UL		LL	UL
H5N1-001 18-60 yrs	Pre	H5N1 whole	27	-	48	5.6	4.7	6.7	-	-	-	-	-	-	2.1	0.0	11.1
		H5N1 whole	15	-	47	5.1	4.9	5.3	-	-	-	-	-	-	0.0	0.0	7.5
		H5N1 whole	7.5	-	48	5.1	4.9	5.5	-	-	-	-	-	-	0.0	0.0	7.4
		H5N1 whole	3.8	-	49	5.0	5.0	5.0	-	-	-	-	-	-	0.0	0.0	7.2
		H5N1 whole	27	Al	49	5.6	4.8	6.5	-	-	-	-	-	-	4.1	0.5	14.0
		H5N1 whole	15	Al	48	5.5	4.8	6.2	-	-	-	-	-	-	2.1	0.0	11.1
		H5N1 whole	7.5	Al	49	5.5	5.0	6.1	-	-	-	-	-	-	0.0	0.0	7.2
		H5N1 whole	3.8	Al	49	5.8	5.0	6.8	-	-	-	-	-	-	2.0	0.0	10.8
	Post I (D21)	H5N1 whole	27	-	48	39.4	22.6	68.7	7.0	4.1	12.2	54.2	39.2	68.6	56.3	41.2	70.5
		H5N1 whole	15	-	47	23.2	14.6	36.7	4.5	2.9	7.1	46.8	32.1	61.9	46.8	32.1	61.9
		H5N1 whole	7.5	-	48	24.0	13.8	41.5	4.7	2.7	8.0	43.8	29.5	58.8	43.8	29.5	58.8
		H5N1 whole	3.8	-	49	18.5	11.2	30.4	3.7	2.2	6.1	32.7	19.9	47.5	32.7	19.9	47.5
		H5N1 whole	27	Al	49	80.6	46.4	139.7	14.5	8.3	25.4	69.4	54.6	81.7	73.5	58.9	85.0
		H5N1 whole	15	Al	48	32.7	19.3	55.4	6.0	3.5	10.1	47.9	33.3	62.8	50.0	35.2	64.8
		H5N1 whole	7.5	Al	49	31.7	19.4	51.7	5.7	3.5	9.4	49.0	34.4	63.7	53.1	38.3	67.5
		H5N1 whole	3.8	Al	49	37.5	21.4	65.7	6.4	3.7	11.3	49.0	34.4	63.7	55.1	40.2	69.3
	Post II (D42)	H5N1 whole	27	-	48	72.8	42.3	125.4	13.0	7.1	23.6	70.8	55.9	83.0	70.8	55.9	83.0
		H5N1 whole	15	-	47	55.7	36.1	86.1	10.9	7.1	16.8	70.2	55.1	82.7	70.2	55.1	82.7
		H5N1 whole	7.5	-	48	40.9	24.1	69.3	7.9	4.7	13.4	58.3	43.2	72.4	58.3	43.2	72.4
		H5N1 whole	3.8	-	49	28.7	17.5	46.9	5.7	3.5	9.4	51.0	36.3	65.6	51.0	36.3	65.6
		H5N1 whole	27	Al	49	180.4	114.6	283.9	32.4	19.6	53.6	89.8	77.8	96.6	89.8	77.8	96.6
		H5N1 whole	15	Al	48	67.7	40.2	114.1	12.4	7.1	21.8	70.8	55.9	83.0	70.8	55.9	83.0
		H5N1 whole	7.5	Al	49	50.5	29.9	85.3	9.1	5.4	15.6	63.3	48.3	76.6	63.3	48.3	76.6
		H5N1 whole	3.8	Al	49	61.6	37.0	102.6	10.5	6.2	18.0	67.3	52.5	80.1	69.4	54.6	81.7

SCF: seroconversion factor (i.e ratio of the post-vaccination GMT and the pre-vaccination GMT); SCR: seroconversion rate (i.e proportion of subjects who were either seronegative at pre-vaccination and have a protective post-vaccination titre of ≥ 1:40, or who were seropositive at pre-vaccination and have a 4-fold increase in titre); SPR: seroprotection rate (i.e proportion of subjects with HI titre ≥ 1:40)

Clinical studies in special populations

- **Eldery population: Flu-059**

Study Flu-059 was an open, randomised, comparative, multicentre trial with seven groups to evaluate the humoral immune response of various formulations of a monovalent H9N2 whole virus vaccine.

Three hundred eighty five (385) healthy elderly subjects above 60 years of age and patients with well-controlled underlying disease in the same age group were enrolled. Subjects were randomised to receive vaccination with a monovalent whole virus formulation (A/H9N2) containing 1.9, 3.8, 7.5 or 15 µg HA per dose without aluminium adjuvant or a monovalent aluminium-adjuvanted whole virus vaccine (A/H9N2) containing 1.9, 3.8 or 7.5 µg HA per dose according to a 2-dose schedule (day 0, day 21).

Results: Ten days after administration of the first dose, seroconversion and seroprotection rates ranged from 1.9%-21.8% and 11.1%-34.5% respectively in the plain H9N2 vaccine groups, as compared to 15.7%-25.9% and 21.6%-37.0% respectively in the adjuvanted H9N2 vaccine groups.

At day 42, a seroconversion factor above 2 was obtained in all H9N2 vaccine groups. GMTs tended to be higher with the aluminium-adjuvanted formulation as compared to the plain formulation for the 7.5 µg HA dosage in particular. In initially seronegative subjects who received the H9N2 aluminium adjuvanted vaccine containing 7.5µg HA, seroprotective levels of 56.4% were found.

Results obtained meet the required CHMP criteria for seroconversion (>30%) and geometric mean titre increase (>2.0). However seroprotective levels at day 42 of 56.4% did not meet the pre-defined CHMP requirement for elderly subjects (>60%).

Supportive studies

- **Flu-045**

Study Flu-045 was an open, randomised, comparative, monocentre trial with two groups to evaluate the immunogenicity (humoral, cell mediated) of a monovalent H9N2 vaccine according to two vaccination schedules.

Fifty subjects aged 24.7 ± 2.45 years old (mean ± SD) were enrolled. Subjects were randomised to receive vaccination with a monovalent aluminium-adjuvanted whole virus formulation (A/H9N2) containing 3.8 µg HA per dose according to two different schedules: a 0, 10 day or 0, 63 day schedule.

Results: Ten days after administration of the first dose, seroconversion and seroprotection rates were 12.0% and 16.0% respectively following the 0, 10-day schedule, and 25.0% for both values following the 0, 63 day schedule.

Eleven days after administration of the second dose in the 0, 10-day group (Day 21), seroconversion and seroprotection rates increased up to 80.0% and 84.0% respectively.

In the 0, 63 day group, 25.0% of subjects had protective levels of HI titres prior to administration of the second dose (Day 63) as compared to 87.5% eleven days after the second dose (Day 74).

In both groups, all CHMP criteria were fulfilled eleven days after the second dose.

The HI GMT at day 74 indicated that higher antibody levels were obtained if two doses of vaccine were administered several weeks apart instead of several days apart. While acknowledging the limitations of the CMI analysis (existing pre-vaccination CMI response, heterogeneity of responses between groups, small sample size), an increase in the cellular immune response in terms of both lymphoproliferation and IFN-γ secretion was observed post vaccination and remained stable over time. However, no boosting effect on the CMI response was observed irrespective of the schedule used.

Discussion on clinical efficacy

The development of Daronrix benefits from the experience with Fluarix, GSK Bio's interpandemic influenza vaccine. Fluarix is an inactivated split influenza vaccine containing 15 µg haemagglutinin (HA) of each of the three influenza virus strains (A/H1N1, A/H3N2 and B). In order to increase

vaccine supply opportunities in case of influenza pandemic, several modified vaccine formulations have been investigated.

Initial dose finding studies with an H2N2 vaccine established the dose of 3.8 µg HA to achieve adequate seroprotection in the age group 18 to 30 years and to fulfill all three criteria defined by CHMP (CPMP/BWP/214/96). This was confirmed for the H9N2 strain. However subsequent investigation of the immunogenicity of this dosage in the age group 30 to 60 revealed that only borderline values for seroprotection were achieved, although the required CHMP criteria for seroconversion (≥40%) and geometric mean titre increase (≥2.5) were largely exceeded. From the individual serology data, it becomes apparent, that many of the vaccinees included in the clinical trials show some pre-existing immunity against the H9N2 antigen. Thus, these subjects may not be regarded as immunologically naïve and allow no conclusion on the immunogenicity of the vaccine in a pandemic situation, where a really 'new' influenza virus strain/antigen is circulating.

Subsequently, the dossier was shifted to a H5N1 mock-up vaccine and further data were provided for this vaccine in order to establish efficacy of the mock-up vaccine.

For H5N1, more than 90% of the vaccinees were seronegative prior to vaccination. All three CHMP criteria are fulfilled by vaccines containing 27 or 15 µg hemagglutinin (HA). For 7.5 and 3.8 µg HA adjuvanted with aluminium, seroconversion rate and seroconversion factor are in compliance with CHMP requirements, while seroprotection rate with 63.3%, and 69.4% respectively, slightly fail the set requirement of 70%. On that basis, a pandemic mock-up vaccine containing 15 µg HA, achieving a seroprotection rate of 70.8% could be approved. However, it has to be noted that 27 µg HA achieve a superior protection rate of nearly 90% and in terms of vaccine efficacy may be the preferred option.

The applicant commits to provide the data on neutralising antibodies and CMI with the final study report of study H5N1-001.

No data are provided with respect to the immunogenicity and safety of a H5N1 mock-up vaccine in the elderly. However, in study Flu-059 the humoral immune response of various formulations of a monovalent H9N2 whole virus vaccine was evaluated in healthy elderly subjects above 60 years of age. Results obtained in this study meet the required CHMP criteria for seroconversion (>30%) and geometric mean titre increase (>2.0). For initially seronegative subjects who received the H9N2 aluminium adjuvanted vaccine containing 7.5µg HA, seroprotective levels of 56.4% were obtained at day 42, which did not meet the pre-defined CHMP requirement for elderly subjects (>60%). The small sample size limits the significance of these results.

Clinical safety

Study H5N1-001

Patient exposure

Overall 400 subjects were enrolled and vaccinated in the study. The primary analysis of safety and reactogenicity was based on the total vaccinated cohort. No subject was excluded from the ATP cohort for analysis of safety.

Adverse events

A tabulation of solicited local and general symptoms can be found in the following tables:

The percentage of doses followed by solicited local symptoms (pain, redness, swelling, ecchymosis, induration) including those of grade 3 intensity (Total vaccinated cohort)

Study (schedule)			Pain			Redness			Swelling			Ecchymosis			Induration		
	N		%	95%CI		%	95%CI		%	95%CI		%	95%CI		%	95%CI	
Group				LL	UL		LL	UL		LL	UL		LL	UL		LL	UL
H5N1-001 (2 dose schedule at 0, 21 days) in 18 to 60 years old																	
H5N1 whole	98	Total	51.0	40.7	61.3	17.3	10.4	26.3	8.2	3.6	15.5	0.0	0.0	3.7	9.2	4.3	16.7
(HA 27µg)		Grade 3	0.0	0.0	3.7	0.0	0.0	3.7	0.0	0.0	3.7	0.0	0.0	3.7	0.0	0.0	3.7
H5N1 whole	98	Total	45.9	35.8	56.3	13.3	7.3	21.6	7.1	2.9	14.2	3.1	0.6	8.7	11.2	5.7	19.2
(HA 15µg)		Grade 3	1.0	0.0	5.6	0.0	0.0	3.7	0.0	0.0	3.7	0.0	0.0	3.7	0.0	0.0	3.7
H5N1 whole	100	Total	40.0	30.3	50.3	11.0	5.6	18.8	8.0	3.5	15.2	2.0	0.2	7.0	6.0	2.2	12.6
(HA 7.5µg)		Grade 3	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6
H5N1 whole	100	Total	29.0	20.4	38.9	12.0	6.4	20.0	2.0	0.2	7.0	2.0	0.2	7.0	4.0	1.1	9.9
(HA 3.8µg)		Grade 3	0.0	0.0	3.6	1.0	0.0	5.4	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6
H5N1 whole	102	Total	55.9	45.7	65.7	10.8	5.5	18.5	6.9	2.8	13.6	0.0	0.0	3.6	6.9	2.8	13.6
(HA 27µg/ Al)		Grade 3	2.0	0.2	6.9	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6
H5N1 whole	98	Total	57.1	46.7	67.1	19.4	12.1	28.6	9.2	4.3	16.7	1.0	0.0	5.6	8.2	3.6	15.5
(HA 15µg/ Al)		Grade 3	0.0	0.0	3.7	0.0	0.0	3.7	0.0	0.0	3.7	0.0	0.0	3.7	0.0	0.0	3.7
H5N1 whole	100	Total	64.0	53.8	73.4	13.0	7.1	21.2	10.0	4.9	17.6	3.0	0.6	8.5	16.0	9.4	24.7
(HA 7.5µg/ Al)		Grade 3	1.0	0.0	5.4	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6	1.0	0.0	5.4
H5N1 whole	101	Total	45.5	35.6	55.8	12.9	7.0	21.0	10.9	5.6	18.7	3.0	0.6	8.4	14.9	8.6	23.3
(HA 3.8µg/ Al)		Grade 3	1.0	0.0	5.4	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6

N = number of doses followed by at least one solicited symptom sheet completed;
% = percentage of doses followed by a report of the specified symptom;
95% CI = exact 95% confidence interval; L.L. = lower limit, U.L. = upper limit
Grade 3 pain = severe (pain that prevents normal activity) ; Grade 3 redness, swelling, induration = largest surface diameter >50mm

The percentage of doses followed by solicited general symptoms (fatigue, fever, headache, myalgia, shivering) including those of grade 3 intensity and those considered to be related to vaccination (Total vaccinated cohort)

Study (schedule) Group	N	Relationship to vaccination	Fatigue %	95%CI LL	95%CI UL	Fever %	95%CI LL	95%CI UL	Headache %	95%CI LL	95%CI UL	Myalgia %	95%CI LL	95%CI UL	Shivering %	95%CI LL	95%CI UL
H5N1-001 (2 dose schedule at 0, 21 days) in 18 to 60 years old																	
H5N1 whole (HA 27µg)	98	Total	13.3	7.3	21.6	2.0	0.2	7.2	12.2	6.5	20.4	17.3	10.4	26.3	9.2	4.3	16.7
		Grade 3	0.0	0.0	3.7	0.0	0.0	3.7	0.0	0.0	3.7	0.0	0.0	3.7	0.0	0.0	3.7
		Related	5.1	1.7	11.5	2.0	0.2	7.2	3.1	0.6	8.7	8.2	3.6	15.5	4.1	1.1	10.1
H5N1 whole (HA 15µg)	98	Total	11.2	5.7	19.2	2.0	0.2	7.2	20.4	12.9	29.7	11.2	5.7	19.2	4.1	1.1	10.1
		Grade 3	0.0	0.0	3.7	1.0	0.0	5.6	1.0	0.0	5.6	1.0	0.0	5.6	0.0	0.0	3.7
		Related	3.1	0.6	8.7	0.0	0.0	3.7	5.1	1.7	11.5	5.1	1.7	11.5	2.0	0.2	7.2
H5N1 whole (HA 7.5µg)	100	Total	15.0	8.6	23.5	2.0	0.2	7.0	26.0	17.7	35.7	18.0	11.0	26.9	3.0	0.6	8.5
		Grade 3	1.0	0.0	5.4	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6	1.0	0.0	5.4
		Related	7.0	2.9	13.9	1.0	0.0	5.4	7.0	2.9	13.9	8.0	3.5	15.2	1.0	0.0	5.4
H5N1 whole (HA 3.8µg)	100	Total	19.0	11.8	28.1	1.0	0.0	5.4	25.0	16.9	34.7	14.0	7.9	22.4	4.0	1.1	9.9
		Grade 3	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6
		Related	8.0	3.5	15.2	1.0	0.0	5.4	9.0	4.2	16.4	5.0	1.6	11.3	2.0	0.2	7.0
H5N1 whole (HA 27µg/ Al)	102	Total	22.5	14.9	31.9	4.9	1.6	11.1	24.5	16.5	34.0	25.5	17.4	35.1	12.7	7.0	20.8
		Grade 3	2.9	0.6	8.4	0.0	0.0	3.6	2.0	0.2	6.9	2.9	0.6	8.4	1.0	0.0	5.3
		Related	9.8	4.8	17.3	4.9	1.6	11.1	8.8	4.1	16.1	8.8	4.1	16.1	8.8	4.1	16.1
H5N1 whole (HA 15µg/ Al)	98	Total	11.2	5.7	19.2	2.0	0.2	7.2	11.2	5.7	19.2	7.1	2.9	14.2	3.1	0.6	8.7
		Grade 3	1.0	0.0	5.6	0.0	0.0	3.7	1.0	0.0	5.6	0.0	0.0	3.7	0.0	0.0	3.7
		Related	7.1	2.9	14.2	1.0	0.0	5.6	5.1	1.7	11.5	3.1	0.6	8.7	1.0	0.0	5.6
H5N1 whole (HA 7.5µg/ Al)	100	Total	13.0	7.1	21.2	2.0	0.2	7.0	10.0	4.9	17.6	23.0	15.2	32.5	6.0	2.2	12.6
		Grade 3	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6	0.0	0.0	3.6
		Related	7.0	2.9	13.9	0.0	0.0	3.6	1.0	0.0	5.4	12.0	6.4	20.0	0.0	0.0	3.6
H5N1 whole (HA 3.8µg/ Al)	101	Total	21.8	14.2	31.1	2.0	0.2	7.0	24.8	16.7	34.3	18.8	11.7	27.8	11.9	6.3	19.8
		Grade 3	3.0	0.6	8.4	0.0	0.0	3.6	3.0	0.6	8.4	2.0	0.2	7.0	1.0	0.0	5.4
		Related	6.9	2.8	13.8	0.0	0.0	3.6	5.0	1.6	11.2	7.9	3.5	15.0	2.0	0.2	7.0

N = number of doses followed by at least one solicited symptom sheet completed; % = percentage of doses followed by a report of the specified symptom;
95% CI = exact 95% confidence interval; L.L. = lower limit, U.L. = upper limit
Grade 3 = severe (symptom that prevents normal activity); Grade 3 fever = >39°C

(cont'd): The percentage of doses followed by solicited general symptoms (sweating increase, arthralgia) including those of grade 3 intensity and those considered to be related to vaccination (Total vaccinated cohort)

Study (schedule) Group	N	Relationship to vaccination	Sweating increase %	95%CI LL	95%CI UL	Arthralgia %	95%CI LL	95%CI UL
H5N1-001 (2 dose schedule at 0, 21 days) in 18 to 60 years old								
H5N1 whole (HA 27µg)		Total	5.1	1.7	11.5	5.1	1.7	11.5
		Grade 3	0.0	0.0	3.7	0.0	0.0	3.7
		Related	3.1	0.6	8.7	2.0	0.2	7.2
H5N1 whole (HA 15µg)		Total	2.0	0.2	7.2	5.1	1.7	11.5
		Grade 3	0.0	0.0	3.7	1.0	0.0	5.6
		Related	0.0	0.0	3.7	2.0	0.2	7.2
H5N1 whole (HA 7.5µg)		Total	4.0	1.1	9.9	6.0	2.2	12.6
		Grade 3	0.0	0.0	3.6	0.0	0.0	3.6
		Related	1.0	0.0	5.4	3.0	0.6	8.5
H5N1 whole (HA 3.8µg)		Total	8.0	3.5	15.2	7.0	2.9	13.9
		Grade 3	0.0	0.0	3.6	0.0	0.0	3.6
		Related	0.0	0.0	3.6	4.0	1.1	9.9
H5N1 whole (HA 27µg/ Al)		Total	7.8	3.4	14.9	17.6	10.8	26.4
		Grade 3	1.0	0.0	5.3	2.9	0.6	8.4
		Related	5.9	2.2	12.4	7.8	3.4	14.9
H5N1 whole (HA 15µg/ Al)		Total	3.1	0.6	8.7	4.1	1.1	10.1
		Grade 3	0.0	0.0	3.7	0.0	0.0	3.7
		Related	1.0	0.0	5.6	3.1	0.6	8.7
H5N1 whole (HA 7.5µg/ Al)		Total	8.0	3.5	15.2	13.0	7.1	21.2
		Grade 3	0.0	0.0	3.6	0.0	0.0	3.6
		Related	4.0	1.1	9.9	7.0	2.9	13.9
H5N1 whole (HA 3.8µg/ Al)		Total	5.0	1.6	11.2	9.9	4.9	17.5
		Grade 3	1.0	0.0	5.4	1.0	0.0	5.4
		Related	3.0	0.6	8.4	4.0	1.1	9.8

N = number of doses followed by at least one solicited symptom sheet completed; % = percentage of doses followed by a report of the specified symptom;
95% CI = exact 95% confidence interval; L.L. = lower limit, U.L. = upper limit
Grade 3 = severe (symptom that prevents normal activity)

During the 7-day follow up period, pain at the injection site was the most commonly reported solicited local symptom in all vaccine groups. There was a trend for a higher incidence of pain with the adjuvanted formulations as compared to the plain ones. Overall, only five cases of grade 3 pain were observed (one in the plain vaccine group containing 15µg HA, two in the adjuvanted group with 27µg HA and one in each of the adjuvanted groups containing 7.5µg HA and 3.8µg HA). Incidences of redness, swelling, ecchymosis and induration were reported with lower frequencies. Symptoms of grade 3 intensity were rarely reported: only one case of grade 3 redness was observed in the plain vaccine group containing 3.8µg HA, and one case of grade 3 induration in the adjuvanted vaccine group containing 7.5µg HA.

Regarding the incidence of solicited general symptoms during the 7-day follow-up period, fatigue, myalgia and headache were the most commonly reported adverse events. These symptoms were uncommonly graded as severe. A trend for a higher incidence of grade 3 cases was observed in the adjuvanted vaccine groups as compared to the plain ones. Eight cases of grade 3 fatigue (one with 7.5µg HA, 3 with 27µg HA/Al, 1 with 15µg HA/Al and 3 with 3.8µg HA/Al), 7 cases of grade 3 headache (one with 7.5µg HA, 2 with 27µg HA/Al, 1 with 15µg HA/Al and 3 with 3.8µg HA/A) and 6 cases of grade 3 myalgia (one with 7.5µg HA, 3 with 27µg HA/Al and 2 with 3.8µg HA/Al) were reported overall. Other general solicited symptoms were mild or moderate in intensity. Of note, fever was reported with a frequency ≤ 2.0% in all vaccine groups (except in the aluminium adjuvanted group containing 27µg HA where the incidence was 4.9%) with only one case of grade 3 fever in the plain vaccine group (7.5µg HA).

Unsolicited symptoms of any or grade 3 intensity, or considered as causally related to vaccination were reported with similar frequencies with the plain and adjuvanted formulations. In the plain vaccine groups, 10.2% to 22.0% of doses were followed by unsolicited symptoms, as compared to 13.0% to 20.4% of doses in the adjuvanted vaccine groups. Unsolicited symptoms graded as severe or considered as causally related to vaccination were observed following 0.0% to 4.0% and 0.0%-1.0% of doses respectively in the plain groups, as compared to 1.0% to 4.0% and 0.0%-4.0% of doses respectively in the adjuvanted groups.

No data on co-administration of the mock-up vaccine with other vaccines are available. This is reflected in the SPC under section 4.5.

Adverse events and serious adverse events/deaths

In study H5N1-001, two serious adverse events (SAEs) were reported during the study period until Day 51 post vaccination. Both SAEs were reported in the plain vaccine groups (one with 15µg HA and one with 7.5µg HA) and were assessed as not related to vaccination.

Safety in special populations

No safety data are available for patients at particular risk for influenza complications (children, adults with poorly controlled underlying diseases).

Supportive studies

In total 1329 subjects were vaccinated with a monovalent vaccines containing H2N2, H9N2 or H3N2. About 735 vaccinees received the adjuvanted vaccine.

The adjuvanted whole virus formulation with the final 3.8µg antigen (H2N2) content was studied in the study Flu-038, and local solicited signs were reported more frequently than general solicited symptoms. There was an age-dependent decrease in the frequency of reporting.

Pain was the predominant solicited local AE reported by 75% of subjects below 30 years and 38% above 30 years of age. Only one dose was followed by a grade 3 pain. No severe (grade 3) induration, redness or swelling was reported.

Fatigue (8% to 28 %), headache (6% to 23%), malaise (0% to 22%), myalgia (11% to 25%) and pain in limb (6% to 13%) were the predominant solicited general AEs. None of these AEs were considered causally related to the vaccination and there was no general symptom with a grade 3.

The adjuvanted H9N2 whole virus vaccine with the final 3.8µg antigen content was studied in the 18 to 60 years old population (Flu-41) and in an elderly population above 60 years (Flu-059). The results found with H9N2 strain showed similar results than the ones generated for the H2N2 strain and local solicited signs were reported more frequently than general solicited symptoms). There was also an age-dependent decrease in the frequency of reporting.

Pain was the predominant solicited local AE reported after 10.9% to 62% of doses in all subjects. Redness, swelling and induration were reported following 8.2% to 26%, 7.3% to 28% and 18.8% to 32% of doses, respectively. Only one dose was followed by a grade 3 pain. No severe (grade 3) induration, redness or swelling was reported.

Fatigue (4.5% to 22%), headache (6.4% to 10%), malaise (8% to 10%), myalgia (4.5% to 36%) and pain in limb (6% to 16%) were the predominant solicited general AEs. The incidence of general symptoms related to vaccination was low (8% or less), except for myalgia (28% in 18-30 year old subjects). Three general symptoms (i.e. myalgia, fatigue, malaise) were reported with a grade 3 score.

A total of 11 subjects reported SAEs. All SAEs except one were considered to be unrelated to vaccination. One SAE (faciocephalgia) was assessed by the investigator as unlikely related to vaccination. The study vaccination was discontinued and the subject was withdrawn from the study.

- Post marketing experience

Data from post-marketing surveillance with interpandemic trivalent vaccines are included in the SPC as per the requirement of core SPC for pandemic influenza vaccines

Discussion on clinical safety

An increased local reactogenicity (specifically for pain) was observed with the adjuvanted formulations as compared to the plain vaccines. However, incidences of general adverse events were usually comparable between these formulations except for arthralgia which tended to be more frequently reported in the adjuvanted vaccine groups containing 27µg HA and 7.5µg HA, as compared to the respective non adjuvanted formulations. Symptoms of grade 3 intensity (local or general), and general symptoms related to vaccination were reported with low frequencies. Differences observed between adjuvanted and plain vaccine groups were therefore considered to be clinically acceptable.

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 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.

- **Summary of the risk management plan for Daronrix**

Safety issue	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Neurological adverse events (e.g. GBS)	- Active surveillance in pandemic cohorts - Passive surveillance (via spontaneous reporting of adverse events – GSKBio's routine pharmacovigilance system)	Mentioned as occurring as a class effect in trivalent vaccines in section 4.8 of the SPC.

Safety profile of the final pandemic vaccine	- Pandemic cohorts - Special PSUR reporting requirements in the pandemic situation	NA
Immunogenicity of the final pandemic vaccine	Pandemic cohorts (subset of subjects)	NA
Limited safety data in children, pregnant women, individuals with clinically severe underlying medical conditions and immunocompromised individuals	- Routine pharmacovigilance - Active surveillance as part of pandemic cohort study - Plan of clinical trial programme to investigate these groups is being provided as a FUM.	Appropriate information on lack of data in these groups in sections 4.2, 4.6 and 5.1 of the SPC

6. Overall conclusions and benefit/risk assessment

Quality

The application is for a core pandemic dossier, based on data generated with 3 mock-up strains: H5N1, H9N2 and H2N2. The data with the two latter strains is supportive to the data generated with the H5N1 reverse genetics strain, derived from A/Viet Nam/1194/2004.

The manufacture of the drug substance, the monovalent antigen bulk, has been validated at full-scale with the H5N1 antigen. The specifications set for the bulk antigen and the final vaccine are appropriate. The stability of the monovalent H5N1 antigen bulk is 3 months at 2-8 °C and of the drug product is 12 months at 2-8 °C.

Due to the constrains in a pandemic situation the applicant provided additional information with regard to the optimisation of the manufacturing process, alternative testing for mycoplasma, the supply of SPF and production eggs and measures already in place to comply with BSL2+ containment.

Non-clinical pharmacology and toxicology

The applicant submitted a reduced non-clinical package for Daronrix, this is in accordance with the Guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application (CPMP/VEG/4717/03).

Animal studies with inactivated adjuvanted virus vaccines (e.g. RSV, measles virus) have shown evidence of vaccine-enhanced disease (treatment with vaccine and subsequently challenged with live virus). Aluminium adjuvanted inactivated virus vaccines may induce too strong a Th2 response, which, upon challenge with live virus, results in vigorous T-cell recall producing immune-enhanced disease. There is therefore a theoretical concern that alum adjuvanted whole virus vaccines, when given to naïve populations (e.g. infants, young children) might predispose them to (even) more serious influenza disease during a pandemic (immune enhancement).

The applicant will address this issue in the ferret challenge model to evaluate the protective efficacy of candidate flu vaccines. The applicant commits to submit the non-clinical reports (immunogenicity study in naïve mice and challenge studies in naïve ferrets) when they become available and submits a variation to amend the product information, e.g. if the outcome of animal studies would raise any concerns for the use of this vaccine in children

Efficacy

For H5N1, more than 90% of the vaccinees were seronegative prior to vaccination. All three CHMP criteria are fulfilled by vaccines containing 27 or 15 µg haemagglutinin (HA). For vaccines containing 7.5 and 3.8 µg HA adjuvanted with aluminium, seroconversion rate and seroconversion factor are in compliance with CHMP requirements, while seroprotection rate with 63.3%, and 69.4% respectively, slightly fail the set requirement of 70%. On that basis, a pandemic mock-up vaccine containing 15 µg HA, achieving a seroprotection rate of 70.8% could be approved. However, it has to be noted that 27

µg HA achieve a superior protection rate of nearly 90% and in terms of vaccine efficacy may be the preferred option.

No data are provided with respect to the immunogenicity and safety of a H5N1 mock-up vaccine in the elderly. However, in study Flu-059 the humoral immune response of various formulations of a monovalent H9N2 whole virus vaccine was evaluated in healthy elderly subjects above 60 years of age. Results obtained in this study meet the required CHMP criteria for seroconversion (>30%) and geometric mean titre increase (>2.0). For initially seronegative subjects who received the H9N2 aluminium adjuvanted vaccine containing 7.5µg HA, seroprotective levels of 56.4% were obtained at Day 42, which did not meet the pre-defined CHMP requirement for elderly subjects (>60%). The small sample size limits the significance of these results.

The CHMP concluded that in absence of data with the H5N1 mock-up vaccine in subjects over 60 years of age, it is not possible to establish a posology in elderly. However, from Public Health considerations, the CHMP did not consider it appropriate to restrict the indication to adults from 18-60 years.

Regarding the use in children, there are some remaining (theoretical) concerns on possible disease enhancement in a naïve population primed with a whole virus aluminium adjuvanted vaccine. This theoretical risk of immune enhancement has not yet been studied.

The applicant reflects these issues in the SPC (general indication in section 4.1, no dose recommendations for elderly and children in section 4.2 and data presented in section 5.1).

Furthermore the applicant commits to perform immunogenicity and safety studies in elderly (>60 years of age) and children (3 to 9 years of age) with Daronrix. The applicant will update the CHMP on an annual basis of the study progress.

Safety

During the 7-day follow up period, pain at the injection site was the most commonly reported solicited local symptom. Incidences of redness, swelling, ecchymosis and induration were reported with lower frequencies. Regarding the incidence of solicited general symptoms during the 7-day follow-up period, fatigue, myalgia and headache were the most commonly reported adverse events.

The safety profile of the vaccine, as it is revealed by the clinical studies performed, is satisfactory, especially in a pandemic situation.

From the safety database all the adverse reactions reported in clinical trials and post-marketing data from the interpandemic trivalent vaccines 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

The applicant performed a readability testing on the English version of the package leaflet (PL) in 10 persons using a semi-structured questionnaire.

In conclusion, the main objectives of the user consultation have been achieved, namely to assess the readability of the PL, to identify problems regarding comprehensibility and usefulness of the information and to describe possible changes to the PL to improve readability.

Benefit/risk assessment

An influenza pandemic is a global outbreak of influenza disease that occurs when a new type A influenza strain emerges in the human population, causes serious illness, and then spreads easily from person to person worldwide. Though there may be no vaccines available at the beginning of a pandemic, efficacious and safe vaccines are regarded as an important tool to counteract this severe threat to public health, allowing protection from (severe) disease or death. The formulation of a pandemic vaccine has to take into account that, in contrast to seasonal influenza, all people will be immunologically naïve for the circulating pandemic strain. This naivety is expected to make it more difficult to elicit a protecting immune response in vaccinees. However, it may deserve some further

discussions, what serological status and background immunity against new haemagglutinins may be characteristic for a naïve population.

With this background the applicant developed Daronrix, a monovalent, whole virion, inactivated and adjuvanted vaccine, containing 15 µg haemagglutinin (HA) from the influenza strain A/Vietnam /1194/2004 (H5N1) per 0.5 ml dose. In the submitted core pandemic dossier, the applicant reported, as required according CPMP/VEG/4717/03, the manufacturing experience and testing and the findings from non-clinical tests and clinical trials using the NIBRG 14 (H5N1) mock-up strain.

Clinical trials on protective efficacy for the mock-up vaccine cannot be performed. Therefore a detailed characterisation of the immunological response to the mock-up vaccines is required. The vaccine virus strains chosen for these studies should allow simulating a situation where the target population for vaccination is immunologically naïve. The evaluation of the clinical efficacy is mainly based on the quantification of HI titres in vaccinees and subsequent analysis of the derived parameters seroprotection rate, seroconversion rate and factor, what generally is accepted as surrogate markers for efficacy of influenza vaccines. The criteria for these studies are laid down the Guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application, CPMP/VEG/4717/03. All three CHMP criteria are fulfilled with the vaccine containing 15 µg haemagglutinin (HA) from the influenza strain A/Vietnam /1194/2004 (H5N1) per 0.5 ml dose. The initial studies with whole virion, inactivated and adjuvanted H2N2 and an H2N9 vaccines are supportive to the clinical trial done with the H5N1 vaccine.

The safety profile of the mock-up vaccine is acceptable.

From an epidemiological point of view it is very unlikely that influenza strain A/Vietnam /1194/2004 would be the next pandemic strain, since the virus will either undergo further antigenic drift or the pandemic will be caused by another subtype of influenza vaccines (antigenic shift). Antigenic shift and drift are natural phenomenona related to all influenza viruses. For example, additional mutations will be required to enable the virus to transmit effectively from human to human. It is highly unlikely, therefore, that Daronrix containing the strain derived from A/Vietnam /1194/2004 will provide protection when using during a pandemic. In line with the developed core dossier concept, a variation would have to be submitted to introduce the WHO/EU recommended strain prepared from the influenza virus causing the pandemic, prior to use of Daronrix in a pandemic. This will assure that the vaccine will induce a satisfactory immune response to the pandemic influenza virus.

For the same scientific reasons, and in absence of any studies demonstrating that antibodies elicited by Daronrix (containing the strain derived from A/Vietnam /1194/2004) will react with other H5N1 subtypes (in the neutralising antibody assay), this vaccine has not been demonstrated to have a role in use in the prepandemic period. No predictions can be made of the immunogenicity of Daronrix against strains other than A/Viet Nam/1194/2004.

During the pandemic, the applicant will collect safety and effectiveness data of the pandemic vaccine and submit this information to the CHMP for evaluation (specific obligation).

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

During the pandemic, the applicant will conduct a prospective cohort study as identified in the pharmacovigilance plan (specific obligation).

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by concensus that the risk-benefit balance of Daronrix for the prophylaxis of influenza in an officially declared pandemic situation was favourable and therefore recommends the granting of the marketing authorisation under exceptional circumstances.

The CHMP recommends granting this marketing authorisation for Daronrix under exceptional circumstances, because in the present stage of knowledge comprehensive scientific information required for the vaccine containing the actual pandemic strain cannot be gathered.
The missing scientific information relates to the safety and effectiveness of the pandemic vaccine. These data can only be obtained once the actual strain causing the pandemic is included in the vaccine and during actual use of the vaccine. Therefore the company has agreed the following specific obligations:

- To collect, during the pandemic, clinical safety and effectiveness data of the pandemic vaccine and submit this information to the CHMP for evaluation.
- To conduct, during the pandemic, a prospective cohort study as identified in the Pharmacovigilance plan.