EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Assessment report

Pumarix

Common name: Pandemic influenza vaccine (H5N1) (split virion, inactivated, adjuvanted)

Procedure No. EMEA/H/C/001212

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

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1. Background information on the procedure

1.1. Submission of the dossier

The applicant GlaxoSmithKline Biologicals S.A. submitted on 10 July 2009 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Pumarix, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 29 January 2008.

The applicant applied for the following indication:

Prophylaxis of influenza in an officially declared pandemic situation. Pandemic influenza vaccine should be used in accordance with official guidance.

The legal basis for this application refers to:

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

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

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included EMA Decision P/81/2009 for the following condition:

- Influenza infection caused by an influenza strain contained in the vaccine or related to a strain contained in the vaccine.

on the agreement of a paediatric investigation plan (PIP).

The PIP is not yet completed.

Information relating to Orphan Market Exclusivity

Similarity

Not applicable.

Market Exclusivity

Not applicable.

Licensing status

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

1.2. Steps taken for the assessment of the product

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

Rapporteur: **Ian Hudson** Co-Rapporteur: **Barbara van Zwieten-Boot**

- The application was received by the EMA on 10 July 2009.
- Accelerated Assessment procedure was agreed-upon by CHMP on 20th-23rd July 2009.
- The procedure started on 06th August 2009.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 12th August 2010. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 14th August 2009. In accordance with Article 6(3) of Regulation (EC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days.
- During the meeting on 17th-20th August 2009, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 24st August 2009.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 25th June 2010.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 3rd September 2010.
- During the CHMP meeting on 20th-23rd September 2010, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP consolidated List of Outstanding Issues on 15th October 2010.
- The Rapporteur circulated the Joint Assessment Report on the applicant's responses to the list of outstanding issues to all CHMP members on 1st November 2010.
- During the meeting on 15th-18th November 2010, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation under exceptional circumstances to Pumarix on 18th November 2010. The applicant provided the letter of undertaking on the specific obligations and follow-up measures to be fulfilled post-authorisation on 17th November 2010.

2. Scientific discussion

2.1. Introduction

An influenza pandemic is a global outbreak of influenza disease that occurs when a type A influenza strain to which most or all humans are immunologically naïve emerges to cause clinically apparent 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 circulating in the world whereas pandemics are caused by new subtypes or by subtypes that have not circulated among people for a long time.

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

Both H and N antigens of influenza A and B viruses may undergo minor antigenic changes (antigenic drift) over time. Antigenic drift necessitates regular (most often annual) updating of the A and B strains that are included in inter-pandemic (seasonal) influenza vaccines.

Major changes (antigenic shift) in haemagglutinins occur much less commonly and only in type A strains but are of great importance to human health. Antigenic shifts may occur *via* serial mutations or by gene re-assortment. The H1N1v strain responsible for the current pandemic is a triple reassortant containing genes variably derived from avian, swine and human adapted strains.

Specific guidance has been developed for the fast track assessment procedure for pandemic influenza vaccines[1], which can only be used once WHO/EU have officially declared the pandemic. The procedure involves the submission and evaluation of a core pandemic dossier during the inter-pandemic period, followed by a fast track assessment of the data for replacing the mock-up vaccine strain with the recommended pandemic strain as a variation to the MAA. GlaxoSmithKline Biologicals has submitted a Marketing Authorisation Application (core pandemic dossier) for Pumarix in line with the above mentioned guidelines.

Pumarix is a split virion inactivated influenza vaccine, containing the mock-up strain H5N1 derived by reverse genetics from the avian influenza virus A/Indonesia/05/2005 (H5N1) The final formulation contains 3.75 µg haemagglutinin (HA) per 0.5 ml dose adjuvanted by AS03. Pumarix is indicated for prophylaxis of influenza in an officially declared pandemic situation. Pandemic influenza vaccine should be used in accordance with official guidance.

From an epidemiological point of view it is very unlikely that influenza strain A/Indonesia/05/2005 (H5N1) would be the next pandemic strain, since the H5N1 virus continues to undergo antigenic drift. It is also possible that the next pandemic will not be caused by a H5N1 virus but will be due to another subtype of influenza virus (e.g. with haemagglutinin of type H2, H7 or H9). In line with the 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 Pumarix in a pandemic. Pumarix is not indicated for prophylactic use during the prepandemic period.

Using immune parameters to predict protection

Influenza virus infection elicits host production of antibody against several viral components but the major antigens are the haemagglutinin and neuraminidase molecules. Antibody to haemagglutinin

[1] *Guideline on Submission of Marketing Authorisation Applications for Pandemic Influenza Vaccines through the Centralised Procedure* (CPMP/VEG/4986/03).
Guideline on Dossier Structure and Content for Pandemic Influenza Vaccine Marketing Authorisations Application (CPMP/VEG/4717/03).

(anti-HA) is thought to play a major role in immunity and is effective in neutralising virus infectivity. The role of anti-neuraminidase antibody in protection is less clear and this is rarely measured except for specific experimental reasons. Serum neutralising antibody is functional but is not routinely measured because these assays are labour intensive and require use of cell cultures.

There are data to indicate that HI titres of at least 1:40 and SNA titres of at least 1:8 may correlate with protection against seasonal influenza in young healthy people. HI titres of 1:10 – 1:20 are associated with lesser degrees of protection. Some authors propose that HA titres of 1:65 and SNA titres of 1:80 are required for good protection, especially in the elderly. The data seem to go back to Meikeljohn *et al*. (1952), Hobson *et al*. (1972) and Potter and Oxford (1979). In particular the latter study suggested that HI titres of about 1:30-1:40 correlated with about 50% protection (at least in healthy adults) against challenge with the then current seasonal strain. Overall, the basis for these cut-offs for predicting protection against seasonal influenza cannot be considered to be definitive and may not be applicable in a pandemic situation.

The clinical studies presented in the application have assessed the safety and immunogenicity of Pumarix ("Q-Pan H5N1", manufactured in Saint-Foy, Quebec, Canada) in adults. One of these studies directly compared Pumarix with Pandemic influenza vaccine (H5N1) (split virion, inactivated, adjuvanted) GlaxoSmithKline Biologicals ("D-Pan H5N1", manufactured in Dresden, Germany).

2.2. Quality aspects

2.2.1. Introduction

Pumarix is a split inactivated influenza vaccine. The final formulation contains 3.75 µg haemagglutinin (HA) of A/Indonesia/05/2005/PR8-IBCDC-RG2 (H5N1) per 0.5 ml dose adjuvanted by AS03.

The reference virus for Pumarix used in the clinical development programme is A/Indonesia/05/2005/PR8-IBCDC-RG2 (H5N1), which was developed by the US Centre for Disease Control (CDC) using reverse genetics. The reassortment strain combines the H5 and N1 segments to the PR8 strain backbone. In addition the H5 was engineered to eliminate the polybasic stretch of amino-acids at the HA cleavage site that is responsible for high virulence of the original strains. The virus is propagated in fertilised hens eggs.

The vaccine consists of a suspension vial with the H5N1 antigen and an oil-in-water emulsion vial with the AS03 adjuvant, which are mixed extemporaneously. Thiomersal, at a final concentration of 10 µg/ml (5 µg per dose) in the adjuvanted vaccine, is added because of the multi-dose presentation.

The composition of antigen and adjuvant vial can be found in tables 1 and 2 respectively:

Table 1: Composition of a unit dose of inactivated split virion H5N1 drug product - antigen component

Ingredients	Quantity (per dose 0.25 mL)	Function	Reference
Active substance			
Split-virion Monovalent, A/H5N1, A/Indonesia/5/2005	3.75 µg HA (15µg /mL)	Immunogen	GSK Monograph
Excipients			
Thiomersal	5µg (20µg/mL)	Preservative	USP
Phosphate Buffered Saline, pH 7.2 composed of[1]:			
Sodium chloride	2.125mg	Buffer component	USP
Potassium Chloride	0.05mg		USP
Disodium Hydrogen Phosphate (Na_2HPO_4)	0.363mg		USP
Potasium Dihydrogen Phosphate (KH_2PO_4)	0.05mg		NF
Water for injections	q.s 0.25mL		USP

NF= National Formulary; USP= United States Pharmacopeia
[1]Quantities presented per 0.25mL PBS

Table 2: Composition of AS03 used for extemporaneous formulation of Pandemic influenza Vaccine

Ingredients	Quantity (per 0.25 ml dose)	Function	Reference
OIL PHASE	**0.025 ml**		
Squalene	10.69 mg	Oil phase constituent	GSK Monograph
D,L-α-tocopherol	11.86 mg	Oil phase constituent	Ph. Eur. 0692

		and immunostimulant	and USP
AQUEOUS PHASE	**0.225 ml**		
Polysorbate 80	4.86 mg	Surfactant	Ph. Eur. 0428
Sodium Chloride (NaCl)	1.77 mg (121 mM)	Tonicity adjuster	Ph. Eur. 0193
Potassium Chloride (KCl)	0.04 mg (2.38 mM)	Tonicity adjuster	Ph. Eur. 0185
Disodium phosphate (Na_2HPO_4)	0.25 mg (7.14 mM)	Buffering agent	Ph. Eur. 0118
Potassium dihydrogen phosphate (KH_2PO_4)	0.04 mg (1.3 mM)	Buffering agent	Ph. Eur. 0920
Water for injection	q.s. ad 0.225 ml	Solvent	Ph. Eur. 0169

Ph. Eur. = European pharmacopoeia; Mn = monograph

2.2.2. Active Substance

Manufacture

The manufacturing process for the monovalent bulks is developed from the applicant's experience gained in production of its seasonal vaccines Flulaval and Fluviral, which are licensed in USA and Canada but not in the EU. The manufacturing process for the monobulks is different to the process reviewed and approved for the Pandemrix/Prepandrix licences (the prepandemic and pandemic adjuvanted vaccines produced at the applicant's German site in Dresden). The manufacturing process can be divided into five main parts:

- Propagation of the working seed in fertilised hen's eggs, harvesting and pooling of infected allantoic fluids
- Inactivation of the monovalent split virus using UV and formaldehyde/thiomersal
- Concentration and purification of the whole virus bulk
- Splitting of the monovalent with sodium deoxycholate
- Homogenisation and sterile filtration

The production process for monovalent bulks is adequately described.

Control of Materials

Control of starting materials (virus seed lots, eggs and raw materials) is acceptable. The CDC working seed release package has been provided and includes the results of pathogenicity testing in chickens and ferrets and monitoring of plaque formation on chicken embryo fibroblast cells. Data to confirm the sequence of H and N genome segment of the strain to the CDC reference strain have been provided. Two working seeds have been validated for use: V7/E2/E3 and V7/E2/E6. The company has committed to provide further details on the materials, especially animal-derived materials, used for derivation of the reverse genetics strain in accordance with guideline on Dossier Structure and Content for pandemic Influenza Vaccine Marketing Authorisations Applications (CPMP/VEG/4717/03). Also, the full validation report for the PCR method used for detection of mycoplasmas for the working seeds will be provided post-authorisation (see section 2.7).

Process validation

Critical steps and one intermediate (the inactivated whole virion monovalent bulk) have been identified and are sufficiently controlled. Proposed hold periods are specified and validated. Specifications for inactivated whole virion monovalent bulk intermediate are acceptable. Nine data sets from the 2006, 2007 and 2008 A/Indonesia/5/2005 drug substance production campaigns were used to illustrate the robustness and consistency of the Quebec H5N1 drug substance manufacturing process. The results provided demonstrate the consistency of the Quebec H5N1 drug substance production process, irrespective of the upstream facility used and egg-processing scale.

The capability of the UV/formaldehyde/thiomersal inactivation steps for batches of A/Indonesia/05/2005 virus have been provided and demonstrate satisfactory inactivation. Ability of the manufacturing process to inactivate avian leucosis virus and mycoplasma has been demonstrated. The structure of the inactivated split monovalent bulks was studied by transmission electron microscopy and confirmed the predominance of disrupted particles after splitting. Further evidence to confirm virus splitting will be provided at the time of the next manufacturing campaign (see section 2.7).

Specifications

The structure of the inactivated split monovalent bulks was studied by transmission electron microscopy and confirmed the predominance of disrupted particles after splitting.

Relevant impurities have been specified and are controlled. Release specifications for the monovalent bulk of the active substance include controls for appearance, HA content, neuraminidase identity, sterility, bacterial endotoxins, test for residual infectious viruses, residual sodium deoxycholate, residual formaldehyde and test for fragmentation (not routine) and are in line with PhEur monograph 0158. Overall, methods of analysis are acceptable. Specific requalification data for the neuraminidase identity test and SRD method for HA quantification will be provided post-authorisation (see section 2.7).

Container-closure and stability

The monovalent bulks are filled and stored in 1L, 10L or 20L bags of two different types. Compliance of the construction materials of both bags to guideline CPMP/QWP/4359/03 has been provided.

Stability

Data currently support 18 to 24 months stability at 2-8°C for bulks depending on the bag's type used for storage.
In accordance with EU GMP guidelines[2], any confirmed out of specification result, or significant negative trend, should be reported to the Rapporteur and the EMA.

2.2.3. Finished Medicinal Product

The drug product is described in three parts: The drug product containing H5N1 antigen, the AS03 adjuvant and the mixed AS03 adjuvanted H5N1 influenza vaccine which is the preparation to be administered within 24 hours.

Drug Product (H5N1 vial)

Pharmaceutical Development

Developmental changes implemented since the first clinical studies have been stated and clinical studies have provided reassurance of product remaining comparable.

Manufacture of the product

Manufacture for the antigen component of the drug product is relatively simple and consists of aseptic formulation of the final bulk with the excipients followed by filling into final containers.
An overage of 20% for the HA content will be applied at formulation of the commercial lots. Supporting data and satisfactory justification have been provided.
Antigen bulk received is sterile. Bioburden is controlled throughout the manufacturing process.

[2] 6.32 of Vol. 4 Part I of the Rules Governing Medicinal Products in the European Union

Product Specification

Specifications for excipients and analytical procedures are in line with USP and/or NF. The applicant has assessed the differences between the USP and PhEur requirements for specifications and methods relevant to the excipients used in Pumarix and presented an acceptable strategy for testing in the future.

Controls of final bulks (sterility, HA, total protein, residual ovalbumin, thiomersal, residual formaldehyde and residual sucrose) and final containers (sterility, bacterial endotoxins, pH, thiomersal, appearance, osmolality and HA) of the antigen vial are acceptable (in line with PhEur). Methods are either in line with PhEur or are validated

Compliance with the product specifications has been shown on a number of batches representatives of the final formulation and commercial scale manufacture. There are no final products process-related or degradation impurities.

Container Closure system

Adequate data are provided to affirm the quality of the container closure system. HA content, appearance, sterility, thiomersal content and pH are measured as stability-indicating parameters as part of the stability studies. Stability test methods and specifications are identical to those at release. 18 months stability data are provided for lots AFLPA109A-112A and demonstrate that specifications are met. An 18 month shelf-life for final containers based on the real time data submitted is acceptable.

Drug Product (AS03 adjuvant vial)

AS03 is an oil-in-water emulsion in 3mL multi-dose (10 dose) glass vials. Squalene and DL-α-tocopherol) form the oily phase (10%v/v) and phosphate buffered saline the aqueous phase (90%v/v). Polysorbate 80 is used as a surfactant to stabilise the oil/water interface.

Pharmaceutical Development

Developmental changes implemented since the first clinical studies have been stated and non-clinical and clinical studies have provided reassurance of product remaining comparable.

Manufacture of the AS03 adjuvant vial

Formulation of the AS03 adjuvant consists of the preparation of the bulk followed by filling into glass vials. Process parameters are identified. No routine in-process tests are conducted. Bioburden is adequately controlled throughout the manufacturing process.

Specifications of the AS03 adjuvant

With the exception of squalene, all excipients are described and controlled in line with the Ph.Eur. Adequate quality control of squalene is performed by the supplier and by GSK (according to an internal GSK monograph which is in line with the Ph.Eur. monograph for squalane).

Emulsion bulk and AS03 final containers are tested at release for Description, Identity and Content of adjuvant components (polysorbate 80, DL-α-tocopherol and squalene), pH, Endotoxin content, Sterility, Particle size, Polydispersity index and Volume (final containers only).

Tests for sterility and bacterial endotoxins are performed in line with the Ph.Eur. and tests for polysorbate 80, DL-α-tocopherol and squalene are validated. The method used for particle size analysis and associated system suitability measurements is acceptable.

Stability of the AS03 adjuvant

Data provided from the stability studies for the bulk emulsion support the proposed shelf life of 2 years. For final AS03 container lots a shelf-life of 36 months has been approved.

Drug Product (mixed H5N1 and AS03 vial)

At the time of vaccine administration the content of the adjuvant vial is withdrawn from the vial with a syringe and is injected into the antigen vial and shaken.

Data from 'withdrawable' volume studies conducted to support the required overfill for both antigen and adjuvant vials have been provided. Results from uniformity of dose studies demonstrate that content of HA, squalene, Polysorbate 80 and tocopherol for each dose of the 10-dose vial remains equivalent.

SDS PAGE and Western blot analysis performed show that HA profiles of the adjuvanted formulation are comparable to the non-adjuvanted formulation and remain unchanged after a period of 24 hours at 25°C. Compatibility between the antigen and adjuvant after 24h at 25°C has been demonstrated by evaluation of appropriate key quality criteria. Preservative efficacy of thiomersal concentration after mixing the content of the antigen container with AS03 adjuvant has been shown in line with Ph.Eur. 5.1.3. The applicant has shown that there is limited (less than 10%) physico-chemical interaction between the Quebec split virion antigen and the adjuvant system and thus, there seems to be no need for controlling antigen/adjuvant interaction for this product as a release test (same as Prepandrix/Pandemrix). There is sufficient evidence that there is little/no effect of the reconstitution conditions on the essential characteristics of the antigen/adjuvant combination.

Current in-use experience with AS03-adjuvanted H1N1v vaccine has demonstrated that the antigen suspension is prone to significant aggregation which is not dispersed on addition of the adjuvant. Data available for Pumarix H5N1 (visual and by nephelometry), show that aggregation appears lower for H5N1 strain when compared against H1N1v strain. The nephelometry method has been validated and is proposed to be used as an IPC and also in stability studies, the specification is not as yet set until further experience is gained, this is acceptable.
It is acceptable that a number of studies regarding aggregation will be addressed by the applicant as post-authorisation commitments (see section 2.7).

In accordance with EU GMP guidelines[3], any confirmed out of specification result, or significant negative trend, should be reported to the Rapporteur and the EMA.

2.2.4. 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 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.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

At the time of the 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 and agreed timeframe.

2.3. Non-clinical aspects

2.3.1. Introduction

Preclinical development of Pumarix was generally in agreement with current guidelines. The antigen is produced in hen's eggs using the same process as that is applied to the applicant's own FluLaval brand of seasonal influenza vaccine approved outside the EU.

[3] 6.32 of Vol. 4 Part I of the Rules Governing Medicinal Products in the European Union

A safety pharmacology study was conducted in rats with intravenous dosing of Quebec-derived A-Wisconsin virus at 60 µg haemagglutinin /ml adjuvanted with AS03.

Two single dose and two repeat dose general toxicity studies have been reported and a further two repeated dose toxicity studies in rabbits are ongoing.
The applicant also presented results from immunogenicity studies in mice and of homologous and heterologous influenza challenge studies in ferrets, each using H5N1 vaccine, manufactured in eggs at the Quebec site. ("Q-Pan")

Good Laboratory Practice (GLP)
The safety studies included in the dossier were all compliant with GLP.

2.3.2. Pharmacology

Two aspects of the pharmacology profile of the AS03-adjuvanted H5N1 Quebec influenza vaccine have been studied: (a) immunogenicity and protection studies in animals, and (b) the mode of action of the AS03 adjuvant system.

- The ability of the vaccine to elicit an immune response in animals. Immunogenicity studies in naïve mice and immunogenicity and efficacy studies in a naïve ferret model were conducted to demonstrate the immunogenicity as well as the efficacy of the adjuvanted vaccine against mortality and morbidity induced by challenge with viral strains homologous and heterologous to the vaccine strain.
- The mode of action of the AS03 adjuvant system. In vitro and in vivo experiments were conducted to explore the mechanism by which AS03 combined with split antigen promotes a robust and persistent humoral and cellular response.

In addition to these studies, a safety pharmacology experiment in rats was conducted to assess the vaccine effect on cardiovascular and respiratory systems

The mechanism of action of the adjuvant has already been assessed and discussed during the MAA procedure for the D-pan vaccine (Pandemrix). Overall the data indicate that AS03 does not simply act as a 'delivery system' for the influenza split antigen but rather works as a stimulant of the adaptive immune response, especially the cellular component. AS03 appears to induce a stronger as well as a broader immunity. This is particularly important for inducing protection of a naïve population against a virus that is prone to antigenic drift, such as pandemic influenza.

Primary pharmacodynamic studies

Two immunogenicity studies were conducted in mice using H5N1 vaccine manufactured at the Quebec facility, adjuvanted with AS03. One used vaccine antigens from A/Vietnam/1194/2004 and the second used vaccine antigens from A/Indonesia/5/2005. Immunogenicity was greater in the presence of the adjuvant by both measures used (quantification of antigen-specific IgG in sera, haemagglutination inhibition tires) and a dose-response relationship was shown between antigen dose and serum IgG concentrations; however, there was no evidence of a dose-relationship using the functional antibody measure.

Vaccine efficacy studies were conducted in ferrets exposed to lethal challenge doses of homologous virus (A/Indonesia/5/2005), or heterologous virus (vaccine prepared from A/Indonesia/5/2005 H5N1 and the challenge virus was A/Hong Kong/156/97 H5N1) and a final experiment where the vaccine was based on H5N1 A/Vietnam/1194/04 and the challenge virus was A/Indonesia/05/2005 (10^5 TCID). All studies indicated that adjuvanted vaccine conferred protection from lethal challenge with influenza virus, whereas without adjuvant, or with a half-dose of adjuvant, vaccine efficacy was compromised. Viral shedding, lung viral load measures and serology results were generally internally consistent. Cross-reactivity was indicated.

Secondary pharmacodynamic studies

Secondary pharmacodynamic studies were not performed. This approach is in accordance with the relevant guidelines, note for guidance on preclinical pharmacological and toxicological testing of

vaccines (CPMP/SWP/465/95) and the guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application, CPMP/VEG/4717/03.

Safety pharmacology programme

A safety pharmacology study (0016/072148) was performed in 8 nine-week-old male Wistar rats. Under surgical anaesthesia, the rats underwent cannulation of the femoral artery, jugular vein, and trachea, and insertion of subcutaneous ECG electrodes. Under continuous anaesthesia by infusion pump, rats were treated by intravenous bolus with 1ml/kg of saline placebo (n = 4) or Quebec-sourced A/Wisconsin/67/05 influenza vaccine adjuvanted with AS03 (n = 4). The final concentration of the influenza antigen was 30µg/ml and the AS03 concentration represented the full human dose. Over 120 minutes after infusion there was a tendency for minute volume to increase in all animals, and a single animal in the actively treated group showed a transient inverted P wave on ECG. Both occurrences are non-specific and often observed in such experiments; and there was no evidence of any treatment-specific changes in cardiorespiratory performance.

The CHMP highlighted that while safety pharmacology studies should be performed in a relevant animal model, the clinical relevance of the rat for an influenza vaccine can be questioned. In addition, The 2h post-dose observation time only covers the very acute effects of vaccination. The current clinical experience with AS03 adjuvanted vaccines do not indicate that vital physiological/ organ systems are affected by the adjuvant. In addition an antigen specific effect on such systems is also not to be expected. Therefore the limited safety pharmacology testing (without the use of the actual antigen) is accepted.

The CHMP pointed out that dose extrapolation on ml/kg base for adjuvants from animals to humans is complicated. However the full human dose was considered sufficient for a safety pharmacology study. Overall, no concerns for human use were raised.

Pharmacodynamic drug interactions

No studies were performed. Since this is an MAA for a vaccine, this was considered acceptable.

2.3.3. Pharmacokinetics

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

2.3.4. Toxicology

The toxicity profile of the AS03-adjuvanted Quebec influenza vaccine has been evaluated in single-dose toxicity, repeat-dose toxicity and local tolerance studies in New Zealand White rabbits.
Reproductive toxicity testing was described in rats.

Single dose toxicity

Two single-dose toxicity studies have been conducted on AS03-adjuvanted vaccines. The first of these used A/H3N2 antigens (A/Wisconsin/67/2005), prepared using the same manufacturing process as the one used for H5N1 antigen. The second used the Quebec-sourced H5N1 (A/Indonesia/5/2005) antigen at a 30µg dose. For both of these studies, local tolerance data were also collected.

Study ID	Species/ Sex/Number/ Group	Test material	Dose	Major findings
1536-06196	NZW rabbit (3/sex/group)	Q-H3N2 + AS03	Saline, AS03, 15µgHA + AS03	**no deaths or clinical signs**
2990/355	NZW rabbit (3/sex/group)	Q-H5N1 (30µg) + AS03	Saline, 30 µg HA + AS03,	**no unscheduled deaths or associated toxicity**

Minor inflammation at the injection site was noted in all groups, which is indicative of the injection method. The adjuvanted vaccines were associated with fasciitis, cellulitis, and in males, granulomatous myositis. However, there was no difference in severity between the adjuvanted seasonal and H5N1 antigens, suggesting that this is associated with the adjuvant and not with the antigen.

Repeat dose toxicity

Four repeat-dose toxicity studies have been conducted on AS03-adjuvanted vaccines, all using New Zealand White rabbits. The first study (1990/956) used repeated doses of A/H5N1 antigens produced in Dresden (using the Fluarix process).

This study has been assessed during the MAA of Pandemrix/ Prepandrix, and the findings in this study in animals receiving AS03 (with or without antigen) (increases in fibrinogen, WBC (neutrophils), spleen weight, large iliac lymph nodes, lymphoid hyperplasia) are indicative of an acute and transient inflammatory response. No delayed systemic effects due to the test vaccine could be observed.

The second study (1536-06194) used Quebec and Dresden-sourced A/H3N2 split influenza viruses (produced using the FluLaval or Fluarix processes) as a surrogate for the A/H5N1 split influenza virus. It should be noted that the Quebec H3N2 antigen is produced according to the same manufacturing process as the Quebec H5N1 antigen and therefore it contains the same levels of impurities and residuals as the H5N1 antigen. This second repeat-dose study was specifically designed to mimic two separate immunization series repeated at a prolonged interval, as might occur in a prime-booster strategy or with annual vaccination.

The study confirmed the absence of overt toxicity by the Q-sourced product, in comparison with the D-sourced product, however with a strain belonging to the 'normal' seasonal vaccines and showed no specific findings.

For the third repeat-dose toxicity study (2990/356) 10 male and 10 female rabbits were dosed intramuscularly with saline or Quebec H5N1 antigen (30 µg HA) + AS03 on days 1, 15 and 29. Five were killed on days 32 (three days after the third injection) and five on day 57 (28 days after the third injection).

For the fourth repeat-dose toxicity study (8550) 20 male and 20 female rabbits were dosed intramuscularly with saline or Quebec H5N1 antigen (3.8 µg HA) + AS03 on days 0, 14 and 28. They were killed on day 31 (3 days after the third inoculation) or 57 (29 days after the third inoculation).

These studies found local toxicity and immune system changes in response to vaccination which were expected, reversible and consistent with previous findings from similar studies carried out with adjuvanted influenza vaccines. The studies submitted for the MAA of Pumarix used the full human dose given intramuscularly to rabbits in a manner sufficient to support the intended clinical dosing.

Genotoxicity

Genotoxicity of the adjuvant alone was assessed in two *in vitro* tests (reverse mutation (Ames) test in bacteria; gene mutation in mouse cells) and one *in vivo* test (micronucleus test in the rat after intravenous administration). The vaccine was not tested. No indication of genotoxicity was evident.

Carcinogenicity

No carcinogenicity studies were performed according to the Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95 guidance) and the Guideline on adjuvants in vaccines for human use (EMEA/CHMP/ VEG/134716/2004).

Reproduction Toxicity

For a reproductive toxicity study (1536-08129) with AS03-adjuvanted Quebec-derived H5N1 vaccine female rats were dosed with one of the following:
1. Phosphate buffered saline (control group);
2. AS03 adjuvant prior to mating; AS03 diluted 1:1 with PBS during gestation and lactation
3. PBS prior to mating; Quebec H5N1 vaccine + AS03 adjuvant during gestation and lactation and
4. Quebec H5N1 vaccine + AS03 adjuvant.

Dosing was as two intramuscular injections into the rear limbs 28 days prior to cohabitation with untreated male rats, on gestation days (GD) 7, 9, 12 and 16 and on postnatal day 7. After confirmation of mating, female rats were divided into two equal cohorts, one of which would be allowed to bear their litters, the other of which would undergo caesarean section on GD 21.

Overall the data indicated that neither the AS03 adjuvant alone or AS03-adjuvanted H5N1 vaccine had any adverse effect on female rats or their offspring.

To address further the use of the vaccine in the first trimester of pregnancy and to supply direct proof of lack of effects claimed in early pregnancy (i.e in rats, on gestation days 1-6), the applicant provided data from a similar study using D-Pan H1N1-AS03 adjuvanted vaccine and AS03 adjuvant. This study was assessed previously for Pandemrix.

For this study (HEY0001), rats were injected intramuscularly with saline, H1N1v/AS03A or AS03A adjuvant on day 0 to 6 after mating. Adult females were examined macroscopically at necropsy on Day 14 after mating and their uterine contents examined.
That assessment concluded that no evidence of a concern for use of the vaccine early in pregnancy.

Local Tolerance

Local tolerance was assessed in the single dose studies in rabbits (1536-06196 and 2990/355), see above.
Local tolerance assessment of AS03 alone and Quebec-manufactured H3N2 antigen at a dose containing 15µg of HA (i.e., approximately 20-fold higher than the intended human dose on a body weight basis) combined with a full human dose of AS03 did not show any adverse clinical observation in rabbits (Bridge GPS Study Number 1536-06196). Dermal responses did not differ between controls and experimental groups. There were no adverse observations noted at necropsy. Minimal or mild subacute inflammation of the subcutaneous and/or epimysial tissue was noted in animals receiving the adjuvant, with or without influenza antigen. There were no microscopic findings specifically associated with the presence of influenza antigen in the test article. In general, a single intramuscular injection of influenza vaccine containing 15µg of HA and a full human dose of AS03 were well tolerated by New Zealand White rabbits.
These results were confirmed in a second study where local tolerance was assessed using AS03 adjuvanted Quebec H5N1 antigen. In this study (Covance Study Number 2990/355), rabbits received one single IM administration of either of three candidate vaccines - two manufactured with Quebec-sourced seasonal antigens (60µg HA/dose) adjuvanted or not with AS03 (human half-dose) and one manufactured with the Quebec H5N1 antigen (30µg HA/dose) adjuvanted with AS03 (human dose) œ or saline control. Minor inflammation was observed in all vaccine and control groups, which is indicative of an effect of the dose method as opposed to any of the vaccine components. The adjuvanted vaccines were associated with fasciitis, cellulitis, and in males, granulomatous myositis. There was no clear difference in severity of these conditions between the two adjuvanted vaccines, indicating that this was an effect of the adjuvant and not the vaccine antigen.

Other toxicity studies

2.3.5. Ecotoxicity/environmental risk assessment

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

2.3.6. Discussion on non-clinical aspects

Single and repeated dose toxicity studies did not reveal any unexpected findings. The local adverse effects are well-known to be associated with the AS03 adjuvant, and the benefit-risk should be weighed in the clinical studies.
The reproduction toxicity studies conducted with a Q-sourced H5N1 vaccine in combination with the AS03 adjuvant given at day 6 and later, did not give rise to any concern. A study that addressed vaccination in early pregnancy, that is, prior and up to implantation of the embryo raised no concerns. The Flulaval data from another study indicate that an unadjuvanted Q-sourced vaccine is also without effect on reproductive endpoints. As a whole the data presented were considered sufficient to support a marketing authorisation for Pumarix.

2.3.7. Conclusion on the non-clinical aspects

The applicant discussed the mode of action of AS03 adjuvant and its use in prophylactic vaccines. The applicant also presented results of immunogenicity studies in mice and of homologous and heterologous influenza challenge studies in ferrets, each using H5N1 vaccine, manufactured in eggs at the Quebec site.

The use of adjuvant is important as it is intended to allow a lower dose of antigen which will allow more subjects to be vaccinated for a given amount of antigen. The mode of action of AS03 is likely to be through an effect to augment the function of antigen presenting cells with release of pro-inflammatory cytokines that cause effects detected in animals in toxicity studies. Such effects are inherent in the mechanisms of action of AS03 adjuvant. The applicant provided numerous publications relating to the mechanism of action of adjuvants and their impact on immune system function, including that of antigen presenting cells. Use of AS03 adjuvant to augment immunogenicity is considered adequately justified.

Two immunogenicity studies were conducted in mice using H5N1 vaccine manufactured at the Quebec facility, adjuvanted with AS03. One used vaccine antigens from A/Vietnam/1194/2004 and the second used vaccine antigens from A/Indonesia/5/2005. Immunogenicity was greater in the presence of the adjuvant by both measures used (quantification of antigen-specific IgG in sera, haemagglutination inhibition tires) and a dose-response relationship was shown between antigen dose and serum IgG concentrations; however, there was no evidence of a dose-relationship using the functional antibody measure.

Vaccine efficacy studies were conducted in ferrets exposed to lethal challenge doses of homologous virus (A/Indonesia/5/2005), or heterologous virus (vaccine prepared from A/Indonesia/5/2005 H5N1 and the challenge virus was A/Hong Kong/156/97 H5N1) and a final experiment where the vaccine was based on H5N1 A/Vietnam/1194/04 and the challenge virus was A/Indonesia/05/2005 (10^5 TCID). All studies indicated that ASO3-adjuvanted vaccine conferred protection from lethal challenge with influenza virus, whereas without adjuvant, or with a half-dose of adjuvant, vaccine efficacy was compromised. Viral shedding, lung viral load measures and serology results were generally internally consistent, although in one experiment there was a lack of concordance between the test facility and the applicant's laboratory results for seroconversion. Cross-reactivity was indicated.

No concerns for human use was suggested by a safety pharmacology study in rats conducted with intravenous dosing of Quebec-derived A-Wisconsin virus at 30µg haemagglutinin /ml adjuvanted with AS03.

Two single dose and four repeat dose general toxicity studies have been reported. Apart from pro-inflammatory changes at the injection site that are related to the primary mode of action of the AS03 adjuvant, there was no toxicity of note. These studies used the full human dose given intramuscularly in a manner sufficient to support the intended clinical dosing.

Reproductive toxicity testing was described in rats. Testing suggested that the Quebec-manufactured vaccines tested did not adversely affect female fertility or pregnancy and no effect was indicated in the F1 generation. A study with H5N1 vaccine did not identify toxicity associated with vaccination in pregnancy animals when dosed from day 6 or pregnancy. A study that addressed vaccination in early pregnancy, that is, prior and up to implantation of the embryo raised no concerns.

Genotoxicity of the adjuvant was tested and indicated no positive findings.

The applicant provided results of a reproductive toxicity study with H5N1-AS03 adjuvanted vaccine in which there was proof of H5N1-antibody exposure; no toxicity was identified.

Overall, the preclinical studies did not raise any concerns in terms of toxicity, and the mainly local reactions observed were expected and reversible.

2.4. Clinical aspects

2.4.1. Introduction

This application dossier of Pumarix (i.e. Q-Pan H5N1)was based primarily on clinical studies that evaluated the safety and immunogenicity of AS03-adjuvanted vaccines containing Quebec sourced antigens from A/Indonesia/5/2005 (H5N1) and some data from a version containing A/Vietnam/1194/2004 (H5N1), manufactured in Dresden, Germany (i.e. D-Pan H5N1). The applicant also reported data from a study that directly compared AS03-adjuvanted vaccine containing antigen from the H5N1 pandemic strain manufactured in Quebec (Q-Pan H5N1, Pumarix) or Dresden (D-Pan H5N1").

Further clinical data on the approved formulation of Pumarix are expected in accordance with agreed timelines as outlined in the Letter of Undertaking.

GCP

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

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

- Tabular overview of clinical studies carried out outside the community:

Trial	Protocol number	Countries
Q-Pan-001	110028	Canada, US
Q-Pan-002	110464	Canada, US
Q-Pan-005	110624	Canada, US
Q-Pan-010	111729	Canada, US
Q-pan-011	111756	Japan

2.4.2. Pharmacokinetics

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

2.4.3. Pharmacodynamics

In relation to vaccines, the pharmacodynamic studies consist of assessments of the immune responses. The data on the immunological response to Arepanrix (H5N1) are described and discussed below.

2.5. Clinical efficacy

In the initial application dossier the Applicant provided data from two studies with Q-Pan H5N1 as follows:

Study ID	Study centres	Study groups	Entered *(Completed)*	Study design	Study duration	Inclusion criteria
Q-Pan-001 H5N1 A/Indonesia	10 centres US Canada	**Total** **H5N1 split Quebec** 3.8µg/AS03 **H5N1 split Quebec** 3.8µg/half AS03 **H5N1 split Quebec** 3.8µg no AS03 **H5N1 split Dresden** 3.8µg/AS03 **H5N1 split Dresden** 3.8µg/half AS03	**680 *(662)*** 152 *(148)* 151 *(150)* 78 *(75)* 151 *(148)* 148 *(141)*	Observer-blind, randomized, phase I/II 2 doses at 0, 21 days	Approximately 6 months for each subject	Healthy adults 18-64 years old (18-40 years, 41-64 years)
Q-Pan-002 H5N1 A/Indonesia	40 centres US, Canada	**Total** **H5N1 Quebec 3.8µg/AS03** **lot A** **lot B** **lot C** **Placebo**	**4561 (4343)** **3422 (3263)** **1141** **1141** **1140** **1139 (1080)**	Observer-blind, randomized, phase III 2 doses at 0, 21 days	Initially 6 months; amended to approximately 1 year for each subject	Healthy adults At least 18 years old

The applicant provided the following additional data generated with Q-Pan vaccines as shown below.

Study	Primary Objective	Population	Vaccine	Planned safety	Planned immuno
Q-Pan-001	Safety and Immunogenicity	18-64 years	H5N1 A/Indonesia strain	100	100

Study	Primary Objective	Population	Vaccine	Planned safety	Planned immuno
contingent arms			1.9 µg HA/ full AS03 1.9 µg HA/ half AS03 2-dose schedule		
Q-Pan-009	Immunogenicity	18-64 years	H5N1 A/Indonesia strain 3.8 µg HA/ full AS03 Two doses at: - Day 0, Day 21 - Day 0, Day 14 - Day 0, Day 7 - Day 0, Day 0	312	312
Q-Pan-010	Immunogenicity	18-64 years Primed in Q-Pan-001	H5N1 A/Turkey strain 3.8 µg HA/ full AS03 One booster dose (M15)	650	650
Q-Pan-011	Safety and Immunogenicity	Japanese (20-64 years)	H5N1 A/Indonesia strain 3.8 µg HA/ full AS03	100	100

Also provided was one-year follow-up data from study Q-Pan H5N1-002.

In addition the applicant provided data from the following supportive studies carried out with D-Pan H5N1 (Pandemrix H5N1/Prepandrix):

Study	Primary Objective	Population	Vaccine	N safety	N immuno.
D-Pan H5N1-002	Immunogenicity Reactogenicity/ Safety	Unprimed population 18-60 years	Monovalent split virus (H5N1) 3.8 µg HA - 2 lots + full AS03 - 2 lots Monovalent split virus (H5N1) 3.8 µg HA - 2 lots + diluent	961 5	933 236
D-Pan H5N1-007	Immunogenicity Reactogenicity/ Safety	Unprimed population 18-60 years	Monovalent split vaccine (H5N1). 30 µg, 15 µg, 7.5 µg or 3.8 µg HA* - Dresden sourcing, A/Vietnam strain with or without AS03 2-dose schedule 21 days apart	400	394
D-Pan H5N1-008	Reactogenicity/ Safety	Unprimed population > 18 years	Monovalent split vaccine (H5N1) 15 µg HA with AS03 Fluarix (first dose), placebo (second dose) 2-dose schedule 21 days apart	3802 1269	455 154
D-Pan H5N1 -009/-022/-023	Safety and immunogenicity	Children 3-9 years	1.9 µg HA (A/Vietnam/1194/2004 H5N1) / half dose ASO3 Fluarix	100 34	100 34

D-Pan H5N1-010	Imunogenicity/ safety	Unprimed population 61 years and above	Monovalent split virus H5N1/AS03/3.8µg HA single dose (3.8µg) adjuvanted H5N1/3.8µg HA single dose (3.8 µg) non-adjuvanted H5N1/AS03/7.5µg HA double-dose (7.5µg) adjuvanted H5N1/7.5µg HA): double dose (7.5µg) non-adjuvanted 2-dose schedule 21 days apart	152 54 145 44	165 61 159 52
D-Pan H5N1-011	Safety and Immunogenicity	Japanese adults (20-64 years)	Monovalent split virus (H5N1) A/Indonesia strain 3.8 µg HA/ full AS03	100	100
D-Pan H5N1-012	Reactogenicity Immunogenicity	18-60 years	A/Vietnam/1194/2004 or A/Indonesia/5/2005 3.8 µg HA/ full AS03	512	512
D-Pan H5N1-015 (extension study to -007)	Reactogenicity Immunogenicity	19 - 61 years, primed with 2 doses of H5N1 A/Vietnam/ 1194/2004 containing 3.8, 7.5, 15 or 30 µg HA, AS03 adjuvanted / non-adjuvanted	A/Indonesia/5/2005 3.8 µg HA/ full AS03	350	350

The CHMP agreed that data from Study D-Pan H5N1-009/-023 which has previously been assessed for Pandemrix and Arepanrix should be included in the SmPC to support the use in children, and that data from Study H5N1-015 (extension to H5N1-007) should be included in the SmPC to provide information on heterologous booster responses.

Data from Studies D-Pan H5N1-007, -008, - 010, -011 – 012, and D-Pan H5N1-002 has been previously assessed within the MAs for Pandemrix/Prepandrix, however were not considered relevant to be reflected in the PI for Pumarix.

The applicant further expects to report data from the following study in 4Q2010, and study data agreed in the paediatric investigation plan (PIP) as outlined in the Letter of Undertaking.

Q-Pan-005	Safety and Immunogenicity	≥18 years	Priming: 2 doses H5N1 A/Indonesia strain 7.5 µg HA/ half or full AS03 3.8 µg HA/ half or full AS03 Boost: 1 dose H5N1 A/Turkey strain 7.5 µg HA/ half or full AS03 3.8 µg HA/ half or full AS03	840	840

Assays
As in all the previously reported studies with D-Pan and H5N1, sera were forwarded to GSK. Assays for haemagglutination inhibition (HI) and serum neutralising antibody (SNA) were performed as previously described. In brief, these assays were as follows:

HI – The standardised and validated micromethod uses four HI units of the appropriate antigen and a 0.5% horse erythrocyte suspension. Horse erythrocytes contain a high proportion of α2, 3 linkages thus making them more sensitive for the detection of antibody to avian HA in the HI assay. All HI assays were performed in duplicate in the same run along with control sera and each run was judged against acceptance criteria. The validation results of the assay based on the Indonesia strain have been provided.

SNA – The previously described microneutralisation assay was used. A cell suspension, containing a defined amount of Madin-Darby Canine Kidney (MDCK) cells is then added to the pre-incubated mixture of virus and heat-inactivated serum and incubated at 37°C. Virus replication is determined by testing the supernatant of each well in a haemagglutination assay using chicken erythrocytes. All SN assays are run in triplicate in the same run. The assay variability is controlled by the use of control sera included in each run and each run is judged against acceptance criteria. The assay cut-off is defined as 1:28, which results from pre-dilution of the sera and is the first computable ND50 value.

The specificity of the neutralisation assay has been estimated by testing a set of samples from naïve (i.e. previously unvaccinated) children (6 to 9 years) collected before vaccination with the seasonal vaccine. Out of 46 subjects, one was slightly positive. These data are described in the Validation Report. Based on this result, the specificity of the SNA can be estimated to be 98%.

2.5.1. Main studies

Q-PAN H5N1-001

This was a randomised, observer-blind, multi-centre, active-controlled study conducted at 10 sites (7 in the US and 3 in Canada). The primary immunogenicity objective was to demonstrate the adjuvant activity of AS03 by comparing immune responses to Q-Pan H5N1 3.8 µg HA/AS03 at full and half strengths versus Q-Pan HA 3.8 µg alone. The primary immunogenicity endpoint was the Day 42 HI antibody response to homologous virus in subjects receiving two doses of vaccine Superiority of the adjuvanted formulation was declared if the lower bound of the 95% confidence interval (CI) on the GMT ratio exceeded 2.0 and the lower bound of the 95% CI on the difference in SCR exceeded 15%.

Formulations, lots and treatment group allocations were as follows:

Product	Formulation	Lot number	Group(s)
A/Indonesia/5/05 antigen (Quebec)	15 µg/mL	AFLPA009A	A, B, C
A/Indonesia/5/05 antigen (Dresden)	15 µg/mL	DFLSA006A	D, E
AS03	Full strength	DA3BA008A	B, D
AS03	Half strength	DA3AA006A	C, E
Phosphate-buffered saline	--	DD11A003A	A

The following formulations were used in the different groups:

Group A ("Q000ASO3") – Quebec manufactured 3,8 µg HA (A/Indonesia/5/05), no Adjuvant
Group B ("Q100AS03") – Quebec manufactured 3,8 µg HA, full dose Adjuvant
Group C ("Q50AS03") – Quebec manufactured 3,8 µg HA, half dose Adjuvant
Group D ("D100AS03") – Dresden manufactured 3,8 µg HA, full dose Adjuvant
Group E ("D50AS03") – Dresden manufactured 3,8 µg HA, half dose Adjuvant

Four blood samples were to be drawn at D0, D21, D42 and D182.

Subject populations were defined as in previous studies with H5N1 vaccine (i.e. total vaccinated {VC}, according to protocol {ATP} for safety and ATP for immunogenicity).

The sample size was based on the evaluation of superiority of Q-Pan plus adjuvant versus Q-Pan without adjuvant using the SCR and GMT at Day 42, both of which required a statistically significant result. Each test was to have α=0.05 (two-sided) at a power of 95%, yielding an overall power of approximately 90% for the simultaneous tests. Based on the FDA draft guidance on pandemic vaccines of March 2006 a 0.3 log10 mean difference (= a 2-fold difference in GMT ratio) for the HI antibody titres and a 15% difference in SCR were to be regarded as meaningful.

It was planned that if the first step of the analysis based on Day 42 data indicated that:

a) GMTs fulfilled the ≥2-fold criterion for adjuvant effect <u>and</u>
b) Treatment Groups B and C both demonstrated a Day 42 point estimate for the rate of vaccine homologous HI reciprocal titres ≥ 40 of at least 76%

Then two additional groups were to be recruited as follows:

- Group H: Quebec-manufactured A/Indonesia/5/05 H5N1 antigen containing 1.9 µg of HA with full strength ASO3 on Days 0 and 21 (N ≈ 50)
- Group I: Quebec-manufactured A/Indonesia/5/05 H5N1 antigen containing 1.9 µg of HA with half strength AS03 on Days 0 and 21 (N ≈ 50).

These additional groups were recruited but the results were not provided in the initial application dossier.

<u>Study Population</u>

Subject populations were defined as in previous studies with H5N1 vaccine (i.e. total vaccinated {VC} and according to protocol {ATP} cohort for safety and ATP for immunogenicity).

Of 680 vaccinated subjects (68 at each of the 10 study sites) numbers by group were as follows:

	Total		Q000AS03	Q100AS03	Q50AS03	D100AS03	D50AS03
Title	n	%	n	n	n	n	n
Total enrolled cohort	680	-	-	-	-	-	-
Total vaccinated cohort	680	100	78	152	151	151	148
ATP safety cohort	672	98.8	78	149	149	149	147
ATP immunogenicity cohort	648	95.3	75	144	146	140	143

All 680 (100%) enrolled subjects received a study vaccine. The ATP safety (ATP-S) cohort consisted of 672 (99%) of the 680 subjects. The ATP immunogenicity (ATP-I) cohort consisted of 648 (95%) subjects.

The demographic information for the total vaccinated cohort was similar to that of the ATP-I cohort. The mean age of study subjects was 38.6 years, with a minimum age of 18 years and a maximum age of 64 years. A total of 371 (54.6%) subjects were between the ages of 18 and 40, and the remaining 309 (45.4%) subjects were between the ages of 41 and 65. A total of 393 (57.8%) subjects were female and 287 (42.2%) subjects were male. The majority (86.8%) of subjects were Caucasian; of the remaining subjects, 5.6% were African American, 4% were unspecified race, 1.3% were Southeast Asian, and all other races were less than 1%.

HI Results up to D42 for the initial 5 treatment groups

Quebec- versus Dresden-manufactured vaccine

- Group B (Q100AS03) and Group C (Q50AS03) were pooled to form the Quebec group
- Group D (D100AS03) and Group E (D50AS03) were pooled to form the Dresden group.

GMTs for each group were adjusted for age and baseline HI antibody titre and then a ratio of the group GMTs was calculated. For the groups to be considered equivalent the limits of the 95% confidence interval on the ratio were to be between 0.67 and 1.5. This criterion was met for both the A/Indonesia/5/05 antibody (ratio 0.94; 95% CI 0.75-1.17) and the A/Vietnam/1194/04 antibody (ratio 1.16; 95% CI 0.92-1.46) as shown below.

Table 25 Adjusted GMT ratios for subjects receiving Quebec antigen with full or half strength adjuvant compared with subjects receiving Dresden antigen with full or half strength adjuvant at Day 42, by antibody (ATP cohort for immunogenicity)

	Treatment Group				Adjusted GMT ratio (Quebec / Dresden)		
	Quebec		Dresden			95% CI	
Antibody	N	Adjusted GMT	N	Adjusted GMT	Value	LL	UL
A/Indonesia/5/05	290	371.2	282	396.9	0.94	0.75	1.17
A/Vietnam/1194/04	290	36.6	282	31.6	1.16	0.92	1.46

Dresden = D100AS03 and D50AS03
Quebec = Q100AS03 and Q50AS03

Adjuvant activity

The differences between Group B (full adjuvant; Q100AS03) and Group A (no adjuvant; Q000AS03) in HI SCRs to A/Indonesia/5/05 and A/Vietnam/1194/04 met the pre-defined criteria for superiority of adjuvanted vaccine.

Table 18 Comparison of seroconversion rates at Day 42 in subjects receiving Quebec antigen with full strength adjuvant and Quebec antigen with no adjuvant, by antibody and pre-vaccination status (ATP cohort for immunogenicity)

		Treatment Group						Difference in SCR (Q100AS03 minus Q000AS03)		
	Pre-vacc.	Q100AS03			Q000AS03				95% CI	
Antibody	status	N	n	%	N	n	%	%	LL	UL
A/Indonesia/5/05	S-	144	140	97.2	75	13	17.3	79.89	69.36	87.27
	S+	0	-	-	-	-	-	-	-	-
	Total	144	140	97.2	75	13	17.3	79.89	69.36	87.27
A/Vietnam/1194/04	S-	140	89	63.6	71	1	1.4	62.16	52.94	70.00
	S+	4	0	0.0	4	0	0.0	0	-52.33	52.33
	Total	144	89	61.8	75	1	1.3	60.47	51.45	68.30

S- = seronegative subjects (antibody titre < 10 1/DIL) prior to vaccination
S+ = seropositive subjects (antibody titre >= 10 1/DIL) prior to vaccination
N = number of subjects with pre- and post-vaccination results available
n/% = number/percentage of subjects with a vaccine response
95% CI = Standardized asymptotic 95% confidence interval; LL = lower limit, UL = upper limit

Comparison between Group C (half adjuvant; Q50AS03) and Group A (no adjuvant; Q000AS03) showed that Quebec antigen with half-strength adjuvant gave significantly higher SCRs and GMTs for antibody to both strains compared to unadjuvanted Quebec antigen.

Table 20 Comparison of seroconversion rates at Day 42 in subjects receiving Quebec antigen with half strength adjuvant and Quebec antigen with no adjuvant, by antibody and pre-vaccination status (ATP cohort for immunogenicity)

		Treatment Group						Difference in SCR (Q50AS03 minus Q000AS03)		
	Pre-vacc.	Q50AS03			Q000AS03				95% CI	
Antibody	status	N	n	%	N	n	%	%	LL	UL
A/Indonesia/5/05	S-	146	131	89.7	75	13	17.3	72.39	61.04	80.80
	S+	-	-	-	-	-	-	-	-	-
	Total	146	131	89.7	75	13	17.3	72.39	61.04	80.80
A/Vietnam/1194/04	S-	140	82	58.6	71	1	1.4	57.16	47.89	65.31
	S+	6	4	66.7	4	0	0.0	66.67	1.48	90.91
	Total	146	86	58.9	75	1	1.3	57.57	48.57	65.52

Table 21 Comparison of adjusted ratios of GMTs at Day 42 in subjects receiving Quebec antigen with half strength adjuvant and Quebec antigen with no adjuvant, by antibody (ATP cohort for immunogenicity)

	Treatment Group				Adjusted GMT ratio (Q50AS03 / Q000AS03)		
	Q50AS03		Q000AS03			95% CI	
Antibody	N	Adjusted GMT	N	Adjusted GMT	Value	LL	UL
A/Indonesia/5/05	146	311.2	75	10.4	29.96	20.68	43.41
A/Vietnam/1194/04	146	33.5	75	5.8	5.83	4.04	8.84

Comparison of Quebec antigen with full or half strength AS03 showed numerically higher SCRs and GMTs with full strength adjuvant. The differences were not large enough to indicate superiority of full over half strength adjuvant. The results for homologous virus are shown below.

								Difference in seroconversion rate (Q100AS03 minus Q50AS03)		
		Q100AS03			Q50AS03				95% CI	
Antibody	Pre-vaccination status	N	n	%	N	n	%	%	LL	UL
FLU A/IND/05 AB (1/DIL)	S-	144	140	97.2	146	131	89.7	7.50	1.97	13.84
	S+									
	Total	144	140	97.2	146	131	89.7	7.50	1.97	13.84

Q100AS03 = Q100AS03: 3.8 ug Quebec A/Indo Full AS03
Q50AS03 = Q50AS03: 3.8 ug Quebec A/Indo Half AS03

				Adjusted GMT ratio (Q100AS03 / Q50AS03)		
Q100AS03		Q50AS03			95% CI	
N	Adjusted GMT	N	Adjusted GMT	Value	LL	UL
144	450.8	146	311.2	1.45	1.07	1.97

When examined by age (18 - 40 and 41 - 64 years) the criteria for adjuvant effect were fulfilled in both strata but the homologous virus SCR dropped 4% in the younger age group and 12% in the older group when the adjuvant strength was halved. Similarly, the GMT was only slightly affected in the younger age group but there was a 2-fold reduction in GMT in the older age group.

HI data up to D182

The lower bound of the 95% CI for SCRs exceeded 85% at Day 42, but not at D21, in the four groups that received adjuvanted Quebec or Dresden antigen compared to 9.6% in Group A.

Table 22 Seroconversion rates for A/Indonesia/5/05 antibody at Days 21 and 42 (ATP cohort for immunogenicity)

			Seroconversion							
			Day 21				Day 42			
	Pre-vacc				95% CI				95% CI	
Group	status	N	n	%	LL	UL	n	%	LL	UL
Q000AS03	S-	75	5	6.7	2.2	14.9	13	17.3	9.6	27.8
	S+	0	0	-	-	-	0	-	-	-
	Total	75	5	6.7	2.2	14.9	13	17.3	9.6	27.8
Q100AS03	S-	144	60	41.7	33.5	50.2	140	97.2	93.0	99.2
	S+	0	0	-	-	-	0	-	-	-
	Total	144	60	41.7	33.5	50.2	140	97.2	93.0	99.2
Q50AS03	S-	146	60	41.1	33.0	49.5	131	89.7	83.6	94.1
	S+	0	0	-	-	-	0	-	-	-
	Total	146	60	41.1	33.0	49.5	131	89.7	83.6	94.1
D100AS03	S-	139	63	45.3	36.9	54.0	134	96.4	91.8	98.8
	S+	1	1	100	2.5	100	1	100	2.5	100
	Total	140	64	45.7	37.3	54.3	135	96.4	91.9	98.8
D50AS03	S-	141	53	37.6	29.6	46.1	130	92.2	86.5	96.0
	S+	1	1	100	2.5	100	1	100	2.5	100
	Total	142	54	38.0	30.0	46.5	131	92.3	86.6	96.1

Due to the low numbers who were seropositive with respect to A/Indonesia before vaccination the SPRs followed the SCRs. The difference in GMTs between adjuvanted and unadjuvanted vaccines at Day 42 was very large (being 321-480 in the adjuvanted groups and 11 in the non-adjuvanted group. A similar pattern was observed for GMTs for HI antibody to A/Vietnam/1194/04 although the actual GMTs were much lower for the clade 1 Vietnam strain.

The GMFRs increased markedly after the second vaccine dose in the adjuvanted antigen groups to reach 93-95 with full strength adjuvant and 64-69 for half-strength adjuvant at D42 compared to only 2.1 in the unadjuvanted antigen group.

HI data at D182:

Only the groups that had received full-strength adjuvanted vaccine maintained SCRs (based on HI to homologous virus) with lower 95% CI that were ≥ 40%. However, there was little difference between D182 SCRs in groups that received full or half-dose adjuvant and these groups had SCRs that were markedly superior to that in the unadjuvanted group. SCRs based on HI antibody to A/Vietnam/1194/04 were notably lower and were from 0% - 11% and 9% at Day 182.
SPRs were nearly identical to the SCRs. SPRs for HI to A/Vietnam/1194/04 ranged from 1-13%.
The GMFRs were all between 4.5 and 5.6 for the adjuvanted groups compared to 1.1 in the non-adjuvanted antigen group. The difference in GMFR between adjuvanted and unadjuvanted vaccine groups at Day 182 was similar to that observed at Day 21.

NA data up to D182 in a subset
Up to 40% of tested subjects per group were seropositive for NA to the vaccine strain before the first dose and up to 80% were seropositive for NA against A/Vietnam/1194/04.
D42 GMTs were highest in and similar between D-Pan (1497) and Q-Pan (1567) groups with full strength AS03. GMTs were 1242 and 1353 in the half strength AS03 groups and only 184 in the unadjuvanted group. There was less difference in GMTs between the adjuvanted vaccine groups at D182 (between 414 and 456).
At baseline 12.8% to 23.4% per group had titres ≥ 1:80 for the homologous virus. Among 195 subjects across the four adjuvanted vaccine groups all but two were seropositive at Day 21 and all were seropositive at Day 42 and at D182. At D42 all 195 subjects had titres ≥ 1:80 and there was little decline in this proportion by Day 182.
After two doses of vaccine all who were seronegative with respect to A/Vietnam at baseline demonstrated a response in the adjuvanted treatment groups. At Day 182 the two groups that had received full strength AS03 vaccines retained the highest response rates relative to baseline. At Day 182 the proportions with titres ≥ 1:80 were still 16 to 20 percentage points higher in the adjuvanted groups.

NA against the drifted clade 2 strains A/turkey/Turkey/1/05 (a clade 2.2 virus) and A/Anhui/1/05 (a clade 2.3 virus) was measured in sera obtained from recipients of Q-Pan vaccine containing full strength AS03. No subject was seropositive to A/Anhui/1/05 at baseline. At D42, 80.3% had NA titres against A/Anhui of ≥ 40 and 60.6% had titres ≥ 80. However, by D182 only 23.6% were still seropositive. In contrast, the baseline seropositivity rate was 35.7% for NA to A/turkey/Turkey/1/05, at which time approximately 25% had titres ≥ 40. At D42 all subjects were seropositive and 98.6% had titres ≥ 80. In addition, NA persisted such that at D182 60.7% still maintained a response.

Results up to D182 for the contingent arms

It was planned that if Day 42 data indicated that:
a) GMTs fulfilled the ≥2-fold criterion for adjuvant effect and
b) Groups B and C had D42 rates of vaccine homologous HI titres ≥ 40 of at least 76%
Then two additional groups were to be recruited as follows:

- Group H: Quebec-manufactured A/Indonesia/5/05 H5N1 antigen containing 1.9 µg of HA with full strength ASO3 (=$AS03_A$) on Days 0 and 21 (N = 50)
- Group I: Quebec-manufactured A/Indonesia/5/05 H5N1 antigen containing 1.9 µg of HA with half strength AS03 (=$AS03_B$) on Days 0 and 21 (N = 50).

HI seropositivity rates at D0 were 0% - 6% in these additional 100 subjects (about half per age stratum). At D42, GMTs were nearly two-fold higher in the $AS03_A$ group and were higher in the younger age stratum. All CHMP criteria (SPR, SCR and SCF) were met at Day 42 in both adjuvanted groups and in both age strata.

The FDA's Center for Biologics Evaluation & Research (CBER) requirement that the lower bound of the 95% CI for the SCR should meet or exceed 40% was not met by either contingent arm at D21 but was easily met in both groups at D42. Neither group still met the criterion at D182 when the actual SCRs were 49% and 47%. SCRs for HI against A/Vietnam/1194/04, A/Anhui/1/05 and A/turkey/Turkey/1/05 were lower than for A/Indonesia/5/05 but D42 values exceeded the CBER requirement in Group H (QR100AS03). Also, the CBER requirement was exceeded against at D42 against A/turkey/Turkey/1/05 in Group I (QR50AS03).

Table 15 SCRs for A/Indonesia/5/05 antibody at Days 21, 42, and 182 (ATP cohort for immunogenicity)

		Seroconversion rate														
		Day 21					Day 42					Day 182				
	Pre-vaccination				95% CI					95% CI					95% CI	
Group	status	N	n	%	LL	UL	N	n	%	LL	UL	N	N	%	LL	UL
QR100AS03	S-	46	20	43.5	28.9	58.9	46	44	95.7	85.2	99.5	46	23	50.0	34.9	65.1
	S+	3	3	100	29.2	100	3	3	100	29.2	100	3	1	33.3	0.8	90.6
	Total	49	23	46.9	32.5	61.7	49	47	95.9	86.0	99.5	49	24	49.0	34.4	63.7
QR50AS03	S-	50	21	42.0	28.2	56.8	50	42	84.0	70.9	92.8	49	23	46.9	32.5	61.7
	S+	0	0	-	-	-	0	0	-	-	-	0	0	-	-	-
	Total	50	21	42.0	28.2	56.8	50	42	84.0	70.9	92.8	49	23	46.9	32.5	61.7

The D42 SPRs were 84.0% and 95.9% in $AS03_B$ and $AS03_A$ groups, respectively. In the older age stratum an absolute 21% reduction in SPR was observed with half the adjuvant dose (95.8% vs. 75.0%). There was a much smaller difference between adjuvant groups in the younger stratum (92.3% vs. 96.0%). The CBER requirement that the lower bound of the 95% CI for the SPR should meet or exceed 70% was not met at D21 but was met at D42 in both groups (only just in the half dose adjuvant group). The D42 SPRs for A/Vietnam/1194/04, A/Anhui/1/05 and A/turkey/Turkey/1/05 did not attain the CBER criterion.

Table 16 SPR for A/Indonesia/5/05 antibody through Day 182 (ATP cohort for immunogenicity)

				≥ 10 1/DIL				≥ 40 1/DIL			
						95% CI				95% CI	
Antibody	Group	Timing	N	n	%	LL	UL	n	%	LL	UL
A/Indonesia/ 5/05	QR100AS03	PRE	49	3	6.1	1.3	16.9	1	2.0	0.1	10.9
		Day 21	49	27	55.1	40.2	69.3	23	46.9	32.5	61.7
		Day 42	49	48	98.0	89.1	99.9	47	95.9	86.0	99.5
		Day 182	49	33	67.3	52.5	80.1	26	53.1	38.3	67.5
	QR50AS03	PRE	50	0	0.0	0.0	7.1	0	0.0	0.0	7.1
		Day 21	50	29	58.0	43.2	71.8	21	42.0	28.2	56.8
		Day 42	50	43	86.0	73.3	94.2	42	84.0	70.9	92.8
		Day 182	49	28	57.1	42.2	71.2	23	46.9	32.5	61.7

The GMTs at D42 showed a higher value in Group H although the 95% CI overlapped. There was a substantial decline in GMT for both groups by D182, with actual values comparable to those at D21. The GMT pattern observed relative to adjuvant effects was comparable between drifted strains and the vaccine-homologous virus, although actual GMTs were much lower for the drift variant virus strains. The pattern of changes in GMFRs followed the changes in GMTs as noted above.

Table 18 GMFR for A/Indonesia/5/05 antibodies at Days 21, 42, and 182 (post-initial vaccination) for anti-H5N1 (ATP cohort for immunogenicity)

					GMFR			
							95% CI	
Antibody	Group	N	Time point	GMT	Ratio order	Value	LL	UL
A/Indonesia/5/05	QR100AS03	49	PRE	5.6	-	-	-	-
			Day 21	23.0	Day 21/PRE	4.1	2.7	6.2
			Day 42	331.6	Day 42/PRE	59.2	38.0	92.2
			Day 182	32.5	Day 182/PRE	5.8	3.7	9.1
	QR50AS03	50	PRE	5.0	-	-	-	-
			Day 21	20.8	Day 21/PRE	4.2	2.8	6.3
			Day 42	173.9	Day 42/PRE	34.8	21.1	57.3
		49	Day 182	20.0	Day 182/PRE	4.0	2.7	5.9

The contingent group data at D182 were also provided for age strata 18-40 and 41-64 years and for vaccine homologous and heterologous viruses. Within each age cohort the HI seropositivity rates and GMTs were higher in the sub-groups that received full dose adjuvant. The magnitude of the effect of halving the adjuvant did not appear to be greater in the older cohort in this study using half the usual dose of antigen. At D42 and D182 the SPRs in the younger cohort showed only a modest difference according to the adjuvant dose. In the older cohort the effect was more marked at D42 but there was no difference at D182. The SCRs followed the same pattern.

					>= 10 1/DIL				>= 40 1/DIL				GMT		
							95% CI				95% CI			95% CI	
Antibody	Sub-group	Group	Timing	N	n	%	LL	UL	n	%	LL	UL	value	LL	UL
FLU A/IND/05 AB	18-40	QR100AS03	PRE	25	1	4.0	0.1	20.4	0	0.0	0.0	13.7	5.3	4.7	5.9
			PI(D21)	25	18	72.0	50.6	87.9	16	64.0	42.5	82.0	38.9	20.8	73.0
			PII(D42)	25	24	96.0	79.6	99.9	24	96.0	79.6	99.9	605.5	337.8	1085.3
			PII(D182)	25	18	72.0	50.6	87.9	17	68.0	46.5	85.1	45.9	24.6	85.5
		QR50AS03	PRE	26	0	0.0	0.0	13.2	0	0.0	0.0	13.2	5.0	5.0	5.0
			PI(D21)	26	14	53.8	33.4	73.4	10	38.5	20.2	59.4	19.7	10.7	36.2
			PII(D42)	26	25	96.2	80.4	99.9	24	92.3	74.9	99.1	283.8	159.9	503.8
			PII(D182)	25	15	60.0	38.7	78.9	14	56.0	34.9	75.6	23.0	13.3	39.8
	41-64	QR100AS03	PRE	24	2	8.3	1.0	27.0	1	4.2	0.1	21.1	5.9	4.6	7.6
			PI(D21)	24	9	37.5	18.8	59.4	7	29.2	12.6	51.1	13.3	7.4	24.2
			PII(D42)	24	24	100	85.8	100	23	95.8	78.9	99.9	177.1	103.6	302.7
			PII(D182)	24	15	62.5	40.6	81.2	9	37.5	18.8	59.4	22.7	11.9	43.5
		QR50AS03	PRE	24	0	0.0	0.0	14.2	0	0.0	0.0	14.2	5.0	5.0	5.0
			PI(D21)	24	15	62.5	40.6	81.2	11	45.8	25.6	67.2	22.1	12.4	39.5
			PII(D42)	24	18	75.0	53.3	90.2	18	75.0	53.3	90.2	102.3	44.9	232.8
			PII(D182)	24	13	54.2	32.8	74.4	9	37.5	18.8	59.4	17.3	9.8	30.5

					SPR			
							95% CI	
Strain	Sub-group	Group	Timing	N	n	%	LL	UL
FLU A/IND/05 AB	18-40	QR100AS03	PRE	25	0	0.0	0.0	13.7
			PI(D21)	25	16	64.0	42.5	82.0
			PII(D42)	25	24	96.0	79.6	99.9
			PII(D182)	25	17	68.0	46.5	85.1
		QR50AS03	PRE	26	0	0.0	0.0	13.2
			PI(D21)	26	10	38.5	20.2	59.4
			PII(D42)	26	24	92.3	74.9	99.1
			PII(D182)	25	14	56.0	34.9	75.6
	41-64	QR100AS03	PRE	24	1	4.2	0.1	21.1
			PI(D21)	24	7	29.2	12.6	51.1
			PII(D42)	24	23	95.8	78.9	99.9
			PII(D182)	24	9	37.5	18.8	59.4
		QR50AS03	PRE	24	0	0.0	0.0	14.2
			PI(D21)	24	11	45.8	25.6	67.2
			PII(D42)	24	18	75.0	53.3	90.2
			PII(D182)	24	9	37.5	18.8	59.4

The SCFs showed an effect of adjuvant dose in the younger cohort. SCFs were much lower in the older cohort and only at D42 was there any evidence of an adjuvant dose effect.

					SCF		
						95% CI	
Strain	Sub-group	Group	Timing	N	Value	LL	UL
FLU A/IND/05 AB (1/DIL)	18-40	QR100AS03	PI(D21)	25	7.4	4.0	13.7
			PII(D42)	25	114.6	62.6	209.8
			PII(D182)	25	8.7	4.7	16.2
		QR50AS03	PI(D21)	26	3.9	2.1	7.2
			PII(D42)	26	56.8	32.0	100.8
			PII(D182)	25	4.6	2.7	8.0
	41-64	QR100AS03	PI(D21)	24	2.2	1.4	3.6
			PII(D42)	24	29.8	17.0	52.1
			PII(D182)	24	3.8	2.0	7.2
		QR50AS03	PI(D21)	24	4.4	2.5	7.9
			PII(D42)	24	20.5	9.0	46.6
			PII(D182)	24	3.5	2.0	6.1

HI antibody responses to drift-variant viruses were lower overall, but showed similar patterns as reported above for the two age cohorts. Titres were highest against the more closely (serologically) related A/turkey/Turkey and least for the clade 1 representative A/Vietnam.

The pre-vaccination SNA seropositivity rates were quite high in this study despite the very low rates observed with HI data. Both treatment groups demonstrated a neutralising antibody vaccine response (as assessed by a post-vaccination reciprocal titre ≥ 56 for initially seronegative subjects or a ≥ 4-fold increase in reciprocal titre for initially seropositive subjects) at D21, with a further increase at D42. At D42 a neutralising antibody response against A/Indonesia/5/05 was detected in 92% (95% CI 80.4-97.7) and 98% (95% CI 89.4-99.9) of subjects per group. At D182 the response rates had declined in both treatment groups to reach 80% (95% CI 65.7-89.8) and 78% (95% CI 63.4-88.2), but remained slightly above rates at D21.

Table 19 VRRs for A/Indonesia/5/05 neutralizing antibody through Day 182 (ATP cohort for immunogenicity)

		Vaccine response														
		Day 21					Day 42					Day 182				
	Pre-vaccination				95% CI					95% CI					95% CI	
Group	status	N	n	%	LL	UL	N	n	%	LL	UL	N	n	%	LL	UL
QR100AS03	S-	30	24	80.0	61.4	92.3	30	30	100	88.4	100	30	29	96.7	82.8	99.9
	S+	19	9	47.4	24.4	71.1	19	15	78.9	54.4	93.9	19	10	52.6	28.9	75.6
	Total	49	33	67.3	52.5	80.1	49	45	91.8	80.4	97.7	49	39	79.6	65.7	89.8
QR50AS03	S-	26	23	88.5	69.8	97.6	26	26	100	86.8	100	25	24	96.0	79.6	99.9
	S+	24	9	37.5	18.8	59.4	24	23	95.8	78.9	99.9	24	14	58.3	36.6	77.9
	Total	50	32	64.0	49.2	77.1	50	49	98.0	89.4	99.9	49	38	77.6	63.4	88.2

The SNA data against A/Indonesia/05/2005 showed some advantage for full dose adjuvant in the younger subjects but not in the older subjects.

					>= 28 1/DIL				>= 40 1/DIL				GMT		
							95% CI				95% CI			95% CI	
Antibody	Sub-group	Group	Timing	N	n	%	LL	UL	n	%	LL	UL	value	LL	UL
FLU A/IND/05 AB	18-40	QR100AS03	PRE	25	7	28.0	12.1	49.4	5	20.0	6.8	40.7	20.1	15.5	26.2
			PI(D21)	25	25	100	86.3	100	23	92.0	74.0	99.0	210.0	151.1	291.9
			PII(D42)	25	25	100	86.3	100	25	100	86.3	100	2532.3	1694.2	3784.8
			PII(D182)	25	25	100	86.3	100	24	96.0	79.6	99.9	448.2	313.9	640.0
		QR50AS03	PRE	26	14	53.8	33.4	73.4	6	23.1	9.0	43.6	26.6	19.5	36.4
			PI(D21)	26	23	88.5	69.8	97.6	22	84.6	65.1	95.6	138.5	86.7	221.3
			PII(D42)	26	26	100	86.8	100	26	100	86.8	100	1166.2	834.1	1630.3
			PII(D182)	25	25	100	86.3	100	25	100	86.3	100	254.8	192.1	337.9
	41-64	QR100AS03	PRE	24	12	50.0	29.1	70.9	8	33.3	15.6	55.3	36.8	22.0	61.6
			PI(D21)	24	23	95.8	78.9	99.9	21	87.5	67.6	97.3	166.1	106.1	259.8
			PII(D42)	24	24	100	85.8	100	24	100	85.8	100	683.1	442.0	1055.8
			PII(D182)	24	24	100	85.8	100	24	100	85.8	100	263.4	175.7	394.8
		QR50AS03	PRE	24	10	41.7	22.1	63.4	7	29.2	12.6	51.1	26.8	18.6	38.7
			PI(D21)	24	23	95.8	78.9	99.9	23	95.8	78.9	99.9	176.1	124.0	250.0
			PII(D42)	24	24	100	85.8	100	24	100	85.8	100	773.9	512.5	1168.7
			PII(D182)	24	23	95.8	78.9	99.9	23	95.8	78.9	99.9	203.3	143.6	287.9

NA responses against drifted strains were lower. The D42 response rates for A/Vietnam/1194/04 were 35% and 16% while those for A/Anhui/1/05 (74% and 73%) and A/turkey/Turkey/1/05 (85% and 92%) were higher. The D182 rates were 33% and 23% for A/Vietnam/1194/04, 37% and 19% for A/Anhui/1/05 but 79% and 68% for A/turkey/Turkey/1/05.

In line with the response rates the GMTs increased after each dose and were numerically higher in the full dose adjuvant group on D21, D42 and D182.

Table 20 Neutralizing antibody GMTs for A/Indonesia/5/05 antibody through Day 182 (ATP cohort for immunogenicity)

Antibody	Group	Timing	N	GMT	95% CI	
					LL	UL
A/Indonesia/5/05	QR100AS03	PRE	49	27.1	20.3	36.1
		Day 21	49	187.2	143.2	244.7
		Day 42	49	1332.9	946.4	1877.2
		Day 182	49	345.5	263.7	452.6
	QR50AS03	PRE	50	26.7	21.2	33.7
		Day 21	50	155.4	116.6	207.2
		Day 42	50	957.8	738.1	1243.0
		Day 182	49	228.1	183.6	283.5

The pattern observed relative to adjuvant effects and GMTs over time for NA against the drifted strains tested was comparable to that seen with the vaccine-homologous virus although actual GMTs were generally much lower for the drift variant virus strains.

Titres ≥ 1:80 were observed in 16% and 12% at D0. At D42 all subjects were seropositive and only one was seronegative at D182. At Day 21 ≥ 80% had titres ≥ 1:80 against A/Indonesia/5/05, reaching 100% at D42 and persisting at > 90% at D182.

Table 21 Distribution of A/Indonesia/5/05 neutralizing antibody titers (ATP cohort for immunogenicity)

				<28 1/DIL				>=28 1/DIL				>=40 1/DIL				>=80 1/DIL			
						95% CI				95% CI				95% CI				95% CI	
Antibody	Group	Timing	N	n	%	LL	UL	n	%	LL	UL	n	%	LL	UL	n	%	LL	UL
A/Indonesia/5/05	QR100AS03	PRE	49	30	61.2	46.2	74.8	19	38.8	25.2	53.8	13	26.5	14.9	41.1	8	16.3	7.3	29.7
		Day 21	49	1	2.0	0.1	10.9	48	98.0	89.1	99.9	44	89.8	77.8	96.6	41	83.7	70.3	92.7
		Day 42	49	0	0.0	0.0	7.3	49	100	92.7	100	49	100	92.7	100	49	100	92.7	100
		Day 182	49	0	0.0	0.0	7.3	49	100	92.7	100	48	98.0	89.1	99.9	47	95.9	86.0	99.5
	QR50AS03	PRE	50	26	52.0	37.4	66.3	24	48.0	33.7	62.6	13	26.0	14.6	40.3	6	12.0	4.5	24.3
		Day 21	50	4	8.0	2.2	19.2	46	92.0	80.8	97.8	45	90.0	78.2	96.7	40	80.0	66.3	90.0
		Day 42	50	0	0.0	0.0	7.1	50	100	92.9	100	50	100	92.9	100	50	100	92.9	100
		Day 182	49	1	2.0	0.1	10.9	48	98.0	89.1	99.9	48	98.0	89.1	99.9	45	91.8	80.4	97.7

Most subjects had detectable NA against A/Vietnam/1194/04 at baseline and titres ≥ 1:80 were noted in 67% and 74% per group. At Day 21 and Day 42, > 90% per group had titres ≥ 1:80 and > 85% maintained that level at D182. In contrast, most (> 90%) subjects were seronegative at baseline for NA against A/Anhui/1/05. By D42 69% and 53% per group had titres ≥ 1:80 against this strain and at D182 rates had dropped to 25% and 10%. Also, most (75% and 77%) were seronegative at baseline for A/turkey/Turkey/1/05 but at D42 > 90% had titres ≥ 1:80 and 78% and 69% still had these titres at D182.

Discussion on Q-Pan-H5N1-001

Study Q-Pan H5N1-001 set out to examine several different issues. The data supported use of AS03 in conjunction with 3.8 µg HA and a comparable performance of Q-Pan H5N1 and D-Pan H5N1. Administration of two doses was shown to be necessary. Use of half strength AS03 was also acceptable but there were some advantages for full strength adjuvant in older subjects and against drifted variants.

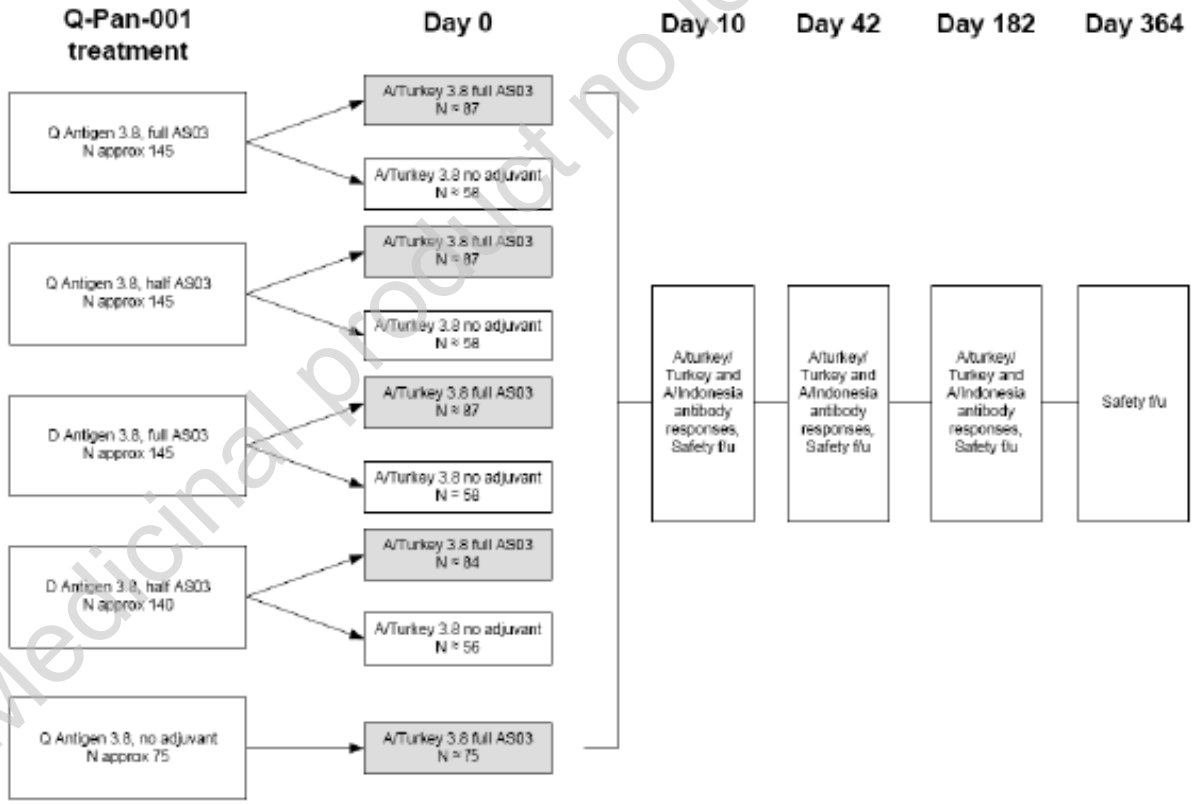
The contingent groups provided additional information on half the HA dose administered with full or half strength AS03. Based on the HI data and the CBER criteria the data suggested that two doses of

vaccine containing half the amount of antigen plus full strength AS03 or half strength AS03 (i.e. Group I) might be satisfactory in healthy adults aged 18-64 years. However, the D42 data suggested (taking into account also the responses to drifted variants) that half the adult dose of HA and AS03 might be a less secure regimen overall, which paralleled the observation made when full and half strength AS03 were administered with 3.8 µg HA in this same study. The contingent group data were not shown separately at D182 for age strata 18-40 and 41-60 years but all other data have suggested that immune responses are likely to be somewhat lower in the latter group and are more sensitive to a reduction in AS03 than in the younger cohort.

When the data were shown according to age cohorts 18-40 and 41-64 years it was notable that HI responses did not show a consistent benefit for full dose adjuvant in the older cohort when AS03 was combined with half the approved dose of antigen. In addition, where some advantage for full dose adjuvant was apparent in the older cohort the magnitude of the effect was not obviously greater than for the younger cohort. These results did not give the same picture by age sub-groups as was noted with the approved HA dose administered in conjunction with full or half dose antigen.

Q-Pan H5N1-010 (booster phase of Q-Pan H5N1-001)

This was the booster phase of **Q-Pan-H5N1-001** in which A/turkey/Turkey vaccine was administered at about 15 months after the initial immunisation series. There were 469 subjects enrolled, representing 69% of the 680 that were randomised into Q-Pan-001. Subjects were randomised to receive adjuvanted or unadjuvanted booster doses in a ratio of 3:2. Adjuvanted vaccine was used to boost Groups A (primed with unadjuvanted vaccine; bottom in diagram), B1, C1, D1 and E1 (grey shaded boxes from top). Unadjuvanted vaccine was administered to Groups B2, C2, D2 and E2 (unshaded boxes from top).



This randomised, observer-blind study assessed immune responses to a booster dose of heterologous H5N1 (A/turkey/Turkey/1/2005) given about 15 months after completion of Q-Pan H5N1-001 (see

above). Subjects (N=469) who agreed to participate in this follow-on study (= 69% of those enrolled in Q-Pan H5N1-001) were randomised to receive adjuvanted (full strength) or unadjuvanted HA booster doses in a ratio of 3:2. Adjuvanted vaccine was used to boost Group A subjects (primed with unadjuvanted vaccine) and a proportion of subjects from each of the other groups (denoted B1, C1, D1 and E1) while the remainder received an unadjuvanted booster (denoted B2, C2, D2 and E2).

The primary objective was to assess whether the booster dose was more immunogenic in subjects primed with two doses of heterologous H5N1 adjuvanted with full dose [A] or half dose [B] AS03 compared to subjects primed with unadjuvanted antigen. HI responses to the booster dose were compared against the CBER criteria for HI SCRs and SPRs at 10 days post-dose.

Pre-booster (i.e. on Day 0 of study 010) 16.3% of subjects primed with unadjuvanted vaccine (Group A) were seropositive to A/Indonesia/5/2005 H5N1 compared to 68.6% primed with AS03A (Groups B1, B2, D1 and D2) and 63.5% primed with AS03B (Groups C1, C2, E1 and E2). Seropositivity against A/turkey/Turkey/1/2005 H5N1 was observed for 10.2% in Group A, 55% primed with AS03A and 49% primed with AS03B.

At D10 post-booster the **SCR** was 96% in Groups B1 + D1 and 91.5% in Group A. The 95% CI around the difference did not meet the target of a lower bound ≥ 15% so the response to an adjuvanted booster dose was not significantly greater in those primed with AS03A vs. those who received an unadjuvanted primary series. The actual difference in SCR was larger for subjects who were seropositive at baseline (14.3% and 5.4%, respectively) but the lower bound of the 95% CI did not meet the target ≥ 15% in either case. In addition, the difference between groups did not meet the target of a lower bound of 95% CI ≥ 15% for either age stratum.

Table 20 Difference between adjuvanted and non-adjuvanted groups (Groups B1 + D1 versus Group A) in percentage of subjects with seroconversion to A/turkey/Turkey/1/2005 antibody at Day 10 by pre-vaccination status in subjects 18 to 64 years of age (ATP cohort for immunogenicity)

Antibody	Pre-vaccination status	B1 + D1			A			Difference in SCR (B1 + D1 minus A)		
									95% CI	
		N	n	%	N	n	%	%	LL	UL
A/turkey/Turkey/1/2005 Ab (1/DIL)	S-	56	55	98.2	42	39	92.9	5.36	-3.36	17.53
	S+	70	66	94.3	5	4	80.0	14.29	-4.01	57.34
	Total	126	121	96.0	47	43	91.5	4.54	-2.59	16.30

A = Q-Pan Indo 3.8 x 2 + Turkey 3.8, AS03A
B1 + D1 = Q-Pan and D-Pan Indo 3.8, AS03A x 2 + Turkey 3.8, AS03A

For the adjusted **GMT ratio** for Groups B1 + D1 / Group A (824 vs. 286) the lower level of the 95% CI was just less than 2 (1.9). Thus, according to the pre-defined criterion the response to an adjuvanted booster dose after priming with HA/AS03A was not superior to that observed after unadjuvanted HA priming.

The adjusted GMT ratio in Groups B1 + D1 (GMT 1206.6) to Group A (GMT 188.8) for subjects aged 18 to 40 years was 6.39 with a 95% CI of 3.53-11.58, which met the target of a LL 95% CI > 2. In contrast, the corresponding comparison for subjects aged 41 to 64 years gave a ratio of 1.50 with 95% CI of 0.85-2.63. Therefore the overall lack of difference between groups in GMT ratio was driven by the results of the older age stratum.

For Groups B1 + D1 the **SCR** (96.0%) showed 95% CI 91.0-98.7%, which exceeded the CBER guidance target of 40%. The CBER guidance target for SCR was exceeded for subjects who were seronegative at baseline (90.4) and those who were seropositive at baseline (86) as well as in each age stratum. In addition, the **SPR** for Groups B1 + D1 was 99.2% (95% CI 95.7-100%), which exceeded the CBER guidance target of 70%. The SPR for each age stratum also exceeded the CBER guidance target.

Responses to the booster dose were also compared between those who were primed with AS03A or AS03B. The **SCRs** were high for all groups and the difference in SCR (B1 + D1 vs. C1 + E1 was -

0.43% (95% CI -5.92 to 5.28%) so it was concluded that there was no difference between responses to the booster according to the amount of adjuvant used for the priming doses. This conclusion also applied regardless of baseline serostatus. Furthermore the difference in SCR for subjects aged 18 to 40 years was 1.67% (95% CI -4.65 to 8.90%) while that for subjects aged 41 to 64 years was -1.69% (95% CI -11.42 to 8.96).

GMTs at Day 10 for subjects in Group A, Groups C1+E1 and Groups B1+D1, respectively, were 229.6, 810.5 and 847.3 with lower values at **Day 42** of 155.4, 699.5 and 652.2. For subjects primed and boosted with adjuvanted vaccines, the GMT for subjects aged 41 to 64 years was approximately half of that seen in younger subjects. For subjects who received unadjuvanted booster there was little difference between the age strata. Older subjects in Group A had GMTs that were nearly double those seen in the younger age stratum.

The adjusted **GMT ratio** (B1 + D1 vs. C1 + E1) was 1.06 (95% CI 0.78 to 1.44). The GMT ratio was 1.04 (95% CI 0.68-1.59) for subjects aged 18 to 40 years and 1.13 (95% CI 0.73-1.75) for subjects aged 41 to 65 years. In those primed without AS03 (Group A), with AS03B (Groups C1 + E1) or with AS03A (Groups B1 + D1) the post-boost (day 10) **SCRs** were 91.5%, 96.5% and 96.0%, respectively. Thus, subjects who received a booster vaccine with AS03A, regardless of whether priming was adjuvanted or unadjuvanted, had high SCRs on Day 10. The corresponding **SCRs at Day 42** were 87.2%, 95.6% and 96.1%, respectively.

In contrast, subjects who received a booster vaccine without adjuvant had lower point estimates of SCRs ranging from 64.6 to 72.9% across groups, regardless of priming condition. SCRs for A/Indonesia/5/2005 were very similar to A/turkey/Turkey/1/2005 results. With the exception of subjects in Group A, there was a trend for lower SCRs for the 41 to 64 years of age stratum. However, the SCR 95% CIs overlap for all vaccine groups given the small sample size. Day 42 SCRs ranged from 57.0% to 96.4% for all groups.

Discussion on Q-Pan-H5N1-010

A single booster dose of A/turkey/Turkey/1/2005 adjuvanted with AS03A elicited an immune response at Day 10 that exceeded CBER guidance targets for HI SCR and SPR against the booster strain and the priming strain (both being clade 2) in subjects primed with A/Indonesia/5/2005/AS03A. Based on the protocol-defined criteria, the SCRs and GMTs indicated that A/turkey/Turkey/1/2005/AS03A was not more immunogenic in those primed with A/Indonesia/5/2005/AS03A compared to those who had received unadjuvanted vaccine in the primary series. The immunogenicity of the adjuvanted booster dose was very similar among recipients of adjuvanted priming regimens without regard to the AS03 formulation (full or half dose) used at priming. All groups that received adjuvanted booster doses had SCRs in excess of 90% but the post-boost GMTs showed a clear trend to be higher in those who had received adjuvanted vaccine for priming.

Priming with adjuvanted vaccine and boosting with unadjuvanted vaccine produced a quantitatively weaker immune response than boosting with adjuvanted vaccine. Those primed with full or half dose AS03 and boosted with AS03 vaccine had the most robust immune response at both Days 10 and 42 after the booster dose. This study did not demonstrate an inhibitory effect on the booster response of unadjuvanted priming as has been noted in some other trials. This may relate to the fact that the booster viral strain used in this study is more closely related to the priming antigen (clade 2.2 vs. 2.1) than has been the case in some other datasets (clade 2.1 vs. clade 1).

The results of this study need to take into account the fact that the priming and boosting strains are closely related (both are clade 2 viruses, with some *in-vitro* cross-reactivity of HI antibodies). Therefore the results may not be extrapolated more broadly to suggest that boosting with AS03-adjuvanted vaccine can be successful regardless of the priming vaccine formulation.

Q-Pan H5N1-002

This randomised, observer-blinded and placebo-controlled study was conducted during 2008 in N. America and included evaluations of lot to lot consistency study and age-specific immune responses. Participants were to be aged 18 to 49 years and in good health or aged > 49 years and in stable health. Subjects in each age stratum were randomly assigned (3:1 ratio) to receive vaccine from one of three lots or placebo.

Subjects were sub-randomised to have samples analysed for primary immunogenicity assessments at D0, D42 and D182.

The study vaccine contained 3.75 µg HA derived from A/Indonesia/5/05 H5N1 plus the same AS03 and thiomersal content as already approved for D-Pan (i.e. before the variation to approve thiomersal-free product).

The assignment of treatment was as follows:

Table 2 Study Groups, by Age Strata and Study Vaccine Lot

Study Arms	Age in Years[1]	Antigen lot	Adjuvant lot	Placebo[2]	Subject (N)	Tested for Immunogenicity		
						Lot consistency	SCR/SPR 18-64 yrs	SCR/SPR >64 yrs
A	18-49	A	1		555	420	1260 (420/lot, combined)	
B	18-49	B	2		555	420		
C	18-49	C	3		555	420		
D	18-49			PBS	555		60	
E	50-64	A	1		555 (185/ lot)		420 (140/lot, combined with arms A, B, & C)	
		B	2					
		C	3					
F	50-64			PBS	185		20	
G	> 64	A	1		1110 (370/lot)			420 (140/lot, combined)
		B	2					
		C	3					
H	> 64			PBS	370			40

Subjects in Groups A-D were to be stratified by age 18-30 years and 31-49 years. Subjects in Groups G & H were to be stratified by age 64-75 years and >75 years.
Placebo consisted of 0.5 ml of sterile preserved isotonic saline for injection administered intramuscularly (IM).

The primary immunogenicity objectives were:

- To demonstrate that HI antibody responses to Q-Pan at D42 met or exceeded the CBER Guidance targets for SCRs and SPRs when tested separately for subjects aged 18 to 64 years and > 64 years.
- To demonstrate lot to lot consistency in subjects aged 18 to 49 years. Equivalence was to be tested for each of the 3 pair wise ratios of HI GMTs based on a 2-sided 95% confidence bounds for all the 3 pair wise ratios falling between the limits 0.67 to 1.5.

The target sample size was approximately 4440 healthy adults aged 18 years or older in 8 dose groups (3330 to receive Q-Pan and 1110 placebo). Subjects were sub-randomised to have samples analysed for primary immunogenicity assessments and a subset of D182 sera was to be analysed.

Of 4561 randomised in the study 3072 were aged 18 to 64 years (2304 vaccine and 768 placebo) and 1489 were aged > 64 years (1118 vaccine and 371 placebo). By D182 there had been 218 subjects withdrawn from the study, mainly due to loss to follow-up (58 Q-Pan and 24 placebo with a complete primary vaccination course; 24 and 13 with an incomplete primary vaccination course).

<u>HI at D42 and D182</u>

Very few subjects were HI seropositive at D0 in either of the age strata. The post-vaccination D42 SCRs were higher in the younger age stratum but the lower 95% CI in subjects in both strata who were seronegative at baseline exceeded the CBER requirements. In the Q-Pan group, the D42 SCRs (and the lower 95% CI around these SCRs) exceeded the CHMP criteria in both age strata.

Table 24 A/Indonesia/5/05 seroconversion rates (SCR) at Day 42 in subjects 18 to 64 years of age and greater than 64 years of age (ATP cohort for immunogenicity)

		SCR 18-64 years					SCR >64 years				
		18-64 years			95% CI		>64 years			95% CI	
Group	Pre-vaccination status	N	n	%	LL	UL	N	n	%	LL	UL
Q-Pan	S-	1566	1422	90.8	89.3	92.2	387	287	74.2	69.5	78.5
	S+	5	5	100	47.8	100	9	6	66.7	29.9	92.5
	Total	1571	1427	90.8	89.3	92.2	396	293	74.0	69.4	78.2
Placebo	S-	76	1	1.3	0.0	7.1	40	1	2.5	0.1	13.2
	S+	0	0	.	.	.	0	0	.	.	.
	Total	76	1	1.3	0.0	7.1	40	1	2.5	0.1	13.2

Q-Pan recipients aged 18-64 years and > 64 years maintained SCRs at D182 that still reached or exceeded the CBER criteria. In each case the lower 95% CI exceeded 50%. In addition, the D182 SCRs in Q-Pan recipients still reached or exceeded the CHMP criteria.

Since so few subjects were seropositive (or seroprotected) before vaccination the D42 the SPRs were almost the same as the SCRs and therefore the conclusions were generally the same as for the D42 SCRs above. In contrast, at D182 the SPRs in Q-Pan subjects no longer met the CBER criteria but were > 60% for both age strata and the lower 95% CI exceeded 55%. At Day 182 the SPR among Q-Pan recipients aged 18-60 years had fallen to 62.0% and was comparable with the SPR for the older cohort (63.5%). Therefore the rate in the younger cohort no longer met the CHMP criterion.

Assessment of lot to lot consistency based on HI GMTs

The D42 GMFRs in both age cohorts met the CHMP criteria after vaccination (51.4 and 17.2, compared to 1.0 in the placebo groups). The D182 GMFRs in vaccinated subjects were 7.4 and 7.8. The 95% CI for the GMT ratios at D42 are shown in the next table. In each case these fell within the pre-specified limits and therefore the applicant concluded that lot to lot consistency was demonstrated.

Table 26 Adjusted ratios of H5N1 GMTs for Q-Pan Lot A and Q-Pan Lot B, Q-Pan Lot A and Q-Pan Lot C, and Q-Pan Lot B and Q-Pan Lot C at Day 42 in subjects 18-49 years of age (ATP cohort for immunogenicity)

	Q-Pan Lot A		Q-Pan Lot B		Q-Pan Lot C	
	N	GMT	N	GMT	N	GMT
Adjusted GMT	394	275.8	379	291.7	394	333.5
Adjusted GMT Ratio (95% CI)						
Q-Pan Lot A and Q-Pan Lot B	0.95 (0.78, 1.15)					
Q-Pan Lot A and Q-Pan Lot C	0.83 (0.68, 1.00)					
Q-Pan Lot B and Q-Pan Lot C	0.87 (0.72, 1.06)					

NA at D42

At baseline, the majority of subjects in the 18 to 64 years age group were seronegative for NA against A/Indonesia/5/05 (72%) and A/Vietnam/1194/04 (60%). At Day 42 all subjects tested in the 18 to 64 years group were seropositive against A/Indonesia/5/05 and all had titres ≥ 1:80 while the seropositivity rate against A/Vietnam/1194/04 was 96.7% and 85.1% had titres ≥ 1:80.

Table 30 Distribution of vaccine-homologous and drift variant H5N1 viruses tested by MN in subjects 18 to 64 years of age (ATP cohort for immunogenicity)

				<28 1/DIL				>=28 1/DIL				>=40 1/DIL				>=80 1/DIL			
						95% CI				95% CI				95% CI				95% CI	
Antibody	Group	Timing	N	n	%	LL	UL	n	%	LL	UL	n	%	LL	UL	n	%	LL	UL
FLU A/IND/05 AB	Q-Pan	PRE	188	136	72.3	65.4	78.6	52	27.7	21.4	34.6	40	21.3	15.7	27.8	25	13.3	8.8	19.0
		DAY 42	188	0	0.0	0.0	1.9	188	100	98.1	100	188	100	98.1	100	188	100	98.1	100
FLU A/VIET/04 AB	Q-Pan	PRE	181	108	59.7	52.1	66.9	73	40.3	33.1	47.9	62	34.3	27.4	41.7	37	20.4	14.8	27.1
		DAY 42	181	6	3.3	1.2	7.1	175	96.7	92.9	98.8	174	96.1	92.2	98.4	154	85.1	79.0	89.9

The majority of subjects aged > 60 years were seropositive at baseline for the two viruses but there was a demonstrable immune response with >90% reaching titres of at least 1:80 against these viruses by D42.

A 4-fold rise in NA (vaccine response) was documented against A/Indonesia/5/05 in 94% of vaccinated subjects in the 18 to 64 years group and 78% of older subjects compared to rates against A/Vietnam/1194/04 of 62% and 27%. Response rates to Q-Pan were higher in subgroups that were seronegative before vaccination.

Correspondingly, the NA GMTs were higher in the elderly for both viruses. For A/Indonesia/5/05 the GMT for the 18 to 64 years age group had increased by 66-fold at D42 while there was a 12-fold increase in the older age group. Increments in GMTs against A/Vietnam/1194/04 were 5-fold and just over 2-fold in respective age groups after two doses of Q-Pan.

D182 neutralising antibody data were to be obtained from a subset of 250 subjects who were pre-selected at the time of randomisation. At D182 for 226 eligible subjects the percentages that still met the criteria for vaccine response were numerically higher in the younger age cohort regardless of baseline serostatus. Also, at D182 the greater GMT for the younger cohort was sustained with non-overlapping 95% CI vs. the older age cohort.

Table 10 GMTs of neutralizing antibody against A/Indonesia/5/05 strain at pre-vaccination and at Days 42 and 182 post first dose by age stratum (ATP cohort for immunogenicity, MN testing random subset)

						GMT		
							95% CI	
Antibody	Sub-group	Group	Timing	N	n	Value	LL	UL
FLU A/IND/05 AB	18-64	Q-Pan	PRE	188	52	22.4	19.9	25.3
			PII (D42)	188	188	1450.6	1266.9	1660.9
			PII (D182)	181	180	318.4	287.1	353.1
	>64	Q-Pan	PRE	46	33	52.4	39.2	69.8
			PII (D42)	46	45	631.1	444.5	896.1
			PII (D182)	45	45	215.9	165.6	281.4

Although 72% of subjects aged 18 to 64 years were seronegative (< 1:28) at baseline the percentage with titres ≥ 1:80 was 13.3%. Among those aged > 64 years only 28% were seronegative at baseline and 45.7% already had titres ≥ 1:80. At Day 42 all subjects aged 18 to 64 years and 95.7% aged > 64 years had titres ≥ 1:80 against A/Indonesia/5/05 and most of these subjects retained at least titres at this level at D182 regardless of age. Comparable results were obtained in the TVC analysis.

Discussion on Study Q-Pan-H5N1-002

The study demonstrated that Q-Pan containing A/Indonesia/5/05 elicited HI responses to homologous virus at D42 that met the CBER and CHMP criteria in the respective age groups. However, the responses in the younger age stratum were significantly higher than in the older age stratum. The SCR criteria and GMFR criteria were still met at D182 in both age strata while the CBER SPR criteria were not met in either of the age strata and the CHMP criteria were met only in the older age stratum. Pre-vaccination NA seropositivity rates were higher than pre-vaccination HI seropositivity rates and were

higher for A/Vietnam than for A/Indonesia. Nevertheless, there was a clear response to vaccination in both age strata at D42 with responses documented with respect to vaccine-homologous virus and A/Vietnam.

Clinical studies in special populations

Study D-Pan H5N1-009, -022, -023

This open label study in three phases (009, 022 and 023) carried out with D-Pan H5N1, in children 3-9 years of age which has also been previously assessed within the Marketing Authorisation for Arepanrix and Pandemrix was divided into three parts as shown below:

Figure 1 Sequential staggered study design of study H5N1-009

	Phase A H5N1-009	Phase B H5N1-022	Phase C H5N1-023
Half Adult Dose HA antigen **Half** Adult Dose AS03	•6-9 yr olds •3-5 yr olds		
Full Adult Dose HA antigen **Half** Adult Dose AS03		•6-9 yr olds •3-5 yr olds	
Full Adult Dose HA antigen **Full** Adult Dose AS03			•6-9 yr olds •3-5 yr olds

1. Full adult dose HA = 3.8 µg, Half adult dose HA = 1.9 µg HA

In Phase A randomisation was to half the adult dose (1.9 µg of HA) + half the AS03 or to *Fluarix*
In Phase B and Phase C randomisation was to (allocation ratio 3:1) full HA/half AS03 (**Phase B**) or to the adult dose (**Phase C**) with a *Fluarix* control group.

Immunogenicity up to D42

Phase A
The pre-vaccination HI GMTs for A/Vietnam/1194/2004 and A/Indonesia/05/2005 were <1:10 and so seropositivity rates were zero. On Day 21, the GMTs against A/Vietnam/1194/2004 strain were slightly increased in the Half HA/Half AS03 group in both age strata and then increased markedly after the second dose (540.3 for 6-9 years; 392.7 for 3-5 years). A similar pattern but lower response was seen against A/Indonesia/05/2005 (60.8 for 6-9 years; 53.5 for 3-5 years).

Table 1 Humoral immune response - H5N1 HI antibodies

H5N1 HI Antibodies against A/Vietnam/1194/2004													
		GMT			SPR			SCR			SCF		
			95% CI			95% CI			95% CI			95% CI	
Timing	N	value	LL	UL	%	LL	UL	%	LL	UL	value	LL	UL
1.9 µg HA / Half AS03 - 3-5 years													
PRE	49	5.0	5.0	5.0	0.0	0.0	7.3						
PI(D21)	49	8.7	6.2	12.3	12.2	4.6	24.8	12.2	4.6	24.8	1.7	1.2	2.5
PII(D42)	49	392.7	280.4	550.2	95.9	86.0	99.5	95.9	86.0	99.5	78.5	56.1	110.0
1.9 µg HA / Half AS03 - 6-9 years													
PRE	43	5.0	5.0	5.0	0.0	0.0	8.2						
PI(D21)	43	12.1	8.4	17.5	30.2	17.2	46.1	30.2	17.2	46.1	2.4	1.7	3.5
PII(D42)	43	540.3	424.5	687.7	100	91.8	100	100	91.8	100	108.1	84.9	137.5
H5N1 HI Antibodies against A/Indonesia/05/2005													
1.9 µg HA / Half AS03 - 3-5 years													
PRE	49	5.0	5.0	5.0	0.0	0.0	7.3						
PI(D21)	49	5.2	4.9	5.6	0.0	0.0	7.3	0.0	0.0	7.3	1.0	1.0	1.1
PII(D42)	49	53.5	35.0	81.7	71.4	56.7	83.4	71.4	56.7	83.4	10.7	7.0	16.3
1.9 µg HA / Half AS03 - 6-9 years													
PRE	43	5.0	5.0	5.0	0.0	0.0	8.2						
PI(D21)	43	5.2	4.8	5.8	2.3	0.1	12.3	2.3	0.1	12.3	1.0	1.0	1.2
PII(D42)	43	60.8	38.7	95.5	74.4	58.8	86.5	74.4	58.8	86.5	12.2	7.7	19.1

1. 3-5y = 3-5 years; 6-9y = 6-9 years
2. GMT = geometric mean antibody titre calculated on all subjects; Seroconversion defined as: For initially seronegative subjects, antibody titre ≥ 40 1/DIL after vaccination; For initially seropositive subjects, antibody titre after vaccination ≥4 fold the pre-vaccination antibody titre, SCF = Seroconversion Factor or geometric mean ratio (mean[log10(POST/PRE)]
3. N = number of subjects with available results, 95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit; PRE = pre-vaccination; PI(D21) = post-vaccination at Day 21; PII(D42) = post-vaccination at Day 42

In the AS03-adjuvanted vaccine group

- By Day 42 the SCRs and the SPRs against the vaccine strain were 95.9% to 100% while SCRs against A/Indonesia/05/2005 were 71.4% to 74.4 %. The ≥ 70% threshold for the lower bound of the 95% CI for seroprotection as defined in the CBER Guidance was only met for HI against A/Vietnam/1194/2004.
- On Day 42 the SCFs against A/Vietnam/1194/2004 were 78.5 and 108.1 while SCFs against A/Indonesia/05/2005 strain were 10.7 and 12.2.

In the Fluarix group no subject seroconverted for HI antibody to A/Vietnam/1194/2004 or A/Indonesia/05/2005 and no subject was seroprotected.

On Day 42 the NA GMTs against the A/Vietnam/1194/2004 in the AS03 group had reached 1155.1 in the 6-9 years age stratum and 1044.4 in the 3-5 years age stratum, whereas the increase from baseline in the control group was very small (104.5 for 6-9 years; 158.4 for 3-5 years). The NA seropositivity rates against A/Vietnam/1194/2004 in the AS03 group increased to 90.7% in the 6-9 years age stratum and to 91.7% in the 3-5 years age stratum on Day 21, with non-overlapping CIs (when compared with Day 0). All subjects in the AS03 group were seropositive for NA at D42 while the seropositivity rates in controls for NA against the vaccine strain on Days 21 and 42 were within the same range (78.6% - 80.0%).

On Day 42 the NA SCR against the vaccine strain in the Half HA/Half AS03 group had reached 100% in the 6-9 years age stratum and 95.6% in the 3-5 years age stratum. In contrast there was no further increment in SCRs in the control group after a second dose of Fluarix.

Phase B

The pre-vaccination HI GMTs for antibody against A/Vietnam/1194/2004 and A/Indonesia/05/2005 were <1:10 in all vaccine groups and age strata except for one subject in the 3-5 years cohort. By Day 42 GMTs for HI against A/Vietnam/1194/2004 in the AS03 vaccine group were 615.8 for 6-9 years and 678.1 for 3-5 years age groups and reached 64.9 to 73.7 against A/Indonesia but were still below the cut-off value in the control group. Seropositivity rates followed the same pattern as the GMTs.

Table 3 Humoral immune response - H5N1 HI antibodies

H5N1 HI Antibodies against A/Vietnam/1194/2004													
		GMT			SPR			SCR			SCF		
			95% CI			95% CI			95% CI			95% CI	
Timing	N	value	LL	UL	%	LL	UL	%	LL	UL	value	LL	UL
3.8 µg HA / Half AS03 - 3-5 years													
PRE	42	5.1	4.9	5.4	0.0	0.0	8.4	-	-	-	-	-	-
PI(D21)	41	22.7	14.6	35.3	48.8	32.9	64.9	48.8	32.9	64.9	4.4	2.9	6.8
PII(D42)	42	678.1	475.7	966.6	97.6	87.4	99.9	97.6	87.4	99.9	132.3	91.8	190.7
3.8 µg HA / Half AS03 - 6-9 years													
PRE	45	5.1	4.9	5.4	0.0	0.0	7.9	-	-	-	-	-	-
PI(D21)	45	22.7	14.6	35.3	42.2	27.7	57.8	42.2	27.7	57.8	4.7	2.9	7.8
PII(D42)	45	678.1	475.7	966.6	97.8	88.2	99.9	97.8	88.2	99.9	123.2	85.8	176.8
H5N1 HI Antibodies against A/Indonesia/05/2005													
3.8 µg HA / Half AS03 - 3-5 years													
PRE	42	5.0	5.0	5.0	0.0	0.0	8.4	-	-	-	-	-	-
PI(D21)	41	5.5	4.9	6.2	0.0	0.0	8.6	0.0	0.0	8.6	1.1	1.0	1.2
PII(D42)	42	73.7	45.2	120.3	76.2	60.5	87.9	76.2	60.5	87.9	14.7	9.0	24.1
3.8 µg HA / Half AS03 - 6-9 years													
PRE	45	5.0	5.0	5.0	0.0	0.0	7.9	-	-	-	-	-	-
PI(D21)	45	5.3	4.9	5.8	0.0	0.0	7.9	0.0	0.0	7.9	1.1	1.0	1.2
PII(D42)	45	64.9	38.7	108.9	68.9	53.4	81.8	68.9	53.4	81.8	13.0	7.7	21.8

1. 3-5y = 3-5 years; 6-9y = 6-9 years
2. GMT = geometric mean antibody titre calculated on all subjects; Seroconversion defined as: For initially seronegative subjects, antibody titre ≥40 1/DIL after vaccination; For initially seropositive subjects, antibody titre after vaccination ≥4 fold the pre-vaccination antibody titre, SCF = Seroconversion Factor or geometric mean ratio (mean[log10(POST/PRE)]
3. N = number of subjects with available results, 95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit; PRE = pre-vaccination; PI(D21) = post-vaccination at Day 21; PII(D42) = post-vaccination at Day 42

In the AS03-adjuvanted vaccine group the Day 42 SCRs and SPRs against the vaccine strain reached 97.8% for subjects aged 6-9 years and 97.6% for subjects aged 3-5 years. SCRs and SPRs against A/Indonesia/05/2005 increased to 68.9% and 76.2% in respective age groups. The ≥70% threshold for the lower bound of the 95% CI as defined in the CBER Guidance was met for HI against A/Vietnam/1194/2004. At Day 42 the SCFs against A/Vietnam/1194/2004 were 123.2 for 6-9 years and 132.3 for 3-5 years. The increments in SCFs against A/Indonesia/05/2005 strain were relatively modest (13.0 and 14.7).

In the Fluarix group no subject seroconverted for HI antibody to A/Vietnam/1194/2004 or A/Indonesia/05/2005 and no subject was seroprotected.

Pre-vaccination NA GMTs were ≥1:28 and were 25.6 to 65.5 while baseline seropositivity rates ranged from 47.1% to 78.6%. On Day 42 GMTs exceeded 1500 in the AS03 group but there was a negligible increase in the control group. The seropositivity rates and seroconversion rates followed the same pattern as the GMTs.

Phase C

Pre-vaccination GMTs for HI antibody against A/Vietnam/1194/2004 and A/Indonesia/05/2005 were <1:10 regardless of age stratum or vaccine group and so seropositivity rates were zero. Day 21 HI

GMTs against A/Vietnam/1194/2004 were slightly increased in the AS03 vaccine group in both age strata and by Day 42 they had reached 883.5 for 6-9 years and 956.4 for 3-5 years. HI GMTs against A/Indonesia/05/2005 in the AS03 group were also much higher at D42 (92.5 for 6-9 years; 167.9 for 3-5 years) compared with D21. Corresponding seropositivity rates followed a similar pattern and by D42 all subjects in both age strata were seropositive against A/Vietnam while rates against A/Indonesia/05/2005 had reached 83.7% in the 6-9 years age stratum and 95.5% in the 3-5 years age stratum.

Table 5 Humoral immune response - H5N1 HI antibodies

H5N1 HI Antibodies against A/Vietnam/1194/2004													
		GMT			SPR			SCR			SCF		
			95% CI			95% CI			95% CI			95% CI	
Timing	N	value	LL	UL	%	LL	UL	%	LL	UL	value	LL	UL
3.8 µg HA / Full AS03 - 3-5 years													
PRE	44	5.0	5.0	5.0	0.0	0.0	8.0	-	-	-	-	-	-
PI(D21)	43	25.0	16.0	39.3	46.5	31.2	62.3	46.5	31.2	62.3	5.0	3.2	7.9
PII(D42)	44	956.4	769.2	1189.3	100	92.0	100	100	92.0	100	191.3	153.8	237.9
3.8 µg HA / Full AS03 - 6-9 years													
PRE	43	5.0	5.0	5.0	0.0	0.0	8.2	-	-	-	-	-	-
PI(D21)	30	27.3	16.2	46.0	56.7	37.4	74.5	56.7	37.4	74.5	5.5	3.2	9.2
PII(D42)	43	883.5	737.3	1058.6	100	91.8	100	100	91.8	100	176.7	147.5	211.7
H5N1 HI Antibodies against A/Indonesia/05/2005													
3.8 µg HA / Full AS03 - 3-5 years													
PRE	44	5.0	5.0	5.0	0.0	0.0	8.0	-	-	-	-	-	-
PI(D21)	43	7.7	6.0	9.8	7.0	1.5	19.1	7.0	1.5	19.1	1.5	1.2	2.0
PII(D42)	44	167.9	121.7	231.5	95.5	84.5	99.4	95.5	84.5	99.4	33.6	24.3	46.3
3.8 µg HA / Full AS03 - 6-9 years													
PRE	43	5.0	5.0	5.0	0.0	0.0	8.2	-	-	-	-	-	-
PI(D21)	30	6.0	5.0	7.2	3.3	0.1	17.2	3.3	0.1	17.2	1.2	1.0	1.4
PII(D42)	43	92.5	59.3	144.2	79.1	64.0	90.0	79.1	64.0	90.0	18.5	11.9	28.8

1. 3-5y = 3-5 years; 6-9y = 6-9 years
2. GMT = geometric mean antibody titre calculated on all subjects; Seroconversion defined as: For initially seronegative subjects, antibody titre ≥ 40 1/DIL after vaccination; For initially seropositive subjects, antibody titre after vaccination ≥4 fold the pre-vaccination antibody titre, SCF = Seroconversion Factor or geometric mean ratio (mean[log10(POST/PRE)]
3. N = number of subjects with available results, 95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit; PRE = pre-vaccination; PI(D21) = post-vaccination at Day 21; PII(D42) = post-vaccination at Day 42

In the AS03 vaccine group the Day 42 SCRs and SPRs were 100% for both age strata against A/Vietnam and 79.1% to 95.5% against A/Indonesia. The ≥ 70% threshold for the lower bound of the 95% CI for seroprotection as defined in the CBER Guidance was met for HI antibody against A/Vietnam/1194/2004 in both age strata and was met against A/Indonesia/05/2005 in the 3-5 year age stratum. On Day 42 the SCFs against A/Vietnam/1194/2004 were 176.7 and 191.3 compared to 18.5 and 33.6 against A/Indonesia/05/2005.

In the control group no subject seroconverted for HI against either strain and none was seroprotected with the exception of one subject with a response to A/Vietnam/1194/2004 on Day 21 only.

Pre-vaccination NA GMTs were ≥1:28 and were generally comparable between the age strata (range 25.6 to 37.3). Despite the low GMTs, the baseline seropositivity rates ranged from 30.8% to 46.7%. By Day 42 NA GMTs against A/Vietnam/1194/2004 increased about 10-fold in the AS03 group in both age strata and all children were seropositive whereas there was no further increase in GMTs in the control group and the seropositivity rates ranged from 61.5% to 87.5%. The seroconversion rates also showed the marked differences between AS03 and control for both age strata.

Comparison between Phases at D42

There was a trend for higher HI GMTs and SCFs against both strains and a higher NA GMT against the vaccine strain with the formulations tested in Phases C and B compared to Phase A. The immune response tended to be higher in Phase C when compared with Phase B. When comparing the formulation used in Phase C or in Phase B with that used in Phase A the difference between A and C was marked whereas the difference between A and B was much less apparent. There were advantages for C over B for HI and NA GMTs and for HI responses to A/Indonesia.

Immunogenicity at Month 6

By Month 6 the HI GMTS had fallen but were still at least 6-fold higher than the pre-vaccination GMTs in the groups that had received AS03 vaccines. Against A/Vietnam the seroprotection rates at Month 6 in children who received the adult dose vaccine in Part C of the study were 82.8% for 3-5 year-olds and 78% for 6-9 year-olds. These rates compare with 56% and 63.6% in respective age groups who received the half/half vaccine in Part A and with 70.2% and 68.9% who received full dose HA and half AS03 in Part B. The 95% CI overlap between Parts A, B and C within each age stratum. The results for the other parameters shown follow a similar pattern.

Vaccine strain homologous (against H5N1 A/Vietnam) immune response persistence in terms of HI antibodies at month 6

		≥ 10 1/DIL			GMT			SPR			SCR			SCF		
			95% CI			95% CI			95% CI			95% CI			95% CI	
Timing	N	%	LL	UL	value	LL	UL	%	LL	UL	%	LL	UL	value	LL	UL
H5N1 HI Antibodies against A/Vietnam/1194/2004																
Half HA/Half AS03 - 3-5 years (Phase A)																
PRE	50	0.0	0.0	7.1	5.0	5.0	5.0	0.0	0.0	7.1						
PII(M6)	50	64.0	49.2	77.1	29.3	19.2	44.6	56.0	41.3	70.0	56.0	41.3	70.0	5.9	3.8	8.9
Half HA/Half AS03 - 6-9 years (Phase A)																
PRE	42	0.0	0.0	8.4	5.0	5.0	5.0	0.0	0.0	8.4						
PII(M6)	44	65.9	50.1	79.5	33.4	21.2	52.7	63.6	47.8	77.6	61.0	44.5	75.8	6.1	3.8	9.7
Full HA/Half AS03 - 3-5 years (Phase B)																
PRE	47	2.1	0.1	11.3	5.1	4.9	5.3	0.0	0.0	7.5						
PII(M6)	47	72.3	57.4	84.4	46.3	29.8	72.0	70.2	55.1	82.7	68.1	52.9	80.9	9.1	5.8	14.1
Full HA/Half AS03 - 6-9 years (Phase B)																
PRE	47	0.0	0.0	7.5	5.0	5.0	5.0	0.0	0.0	7.5						
PII(M6)	45	73.3	58.1	85.4	43.2	27.9	66.8	68.9	53.4	81.8	68.9	53.4	81.8	8.6	5.6	13.4
Full HA/Full AS03 - 3-5 years (Phase C)																
PRE	32	0.0	0.0	10.9	5.0	5.0	5.0	0.0	0.0	10.9						
PII(M6)	29	82.8	64.2	94.2	80.0	47.0	136.4	82.8	64.2	94.2	82.8	64.2	94.2	16.0	9.4	27.3
Full HA/Full AS03 - 6-9 years (Phase C)																
PRE	43	0.0	0.0	8.2	5.0	5.0	5.0	0.0	0.0	8.2						
PII(M6)	41	78.0	62.4	89.4	61.5	38.9	97.3	78.0	62.4	89.4	78.0	62.4	89.4	12.3	7.8	19.5

SPR = percentage with antibody titre ≥ 40 1/DIL; SCR = percentage with antibody titre ≥ 40 1/DIL after vaccination for initially seronegative subjects, or ≥ 4-fold the pre-vaccination antibody titre for initially seropositive subjects; SCF = fold increase in GMTs post-vaccination compared with pre-vaccination; PRE = pre-vaccination; PII(M6) = post-vaccination at Month 6

In the Fluarix groups in each Part of the study there was no difference between the D0 and the Month 6 HI seropositivity rates and GMTs against either A/Vietnam or A/Indonesia in 3-5 year-olds or 6-9 year-olds. Therefore there was no evidence of any augmentation of the HI immune response as a result of intervening natural exposure to cross-reacting antigens between D42 and Month 6.

Against the heterologous A/Indonesia strain 69% of children aged 3 to 5 years who had received the adult dose were seroprotected at Month 6 compared to 6.0% from Part A and 48.9% from Part B of the study. Corresponding rates in children aged 6 to 9 years were 61% versus 4.5% and 26.7%.

Vaccine strain heterologous (against H5N1 A/Indonesia) immune response persistence in terms of HI antibodies at month 6

		≥ 10 1/DIL			GMT			SPR			SCR			SCF		
			95% CI			95% CI			95% CI			95% CI			95% CI	
Timing	N	%	LL	UL	value	LL	UL	%	LL	UL	%	LL	UL	value	LL	UL
H5N1 HI Antibodies against A/Indonesia/05/2005																
Half HA/Half AS03 - 3-5 years (Phase A)																

		≥ 10 1/DIL			GMT			SPR			SCR			SCF		
			95% CI			95% CI			95% CI			95% CI			95% CI	
Timing	N	%	LL	UL	value	LL	UL	%	LL	UL	%	LL	UL	value	LL	UL
H5N1 HI Antibodies against A/Indonesia/05/2005																
PRE	50	0.0	0.0	7.1	5.0	5.0	5.0	0.0	0.0	7.1						
PII(M6)	50	20.0	10.0	33.7	6.9	5.6	8.4	6.0	1.3	16.5	6.0	1.3	16.5	1.4	1.1	1.7
Half HA/Half AS03 - 6-9 years (Phase A)																
PRE	42	0.0	0.0	8.4	5.0	5.0	5.0	0.0	0.0	8.4						
PII(M6)	44	18.2	8.2	32.7	6.6	5.2	8.4	4.5	0.6	15.5	2.4	0.1	12.9	1.2	1.0	1.5
Full HA/Half AS03 - 3-5 years (Phase B)																
PRE	47	0.0	0.0	7.5	5.0	5.0	5.0	0.0	0.0	7.5						
PII(M6)	47	55.3	40.1	69.8	21.7	14.3	33.0	48.9	34.1	63.9	48.9	34.1	63.9	4.3	2.9	6.6
Full HA/Half AS03 - 6-9 years (Phase B)																
PRE	47	0.0	0.0	7.5	5.0	5.0	5.0	0.0	0.0	7.5						
PII(M6)	45	40.0	25.7	55.7	11.9	8.4	16.9	26.7	14.6	41.9	26.7	14.6	41.9	2.4	1.7	3.4
Full HA/Full AS03 - 3-5 years (Phase C)																
PRE	32	0.0	0.0	10.9	5.0	5.0	5.0	0.0	0.0	10.9						
PII(M6)	29	69.0	49.2	84.7	42.5	23.7	76.3	69.0	49.2	84.7	69.0	49.2	84.7	8.5	4.7	15.3
Full HA/Full AS03 - 6-9 years (Phase C)																
PRE	43	0.0	0.0	8.2	5.0	5.0	5.0	0.0	0.0	8.2						
PII(M6)	41	65.9	49.4	79.9	36.8	22.3	60.6	61.0	44.5	75.8	61.0	44.5	75.8	7.4	4.5	12.1

SPR = percentage with antibody titre ≥ 40 1/DIL; SCR = percentage with antibody titre ≥ 40 1/DIL after vaccination for initially seronegative subjects, or ≥ 4-fold the pre-vaccination antibody titre for initially seropositive subjects; SCF = fold increase in GMTs post-vaccination compared with pre-vaccination; PRE = pre-vaccination; PII(M6) = post-vaccination at Month 6

NA against A/Vietnam at Month 6 was reported from Part A of the study (i.e. half adult dose versus Fluarix) and showed that in the AS03 vaccine group the GMTs had dropped to a similar degree in both age strata. As at D42 (GMTs 1026 and 1111) the actual GMTs at D180 were comparable for children aged 3-5 years and 6-9 years (776 and 759). At Month 6 all children who had received the AS03 vaccine had NA titres of at least 1:80.

However, in the Fluarix group the GMTs increased between D42 and D180. In the younger age group (3-5 years) the increment was small (from 166 to 200) but is none the less remarkable since a drop in GMT would usually have been expected. In the older age group (6-9 years) the increase was by 6-fold (from 75 at D42 to 482 at D180). These results suggest that natural exposure to cross-reacting antigens had occurred in the interim period.
As a result the seroconversion rates in the 6-9 year-olds at Month 6 were 95% for the AS03 group and 93% for the Fluarix group. Also, all children aged 6-9 years who received Fluarix had NA titres of at least 1:80 at Month 6, while the corresponding rate in the 3-5 year-olds was 80%.

Percentage with NA titres 1:40 and 1:80 against A/Vietnam/1194/2004 on Day 180 (ATP)

					≥1:40 1/DIL				≥1:80 1/DIL			
							95% CI				95% CI	
Antibodies against	Group	Sub-group	Timing	N	n	%	LL	UL	n	%	LL	UL
A/Vietnam	H5N1/2+AS03/2	3-5y	PRE	48	16	33.3	20.4	48.4	13	27.1	15.3	41.8
			PI(D21)	49	43	87.8	75.2	95.4	37	75.5	61.1	86.7
			PII(D42)	48	48	100	92.6	100	48	100	92.6	100
			PII(M6)	50	50	100	92.9	100	50	100	92.9	100
		6-9y	PRE	43	17	39.5	25.0	55.6	11	25.6	13.5	41.2
			PI(D21)	42	38	90.5	77.4	97.3	33	78.6	63.2	89.7
			PII(D42)	41	41	100	91.4	100	41	100	91.4	100
			PII(M6)	42	42	100	91.6	100	42	100	91.6	100
	Fluarix™	3-5y	PRE	14	2	14.3	1.8	42.8	2	14.3	1.8	42.8
			PI(D21)	15	12	80.0	51.9	95.7	10	66.7	38.4	88.2
			PII(D42)	15	12	80.0	51.9	95.7	12	80.0	51.9	95.7
			PII(M6)	15	12	80.0	51.9	95.7	12	80.0	51.9	95.7

					≥1:40 1/DIL				≥1:80 1/DIL			
							95% CI				95% CI	
Antibodies against	Group	Sub-group	Timing	N	n	%	LL	UL	n	%	LL	UL
		6-9y	PRE	14	2	14.3	1.8	42.8	1	7.1	0.2	33.9
			PI(D21)	13	9	69.2	38.6	90.9	8	61.5	31.6	86.1
			PII(D42)	14	9	64.3	35.1	87.2	8	57.1	28.9	82.3
			PII(M6)	14	14	100	76.8	100	14	100	76.8	100

NA was measured against A/Indonesia/05/2005 at Day 42, Month 6 and Month 12 in children who received half dose HA + half ASO3 (Phase A subjects) or Fluarix (control group). The Day 0 samples were erroneously not tested. These samples will be tested and the results will be submitted as they become available.

There was a significant heterologous immune response at each time point and the comparison with the control group indicates that D-Pan H5N1 elicited cross-reactive immunity. There were decreases in GMTs from D42 to Month 6 and Month 12 but the proportions with NA titres of at least 1/80 remained high in both age groups (89.6% at Month 6 and 87.2% at Month 12 in the 3-6 years group and 90.2% at Month 6 and 82.9% at Month 12 in the 6-9 years group. There was a stark contrast between NA titres in the D-Pan H5N1 group and the Fluarix control group.

Discussion on D-Pan H5N1-009

The CHMP considered that the D42 HI data did not fully discriminate between dose groups, but the Month 6 data indicated a strong advantage for using the full adult dose especially in terms of antibody against the drifted strain. Nevertheless all children in the half adult dose group were later shown to have NA titres of at least 1:80 against A/Vietnam.

The NA titres at Month 6 and Month 12 against A/Indonesia were reported later and gave a markedly different picture to that provided by the HI data against this strain up to Month 6. The CHMP further highlighted that there are no data on the use of Q-Pan H5N1 in children and agreed that data from H5N1-009 and -023 should be included in the SmPC to support the use in children as reflected in sections 4.2 and 5.1.

Supportive studies

Q-Pan H5N1-009

This was an open-label, randomised study in Canadian adults aged 18-64 years in which Q-Pan A/Indonesia/5/2005 plus AS03 adjuvant (single lots) was administered to equal groups as follows:

Group A: One 3.8 µg dose A/Indonesia/5/2005/AS03 on Day 0 and Day 21
Group B: One 3.8 µg dose A/Indonesia/5/2005/AS03 on Day 0 and Day 14
Group C: One 3.8 µg A/Indonesia/5/2005/AS03 on Day 0 and Day 7
Group D: Two 3.8 µg doses A/Indonesia/5/2005/AS03 on Day 0 (one in each arm).

The primary objective was to demonstrate that HI responses to H5N1/AS03 at Day 14 after the second dose (after D0 in Group D) met the CBER criteria for SCR and elicited seroprotective titres in at least 50%. The study was stratified by age 18-40 years and 41-64 years.

The post-dose 2 **SCRs** at D14 were lower when the interval between doses was < 14 days. There was no appreciable difference in SCRs between Groups A and B or between Groups C and D and the lower bound of the 98.75% CI for all treatment groups exceeded the CBER target. SCRs were slightly higher for the 18-40 years age stratum within Groups A and B and for the 41-64 age stratum within Groups C and D. SCRs for Groups C and D were lower than those for Groups A and B. At D21 post-dose 2 the SCRs for Groups A, B, C, and D were 95.2%, 92.8%, 80.6% and 74.3%, respectively, and the lower bound of the 95% CI for all treatment groups exceeded CBER guidance targets.

Table 25 SCR for A/Indonesia/5/2005 measured by HI 14 days after the second dose in subjects 18-64 years of age (ATP cohort for immunogenicity)

Group	Pre-vaccination status	N	Seroconversion rate			
			n	%	98.75% CI	
					LL	UL
Q-Pan A	S-	63	61	96.8	86.5	99.8
	S+	2	2	100	7.9	100
	Total	65	63	96.9	86.9	99.8
Q-Pan B	S-	67	62	92.5	80.7	98.3
	S+	2	2	100	7.9	100
	Total	69	64	92.8	81.2	98.3
Q-Pan C	S-	71	52	73.2	58.2	85.2
	S+	3	1	33.3	0.2	95.4
	Total	74	53	71.6	56.8	83.7
Q-Pan D	S-	70	51	72.9	57.7	85.0
	S+	5	3	60.0	9.0	97.4
	Total	75	54	72.0	57.3	83.9

At 14 days post dose 2 for all treatment groups the **SPR** rates were 96.9%, 92.8%, 74.3% and 74.7% in respective groups. For subjects aged 18-40 years in Groups C and D the CBER criterion was not met (98.75% CI lower limits were 47.4% and 49.1%) but was met for the older cohort. At 21 days post dose 2 the corresponding SPR values were 95.2%, 92.8%, 81.9% and 77.0% and the CBER criterion was met in all treatment groups. SPR values 21 days after the second dose did not differ greatly by age group.

At D14 post-dose 2 the **GMTs** were highest in Group A. GMT values 21 days after the second dose differed by age group, especially in Groups A and B. Overall, GMTs for Groups C and D were lower than those for Groups A and B, regardless of age.

Immune responses to drift-variant virus were lower than for vaccine-homologous virus. Immune responses against A/turkey/Turkey/1/2005 were generally higher than against A/Vietnam/1194/2004. For the A/turkey/Turkey/1/2005 strain at Day 14 and Day 21 after the second dose, Groups A and B reached the CBER target for SCR, the CHMP target for SCR, the 50% target for SPR and the CHMP target for SPR while all treatment groups at these time points reached the CHMP target for GMFR. The trend across treatment groups that was seen for the vaccine-homologous virus was also seen for each drift-variant strain so that immune responses were lower for the groups with short intervals between doses.

There was an increase in antibody titres for all virus strains between the first and second vaccinations. The data suggested that a longer interval between vaccinations is associated with a larger increase in HI antibodies, with the largest increases seen for Group A. This indicates that the HI antibody response continues to increase at least 21 days after a single vaccination.

Table 35 Antibody titers at baseline and before dose 2 in subjects 18-64 years of age (ATP cohort for immunogenicity)

Day/Group	A/INDONESIA/5/2005	A/VIETNAM/1194/2004	A/TURKEY/TURKEY/1/2005
Day 0 Pre-dose All Groups Mean antibody titer (SD)	7.739 (21.027)	6.601 (10.614)	12.468 (60.627)
Day 7 Q-Pan C Mean antibody titer (SD)	16.257 (40.394)	8.432 (9.278)	15.859 (36.405)
Day 14 Q-Pan B Mean antibody titer (SD)	79.353 (178.276)	18.382 (34.533)	20.554 (41.536)
Day 21 Q-Pan A Mean antibody titer (SD)	116.531 (321.600)	36.797 (160.581)	51.029 (100.786)

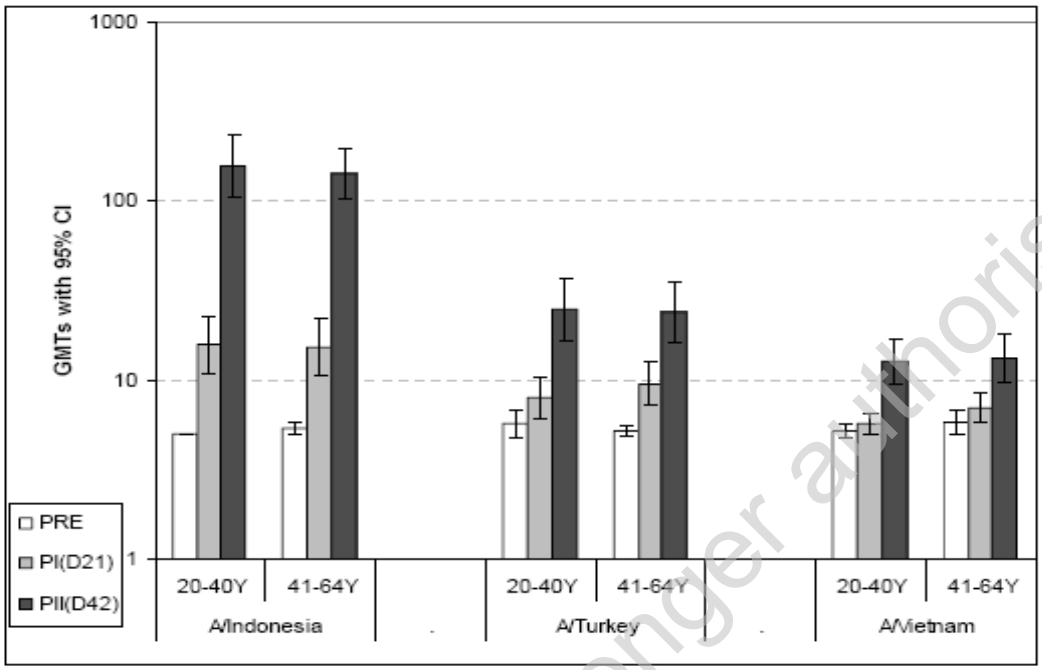
Discussion on Q-Pan-H5N1-009

The data demonstrated that a minimum dose interval of at least 14 days should be applied and that there are likely some advantages for 21 days between doses.

Q-Pan H5N1-011

This was an open-label non-comparative study conducted in Japan with stratification by age (20-40 and 41-64 years; N=50 per age stratum planned and enrolled). HI responses were determined against the vaccine strain (A/Indonesia/5/2005 Clade 2.1) and two drifted strains (A/turkey/Turkey/1/2005 Clade 2.2 and A/Vietnam/1194/2004 Clade 1). NA responses were measured against A/Indonesia/5/2005 and A/Vietnam/1194/2004. Prior to vaccination, 5/100 subjects were seropositive for HI against A/Indonesia/5/2005, 4 for A/turkey/Turkey/1/2005 and 6 for A/Vietnam/1194/2004. Pre-vaccination GMTs were similar between age strata and were < 6.

Figure 1 **GMTs of H5N1 HI antibody titers against A/Indonesia/5/2005, A/turkey/Turkey/1/2005 and A/Vietnam/1194/2004 strains with 95% confidence interval at Days 0, 21 and 42 by age strata 20-40 years and 41-64 years (ATP cohort for immunogenicity)**



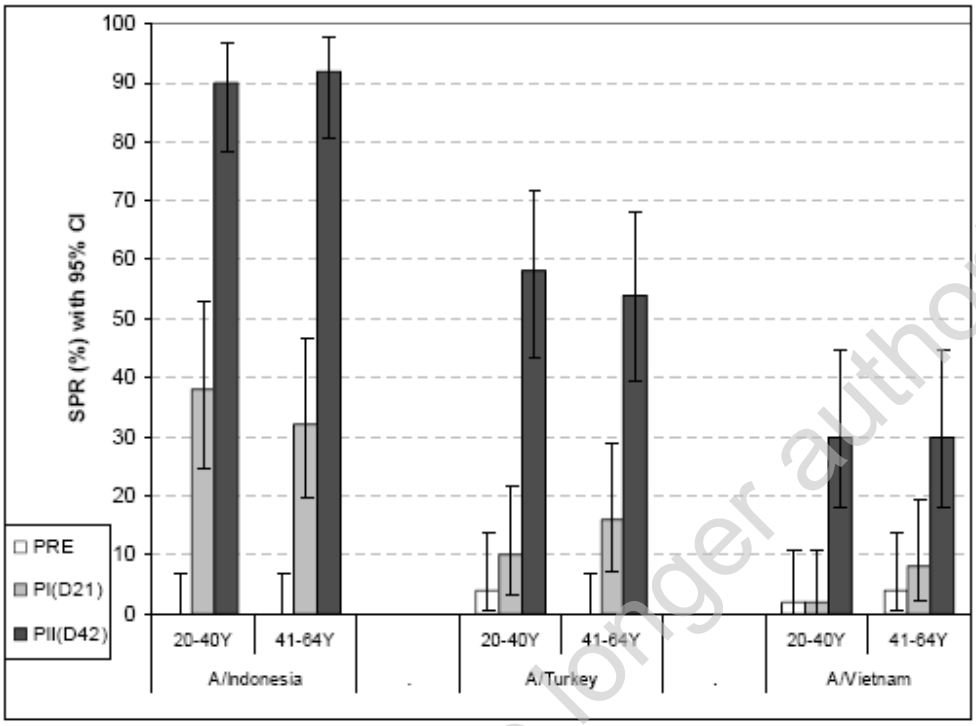
Seropositivity rates were highest (with highest GMTs) for A/Indonesia and lowest for A/Vietnam at D42. On Day 182, the GMTs against all three strains were lower versus Day 42 but higher than values observed at Day 21. In both age strata the GMTs against A/Indonesia/05/2005 were comparable to those against A/turkey/Turkey/01/2005 and higher than observed for A/Vietnam/1194/2004. The seropositivity rates and GMTs against vaccine-homologous and drifted strains tended to be higher in the older age cohort.

The > 40% SCR threshold was exceeded at Day 42 in both age strata for HI antibodies against A/Indonesia/5/2005 and A/turkey/Turkey/1/2005 but not for A/ Vietnam/1194/2004. The SCRs were similar between age strata for all three strains tested. The > 40% SCR threshold was still exceeded on Day 182 for HI antibodies against A/Indonesia/05/2005 and A/turkey/Turkey/01/2005. The threshold was met in both age strata against A/Indonesia/05/2005 but was met only in the older age stratum for A/turkey/Turkey/01/2005.

The ≥ 40% threshold for the lower bound of the 95% CI for seroconversion required by CBER was also still met for HI antibodies against A/Indonesia/05/2005 in both age strata but again only the older age group still met the criterion against A/turkey/Turkey/01/2005. The SCRs for H5N1 HI antibodies against A/Vietnam/1194/2004 were 4.1% in the younger cohort and 20.0% in the older cohort.

The >70% SPR required by the CHMP for adults aged 18-60 years and the CBER criterion were exceeded for HI antibodies against A/Indonesia/5/2005 strain at D42 in both age cohorts but these thresholds were not met for the other two strains in either age stratum.

Figure 4 **Seroprotection rates (SPR) for H5N1 HI antibody titers against A/Indonesia/5/2005, A/turkey/Turkey/1/2005 and A/Vietnam/1194/2004 strains with 95% confidence interval at Days 0, 21 and 42 by age strata 20-40 years and 41-64 years (ATP cohort for immunogenicity)**



At D182 the > 70% SPR threshold was not met against any strain but rates still followed the same pattern by strain as observed at D42. However, the > 70% SPR threshold was still met against A/Indonesia/05/2005 strain in the 41 to 64 years stratum (76.0%) while the CBER criterion was not met. The Day 182 SPRs were still considerably higher compared with those seen D21 but lower than observed at D42. The SPR values against A/Vietnam/1194/2004 were low on Day 182 in both age strata (6.1% for 20-40 years; 24.0% for 41-64 years).

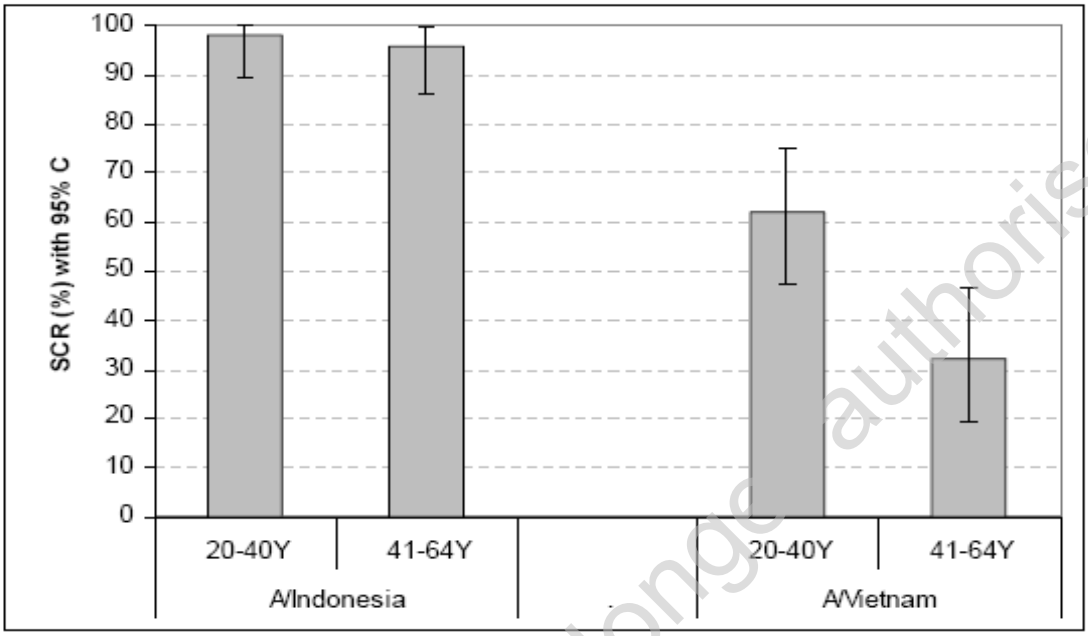
The >2.5 SCF threshold against the A/Indonesia/5/2005 strain was exceeded at D21 (3.0) and reached 28.6 by D42. At D42 the threshold was also reached for A/turkey/Turkey/1/2005 but not for A/Vietnam/1194/2004 in either age stratum. At D182 the > 2.5 SCF threshold was still exceeded in both age strata for A/Indonesia/05/2005 and A/turkey/Turkey/01/2005.

Neutralising antibody (NA) seropositivity rates against A/Indonesia/5/2005 at baseline were low (11/100). By D42 all subjects were seropositive in both age strata. Similarly, baseline GMTs were 14.4 in the 20-40 years and 18.3 in the 41-64 years groups but reached 579.6 and 473.8 by D42. A higher proportion of subjects were already seropositive before vaccination against A/Vietnam/1194/2004 with 70% in the older and 30% in the younger age stratum and GMTs of 61.9 and 24.8, respectively. At D42 the seropositivity rates were 92.0% and 98.0% in respective age groups with GMTs of 106.5 and 154.7.

The D182 GMTs and seropositivity rates for both strains were still high. The GMTs in each age stratum were higher for vaccine-homologous virus. GMTs against A/Indonesia/05/2005 had decreased compared to Day 42 in each age stratum (from 579.6 to 240.5 and from 473.8 to 240.1) but GMTs against A/Vietnam/1194/2004 tended to increase in both age strata (from 106.5 to 173.5 and from 154.7 to 208.0) suggesting some natural boosting effect during the 5 months between samplings.

The NA SCR against both strains increased after the second vaccination and reached 97.0% and 47.0% at Day 42, respectively. The D42 SCRs against A/Indonesia/5/2005 were comparable between age strata. The SCR against A/Vietnam/1194/2004 was higher in the younger group but this reflects the baseline differences in NA titres. On Day 182, the SCRs were 93.9% against A/Indonesia/05/2005 and 58.6% against A/Vietnam/1194/2004 and again showed an age difference.

Figure 6 **Seroconversion rates (SCR) for neutralizing antibody titer against A/Indonesia/5/2005 and A/Vietnam/1194/2004 with 95% confidence interval at Day 42 by age strata 20-40years and 41-64years (ATP cohort for immunogenicity)**



The proportions with NA titres of at least 1:40 and 1:80 did not change substantially between D42 and D182 against A/Indonesia/05/2005 and were comparable between age groups. However, the corresponding percentages for NA against A/Vietnam/1194/2004 showed increments at the 1:80 level between D42 and D182, reflecting the observed increases in GMTs. This phenomenon applied in both age strata and the final rates were comparable between age groups.

Discussion on Q-Pan H5N1-011

In these 100 Japanese subjects immune responses to Q-Pan/AS03 vaccine containing A/Indonesia/5/2005 at D42 fulfilled all CHMP and CBER criteria with no differences between the predefined age strata. The vaccine elicited a high cross-reactive immune response against Clade 2.2 (A/turkey/Turkey/1/2005) and a lesser response to a Clade 1 strain (A/Vietnam/1194/2004) at D42 with no appreciable differences between age strata. In both age strata the CHMP criteria were still fulfilled against A/Indonesia/05/2005 on Day 182 except for SPR in those aged 20 to 40 years. Also, the SCR and SCF criteria were met against A/turkey/Turkey/01/2005 in the older age stratum.

The vaccine elicited NA against Clade 1 and Clade 2 viruses at D42 following the same pattern of relative magnitude as for HI data. The NA SCRs against the A/Vietnam/1194/2004 were lower, particularly in the 41-64 years cohort, reflecting the higher baseline seropositivity rate in this age stratum. Between D42 and D182 there were increases in NA GMTs against A/Vietnam, suggesting that some natural exposure was occurring during the period of the study.

D-Pan H5N1-015

This was a phase II, open, non-randomised study designed to evaluate the reactogenicity and immunogenicity of one or two booster administrations of Q-Pan H5N1 vaccine in adults aged between 19 and 61 years, previously vaccinated with 2 doses of a pandemic candidate vaccine H5N1 A/Vietnam/1194/2004 containing 3.8, 7.5, 15 or 30 µg HA, adjuvanted or not with AS03.

Study 015 was a continuation (boosting phase) of the previously reported dose-finding study 007, assessed in the initial MA application for Pandemrix. One of the eight dose/adjuvant groups in study 007 had been primed at D0 and D21 with 3.8 µg HA (derived from A/Vietnam) + AS03 as in the currently approved version of the prepandemic vaccines. Boosting in study 015 occurred at approximately 14 months after the two priming doses that had been administered in study 007.

In study 015, the four dose groups that had been primed with adjuvanted vaccine in 007 received a single dose of adjuvanted vaccine containing A/Indonesia/05/2005 at Day 0 of study 015. The booster dose consisted of 3.8 µg HA regardless of the dose received for priming (i.e. 3.8, 7.5, 15 or 30 µg HA + AS03 adjuvant). Blood samples were obtained at Days 0, 7, 14 and 21 after the booster.

The four groups that had been primed with non-adjuvanted vaccine in 007 received two doses of adjuvanted vaccine containing A/Indonesia/05/2005 at D0 and D21 of study 015. The booster dose consisted of 3.8 µg HA regardless of the dose received for priming (i.e. 3.8, 7.5, 15 or 30 µg HA without adjuvant). Blood samples were obtained at Days 0, 7, 14, 21, 35 and 42.

An additional control group (no previous doses of H5N1 vaccine) was enrolled into 015. This group received two doses of the same adjuvanted vaccine containing A/Indonesia/05/2005 as used for boosting the eight groups derived from study 007. Doses were given at D0 and D21 and blood samples were obtained at D42.

The pre- and post-boost data in the group that had been primed with two doses of the currently approved version of prepandemic vaccine and then received a single dose of A/Indonesia vaccine (i.e. each dose containing 3.8 µg HA + AS03) are of primary importance.

It was planned that subjects will be followed for up to 24 months with blood samples obtained at 6, 12, 18 and 24 months post-boost. The vaccine administered in study 015 consisted of a single lot of HA and single lot of AS03.

The Primary objectives of study 015 were:

- To assess if the humoral immune response induced 21 days after one booster administration of the pandemic influenza vaccine fulfils the CHMP criteria in subjects primed approximately 14 months earlier with two administrations (21 days apart) of the candidate vaccine formulated from a heterologous strain and adjuvanted with AS03.

- To evaluate the safety/reactogenicity of the candidate vaccine in terms of solicited local and general symptoms, unsolicited symptoms and serious adverse events.

Results

The actual number enrolled was 350 (35 to 50 subjects per group). All were vaccinated while 347 completed the study to Day 51. There were 25 subjects eliminated from the ATP immunogenicity cohort (92.9 %), of which 16 were eliminated because of failure to comply with the blood sampling schedule. The number of subjects per group in the ATP immunogenicity cohort was between 28 and 49. There were 40 subjects enrolled from the initial 3.8 µg HA/AS03 priming dose group and 39/40 were included in the ATP immunogenicity cohort.

The overall mean age of the 350 enrolled into 015 at the time of the first vaccination was 36.3 years (range 19 - 61 years). The male-female ratio was 0.8 and 98.6% were of Caucasian/European origin. There were no major differences between the vaccine groups for any of the demographic parameters.

<u>HI responses</u>

For the main analysis concerning the group primed with 3.8 µg HA + AS03 the CHMP criteria were each exceeded at 21 days following the booster for HI responses to the booster homologous strain i.e. A/Indonesia. The HI responses to a single dose of A/Indonesia vaccine strongly suggested that these subjects had been primed for A/Indonesia by vaccination 14 months earlier with two doses of VT vaccine.

Responses to the booster homologous (**A/Indonesia**) and booster heterologous (**A/Vietnam**) strains only for the groups primed with 3.8 µg HA with or without adjuvant and the control group are presented below.

At D0 in study 015 **GMTs** against A/Indonesia and against A/Vietnam were all low (<10).

- At Day 7 there were significant increases in GMTs against **A/Indonesia** in all except the control group with actual GMTs that were highest in the groups that had been primed with adjuvanted vaccine (118.5 to 193.3).
- At Day 21 the GMTs against A/Indonesia were significantly higher in the four groups primed with adjuvanted vaccine (208.4 - 429.5) compared to those primed with non-adjuvanted vaccine and controls (31 - 77). There were no significant differences between the four dose groups primed with adjuvanted vaccine and no significant differences between the four dose groups primed with non-adjuvanted vaccine. Therefore, priming with adjuvanted vaccine was the important factor and not the HA dose administered.
- The GMT against A/Indonesia in the Control group at Day 42 (443) was significantly higher than the values observed at Day 42 for the four groups that had been primed with non-adjuvanted vaccine (54.3 - 141.4). This unexpected finding was not considered relevant for the current application, which concerns only adjuvanted vaccine, but it is nevertheless of immunological interest and is discussed again below.

- At Day 7, the increases in GMTs against **A/Vietnam** after a single dose of adjuvanted vaccine containing A/Indonesia were significant for the groups primed with A/Vietnam 3.8 µg HA with or without AS03. The actual GMT at day 7 was much higher in the former group (176.8 versus 20.8, respectively) and GMTs in this group remained higher at Day 14 (343.7) and Day 21 (352.8).
- The GMT reached 58.3 at Day 42 (i.e. 21 days after the second dose) in the group primed with non-adjuvanted vaccine.
- The GMT for the control group began to rise after the second dose to reach 27.1 to 31.7 from Day 28 up to Day 42.

In line with the low GMTs at D0 of study 015 the **seropositivity rates** for **A/Indonesia** were low (< 10%) before the booster dose.

- The seropositivity rate with respect to A/Indonesia increased significantly by Day 7 in the four groups primed with adjuvanted vaccine (84.2 % - 93.9 %) and the groups primed with non-adjuvanted vaccine (50.0 % - 64.3 %).
- In the Control group, the seropositivity rates rose significantly from two weeks following the administration of the first dose (from 0.0 % to 46.9 %). At Day 28 (7 days after the second dose) the seropositivity rate was 100%.
- At Day 42 the seropositivity rate in the group primed with 3.8 µg non-adjuvanted vaccine was 67.6% while the rate in the control group was 100 %.

- At D0 the seropositivity rate against **A/Vietnam** was higher in the group primed with A/Vietnam 3.8 µg HA + AS03 (35.9 %) compared with the corresponding non-adjuvanted dose group (5.9%) and Control group (0.0 %).
- The rates increased significantly after the first booster dose so that by Day 21 the rates in respective groups were 94.9 %, 61.8 % and 30.6 %.
- There was no significant increase after the second dose in subjects primed with non-adjuvanted vaccine but the rate in the control group rose significantly (to 73.5 % at Day 28).

The **seroconversion rates** (SCR) against **A/Indonesia** exceeded 40% at Day 7 for the four groups that had been primed with adjuvanted vaccine (84.2 - 93.9 %) and in three groups primed with non-adjuvanted vaccine (44.7 % - 60.7 %). The SCR at Day 7 was 35.3% in the group that had been primed with 3.8 µg HA without AS03 but reached 64.7% by day 14. The Control group achieved a SCR > 40% at Day 21 (59.2 %) and by Day 42 the rate had reached 98.0 %. This compares with a SCR at D42 of about 64% in the group primed with 3.8 µg HA without AS03.

Against **A/Vietnam** the SCR was 81.6% at Day 7 for the group primed with 3.8 µg + AS03 while the corresponding dose group primed with non-adjuvanted vaccine exceeded 40% at Day 14 (61.8 %). The threshold was only reached by the Control group after the second dose (Day 28 rate = 59.2 %).

The **seroconversion factor against A/Indonesia** was > 2.5 in all previously vaccinated groups by day 7. The criterion was also exceeded at Day 14 in the Control group (2.7) and then increased greatly

to 88.6 at Day 42. In contrast the D42 SCF in the group primed with 3.8 µg HA without AS03 was about 10.

Against **A/Vietnam** the > 2.5 criterion was met in all primed groups at day 7 and at Day 28 in the Control group (6.3). SCFs for the group primed with 3.8 µg + AS03 (21.0 - 42.5) were significantly higher than for the corresponding non-adjuvanted dose group (3.7 - 10.3) and the Control group (1.1 - 6.5) at all time points.

The seroprotection rates (SPR) against **A/Indonesia** were all 0-3% at D0. The rate exceeded 70% in the four groups primed with adjuvanted vaccine by Day 7 (84.2 % - 93.9 %). The threshold was reached at Day 14 for groups primed with 7.5 µg HA alone (73.7 %) and 30 µg HA alone (75.0 %), at Day 35 for the group primed with 15 µg alone (71.9 %) and at Day 28 for the Control group (100%). The threshold was not reached for the group primed with 3.8 µg alone at any time-point.

SPRs against **A/Vietnam** were low at D0 (0 % - 10.3 %) but had increased significantly by Day 7 in the groups primed with 3.8 µg HA with or without AS03. A significant increase in SPR was observed on Day 28 in the Control group. However, a SPR > 70% was reached only in the group primed with 3.8 µg HA + AS03 (84.2 % at Day 7 and 89.7 % at Day 21).

Neutralising antibody responses

NA titres were measured against **A/Indonesia/05/2005** in the groups primed with 3.8 µg HA with or without AS03 and in the Control group.

- The D0 GMT was significantly higher in the group primed with 3.8 µg HA + AS03 (157.8) than in the group primed with 3.8 µg HA alone (47.0) and the Control group (19.9).
- At D21 significant increases in GMTs occurred in all groups to reach 3708.9, 692.4 and 307.3 in respective groups.
- At D42 there was a significant increase in GMT in the Control group only (to 1606.4). The actual GMT was higher than that (933.1) in the group primed with 3.8 µg HA alone and the 95% CI only just overlapped. This finding mirrors the unexpected HI findings noted above.

All subjects in the group primed with 3.8 µg HA + AS03 were seropositive at Day 0 and 92% had a titre ≥1:80 compared to 35% in the group primed with non-adjuvanted HA and 6.1% of controls. All subjects in the three groups were seropositive by Day 21, at which time all had titres ≥1:80.

Table 26 Geometric mean titres and seropositivity rates of H5N1 neutralizing antibodies against the A/Indonesia/05/2005 strain at Days 0, 21 and 42 in the H5N1 3.8, H5N1 3.8AD and Control groups (ATP cohort for immunogenicity)

				>= 28 1/DIL				GMT				
						95% CI			95% CI			
Antibodies against	Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
A/Indonesia	H5N1 3.8	PRE	34	25	73.5	55.6	87.1	47.0	34.2	64.6	<28.0	226.0
		PI(D21)	34	34	100	89.7	100	692.4	476.7	1005.7	90.0	11380.0
		PII(D42)	34	34	100	89.7	100	933.1	653.7	1331.9	180.0	9050.0
	H5N1 3.8AD	PRE	38	38	100	90.7	100	157.8	130.3	191.2	28.0	453.0
		PI(D21)	38	38	100	90.7	100	3708.9	2458.6	5594.9	226.0	18100.0
	Control	PRE	49	10	20.4	10.2	34.3	19.9	15.7	25.2	<28.0	453.0
		PI(D21)	49	49	100	92.7	100	307.3	262.5	359.8	113.0	905.0
		PII(D42)	49	49	100	92.7	100	1606.4	1282.7	2011.8	453.0	18100.0

H5N1 3.8 = H5N1 3.8 µg; H5N1 3.8AD = H5N1 3.8 µg + AS03; Control = Control; GMT = Geometric Mean antibody Titre; N = Number of subjects with available results; n/% = number/percentage of seropositive subjects (HI titre >= 1:28); 95% CI = 95% confidence interval, LL = Lower Limit, UL = Upper Limit; MIN/MAX = Minimum/Maximum; PRE = Pre-vaccination at Day 0; PI(D21) = Post-vaccination one at Day 21; PII(D42) = Post-vaccination two at Day 42

Supplement 26 Percentage of subjects with neutralizing antibody titres greater than or equal to 1:40 and greater than or equal to 1:80 against A/Indonesia/05/2005 at Day 0, Day 21 and Day 42 (ATP cohort for immunogenicity)

				>=40 1/DIL				>=80 1/DIL			
Antibodies against	Group	Timing	N	n	%	LL	UL	n	%	LL	UL
A/Indonesia	H5N1 3.8	PRE	34	19	55.9	40.5	70.5	12	35.3	21.8	50.8
		PI(D21)	34	34	100	91.6	100	34	100	91.6	100
		PII(D42)	34	34	100	91.6	100	34	100	91.6	100
	H5N1 3.8AD	PRE	38	37	97.4	88.1	99.9	35	92.1	80.8	97.8
		PI(D21)	38	38	100	92.4	100	38	100	92.4	100
	Control	PRE	49	7	14.3	6.9	25.2	3	6.1	1.7	15.1
		PI(D21)	49	49	100	94.1	100	49	100	94.1	100
		PII(D42)	49	49	100	94.1	100	49	100	94.1	100

Seroconversion rates for NA were all above 85.0 % and did not differ significantly between groups after one vaccination dose.

Seroconversion rate (SCR) for H5N1 neutralizing antibodies against the A/Indonesia/05/2005 strain at Day 21 and Day 42 in the H5N1 3.8, H5N1 3.8AD and Control groups (ATP cohort for immunogenicity)

				SCR			
						95% CI	
Antibodies against	Group	Timing	N	n	%	LL	UL
A/Indonesia	H5N1 3.8	PI(D21)	34	29	85.3	68.9	95.0
		PII(D42)	34	31	91.2	76.3	98.1
	H5N1 3.8AD	PI(D21)	38	35	92.1	78.6	98.3
	Control	PI(D21)	49	44	89.8	77.8	96.6
		PII(D42)	49	49	100	92.7	100

Cell-mediated immune response

- At Day 0, the frequencies of antigen-specific CD4 T-cells directed against A/Indonesia or against A/Vietnam were higher in the group primed with 3.8 µg HA + AS03 compared to the corresponding dose group without adjuvant and the Control group.
- At Day 21, all three groups had a higher frequency of antigen-specific CD4 T-cells compared to D0 (336 - 2721 versus 53 - 1370, respectively).
- Stimulation with the HA peptide pool (A/Indonesia/05/2005 and A/Vietnam/1194/2004) gave the same pattern but the frequencies were lower.
- Responses after stimulation with A/Indonesia/05/2005 split strain, A/Vietnam/1194/2004 split strain or HA peptide pools were similar between groups for each strain.

There was no observable increase in the frequency of antigen-specific CD8 T-cell responses following vaccination. No observable individual differences were detected in any of the groups whether stimulation was by A/Indonesia/05/2005 split strain, A/Vietnam/1194/2004 split strain or HA peptide pool.

- At Day 0, the frequency of memory B-cells directed against A/Indonesia/05/2005 or A/Vietnam/1194/2004 was higher in the group primed with 3.8 µg HA + AS03 compared to the corresponding dose group without adjuvant and the Control group.
- At Day 21, all groups had a higher frequency of memory B-cells compared to D0. The frequency of memory B-cells against both strains was higher in the group primed with 3.8 µg HA + AS03 compared to the corresponding dose group without adjuvant and the Control group.

Results to D180 post-boosting

Of the 350 subjects enrolled and vaccinated in the primary study phase 337 were evaluated at Day 180.

The Month 6 post-boost HI data against **A/Indonesia** showed:

- GMTs for the H5N1 AD groups (42.1 - 82.5) were higher than those observed for the H5N1 non-AD (17.6- 24.3) and Control groups (17.8). See Figure 1.
- SCRs for the H5N1 AD groups (50.0 % - 75.0%) were higher than those observed for the H5N1 non-AD (30.0 %- 41.7%) and Control groups (32.6%). The >40% SCR threshold was exceeded in all H5N1 AD groups and in the H5N1 15µg and H5N1 30 µg groups.
- SPRs for the H5N1 AD groups (50.0 % - 75.8%) were higher than those observed for the H5N1 non-AD (30.0%- 41.7%) and Control groups (32.6%). The >70% SPR threshold was only maintained in the H5N1 7.5AD and H5N1 30AD groups.
- SCFs >2.5 were maintained in all groups but were markedly higher in the H5N1 AD groups (7.9-15) compared to H5N1 non-AD (3.3-4.9) and Control (3.6) groups.

The HI antibody titres against **A/Vietnam/1194/2004** were measured at D180 only in the H5N1 3.8, H5N1 3.8AD and control groups.

- The GMT for group H5N1 3.8AD (192.8) was higher than those for the H5N1 3.8 (27.5) and Control groups (7.8). See Figure 1.
- The SCR for group H5N1 3.8AD (78.9%) was higher than those for the H5N1 3.8 (51.4%) and Control (6.5%) groups. Thus the >40% SCR threshold was exceeded in the H5N1 3.8AD and H5N1 3.8 groups.
- The SPR for group H5N1 3.8AD (84.6%) was higher than those for the H5N1 3.8 (51.4%) and Control (6.5%) groups. Thus the >70% SPR threshold was only exceeded in the H5N1 3.8AD group.
- The SCF threshold was exceeded in the H5N1 3.8 (4.9) and H5N1 3.8AD (21.6) groups but not in the Control group (1.5).

Neutralising antibody titres were measured at D180 against **A/Indonesia/05/2005** in the H5N1 3.8AD, H5N1 3.8 and Control groups.

- The GMT observed for the H5N1 3.8AD (1422.2) group was higher than that observed for the H5N1 3.8 (502.3) and Control (751.3) groups.

- The SCRs for groups H5N1 3.8 and H5N1 3.8AD were 78.4%-82.9% and were lower than the SCR observed in the Control group (95.7%).

Seropositivity rates and geometric means titres (GMTs) of neutralising (MN) antibody titres at Day 0 and Month 6 against the A/Indonesia/05/2005 vaccine strain (ATP cohort for persistence)

				≥ 28 1/DIL				GMT				
						95% CI			95% CI			
Antibodies against	Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
A/Indonesia	H5N1 3.8AD	PRE	37	37	100	90.5	100	155.4	127.9	188.7	28.0	453.0
		PI(M6)	37	37	100	90.5	100	1422.2	916.8	2206.2	71.0	9050.0
	H5N1 3.8	PRE	35	27	77.1	59.9	89.6	50.8	37.4	69.0	<28.0	226.0
		PII(M6)	35	35	100	90.0	100	502.3	349.2	722.5	90.0	3600.0
	Control	PRE	46	9	19.6	9.4	33.9	19.7	15.4	25.3	<28.0	453.0
		PII(M6)	46	46	100	92.3	100	751.3	611.4	923.3	142.0	7200.0

H5N1 3.8AD = H5N1 3.8 µg + AS03; H5N1 3.8 = H5N1 3.8 µg; GMT = Geometric Mean antibody Titre; N = Number of subjects with pre- and post-vaccination results available; n/% = Number/percentage of seroconverted subjects; 95% CI = 95% confidence interval, LL = Lower Limit, UL = Upper Limit; PRE = Pre-vaccination at Day 0; PI(M6) = Post-vaccination one at month 6; PII(M6) = Post-vaccination two at month 6

Seroconversion rate (SCR) for neutralising (MN) antibody titre at Month 6 against the A/Indonesia/05/2005 vaccine strain (ATP cohort for persistence)

				SCR			
						95% CI	
Antibodies against	Group	Timing	N	n	%	LL	UL
A/Indonesia	H5N1 3.8AD	PI(M6)	37	29	78.4	61.8	90.2
	HN3_8	PII(M6)	35	29	82.9	66.4	93.4
	Control	PII(M6)	46	44	95.7	85.2	99.5

H5N1 3.8AD = H5N1 3.8 µg + AS03; H5N1 3.8 = H5N1 3.8 µg; Seroconversion defined as:For initially seronegative subjects, antibody titre >= 56 1/DIL after vaccination; For initially seropositive subjects, antibody titre after vaccination >= 4 fold the pre-vaccination antibody titre; N = Number of subjects with pre- and post-vaccination results available; n/% = Number/percentage of seroconverted subjects; 95% CI = 95% confidence interval, LL = Lower Limit, UL = Upper Limit; PRE = Pre-vaccination at Day 0; PI(M6) = Post-vaccination one at month 6; PII(M6) = Post-vaccination two at month 6

An analysis of CMI responses (influenza-specific CD4/CD8 T-cells expressing different immune markers) was performed on a subset of subjects from the H5N1 3.8AD, H5N1 3.8 and Control groups after 4 type of stimulations.

Cells were stimulated with either a pool of peptides encompassing the haemagglutinin from A/Indonesia/05/2005 or from A/Vietnam/1194/2004 or with the split antigen from H5N1 A/Indonesia/05/2005 or from A/Vietnam/1194/2004 vaccine strains.

At Month 6:
• The frequency of antigen-specific CD4 T-cells was higher in the H5N1 3.8AD and Control groups compared to the H5N1 3.8 group.
• There were no antigen-specific CD8 T-cell responses detected in any of the group with the assay used.
• The frequency of memory B-cells specific to the H5N1 antigen was higher in the H5N1 3.8AD group.

Discussion on clinical efficacy of study 015

The additional data up to D180 post-boost from study 015, support administration of a single dose of the A/Indonesia vaccine to subjects who previously received one or two doses of A/Vietnam vaccine.

The additional data to D180 in study 015 showed that the seroprotection rate against A/Indonesia was just under the 70% threshold at D180 whereas the seroprotection rate against A/Vietnam still exceeded 70%.

Even a single dose of A/Vietnam vaccine had primed for responses to a booster at Month 6 with H5N1/Indonesia vaccine. HI responses to homologous and heterologous booster strains met and exceeded the CHMP criteria at 7 days post-boost with further increments at Month 6 + 21 days.

Therefore, the comparable responses to A/Indonesia vaccine after one or two doses of VT vaccine indicate that the D180 data are very unlikely to be different between groups. The CHMP agreed that tha heterologous booster results should be included in section 5.1 of the SmPC of Pumarix

2.6. Clinical safety

Due to the staggered reporting of studies in this application there is no overall integration of the safety data. Therefore the findings are summarised by study below.

The number of subjects and number of doses evaluated for safety in the two pivotal studies and in supportive studies is shown in the tables below.

Study ID	Formulation	Population (years)	Number of subjects	Number of doses
Q-Pan-001		18-64	680 in total	1345 in total
	Q-Pan full dose AS03 (3.8µg HA)		152	301
	Q-Pan half dose AS03 (3.8µg HA)		151	299
	Q-Pan without AS03 (3.8µg HA)		78	155
	D-Pan full dose AS03 (3.8µg HA)		151	298
	D-Pan half dose AS03 (3.8µg HA)		148	292
Q-Pan-002	Q-Pan AS03 -adjuvanted monovalent split vaccine (H5N1) 3.8 µg HA (3 lots) H5N1 A/ASO3 X H5N1 B/ASO3 Y H5N1 C/ASO3 Z Placebo	18-64	Q-Pan: 2304 Placebo: 768	Q-Pan: 4539 Placebo: 1507
		>60	Q-Pan: 1180 Placebo: 371	Q-Pan: 2208 Placebo: 733
Total	Total of Q-Pan vaccine recipients		3865	7502
	Total of H5N1 vaccine recipients – any formulation		4164	8092

Study ID	Formulation	Population (years)	Number of subjects	Number of doses
H5N1-002	AS03 (2 lots)-adjuvanted monovalent split vaccine (H5N1) 3.8 µg HA (2 lots)	18-60	961 in total	1907 in total
	H5N1 A/ASO3 X		240	478
	H5N1 A/ASO3 Y		239	475
	H5N1 B/ASO3 X		242	477
	H5N1 B/ASO3 Y		240	477
H5N1-007	Monovalent AS03-adjuvanted split vaccine (H5N1) (HA 3.8 µg) Monovalent AS03-adjuvanted split vaccine (H5N1) (HA 7.5 µg) Monovalent AS03-adjuvanted split vaccine (H5N1) (HA 15 µg) Monovalent AS03-adjuvanted split vaccine (H5N1) (HA 30 µg)	18-60	200 in total 50 for each formulation	400 in total 100 for each formulation
H5N1-008	Monovalent AS03-adjuvanted split vaccine (H5N1) (HA 15 µg)	18-60	3397	6664
		>60	405	801
Total	**D-Pan vaccine recipients**	**>18**	**4963**	**9772**

In addition, the MAH presented an Integrated Summary of safety (ISS) based on completed adult trials performed with the D-Pan and Q-Pan vaccines that provided data on 12917 subjects aged >18 years old.

Q-Pan H5N1-001

In this study pain was the most commonly reported solicited local symptom. There was no evidence of increasing local reactogenicity as a function of the second dose. Incidences of solicited local symptoms following vaccination with Q-Pan or D-Pan vaccines with full dose AS03 were comparable.

Solicited local symptoms (per dose) in study Q-Pan-001 (Total vaccinated cohort)

Study (schedule) Group	N	Intensity	Pain %	Pain 95%CI LL	Pain 95%CI UL	Redness %	Redness 95%CI LL	Redness 95%CI UL	Swelling %	Swelling 95%CI LL	Swelling 95%CI UL
H5N1 split Quebec	301	Total	81.7	76.9	85.9	2.3	0.9	4.7	6.0	3.6	9.3
(HA 3.8μg) AS03 full	301	Grade 3	4.0	2.1	6.9	0.0	0.0	1.2	0.0	0.0	1.2
H5N1 split Dresden	298	Total	85.2	80.7	89.1	4.0	2.1	6.9	9.1	6.1	12.9
(HA 3.8μg) AS03 full	298	Grade 3	3.7	1.9	6.5	0.0	0.0	1.2	0.0	0.0	1.2

In the contingent arms pain was the most commonly reported solicited local symptom (96% for Group H and 84% for Group I) but rates were lower after the second doses and rates of severe pain (Grade 3) were 2%. There were no reports of redness or swelling in Group I while the rates were 2% and 8%, respectively, for Group H with no redness or swelling > 100 mm.

Muscle ache was the most commonly reported solicited general symptom and was reported at much higher rates in the groups receiving adjuvanted vaccine (30.9%-41.6%). Rates of Grade 3 muscle ache were 1-4%. Incidences of solicited general symptoms following vaccination with Q-Pan or D-Pan vaccines were comparable when formulated with the same adjuvant content.

Solicited general symptoms (per dose) in Q-Pan-001 (Total vaccinated cohort)

Study (schedule) Group	N	Relationship to Vaccination/ intensity	Fatigue %	Fatigue 95%CI LL	Fatigue 95%CI UL	Fever %	Fever 95%CI LL	Fever 95%CI UL	Headache %	Headache 95%CI LL	Headache 95%CI UL
H5N1 split Quebec	301	Total	29.2	24.2	34.7	1.7	0.5	3.8	31.2	26.0	36.8
(HA 3.8μg) AS03 full	301	Grade 3	2.7	1.2	5.2	0.0	0.0	1.2	3.7	1.8	6.4
	301	Related	28.9	23.8	34.4	1.7	0.5	3.8	27.2	22.3	32.6
H5N1 split Dresden	298	Total	30.2	25.0	35.8	4.0	2.1	6.9	30.2	25.0	35.8
(HA 3.8μg) AS03 full	298	Grade 3	1.3	0.4	3.4	0.0	0.0	1.2	2.3	0.9	4.8
	298	Related	27.5	22.5	33.0	3.7	1.9	6.5	28.2	23.2	33.7

Study (schedule) Group	N	Relationship to vaccination/ intensity	Muscle aches %	95% CI LL	UL	Shivering %	95% CI LL	UL	Sweating %	95%CI LL	UL
H5N1 split Quebec (HA 3.8μg) AS03 full	301	Total	36.5	31.1	42.3	8.3	5.4	12.0	8.3	5.4	12.0
	301	Grade 3	4.0	2.1	6.9	2.0	0.7	4.3	1.0	0.2	2.9
	301	Related	35.5	30.1	41.2	8.3	5.4	12.0	8.3	5.4	12.0
H5N1 split Dresden (HA 3.8μg) AS03 full	298	Total	41.6	36.0	47.4	10.4	7.2	14.4	9.1	6.1	12.9
	298	Grade 3	1.0	0.2	2.9	0.3	0.0	1.9	1.0	0.2	2.9
	298	Related	38.6	33.0	44.4	8.7	5.8	12.5	8.4	5.5	12.1

In the contingent arms muscle ache was the most commonly reported solicited general symptom with rates of 38% for Group H and 42% for Group I but the incidence of Grade 3 muscle ache was low (2% and 0%). Fatigue was reported by 30% in each Group and was severe in intensity (Grade 3) in 2% per group. None of these 100 adults had a documented fever after the first dose and only one (in Group H) had a fever recorded after the second dose. No subject received a prophylactic antipyretic. During the 21-day post-vaccination period after each dose 42% and 38% per group received any antipyretic.

At least one unsolicited AE was reported following 28.4% of doses of non-adjuvanted HA, 32.2% and 30.1% of Q-Pan doses with full and half dose AS03, respectively, and 34.6% and 40.4% of D-Pan doses with full and half dose AS03. No specific AE was reported with > 5% of doses in any vaccine group. The most commonly reported AEs were headache, pharyngolaryngeal pain, nasopharyngitis, upper respiratory tract infection and nausea. Lymph node pain and lymphadenopathy were reported after ≤ 2.7% of doses in any vaccine group.

In the contingent arms at least one unsolicited AE was reported by 58% of subjects in Group H and 46% in Group I. No AE preferred term was reported by > 4 (i.e. 8%) subjects in either treatment group. Vaccine-related unsolicited AEs were reported by 20% in Group H and 12% in Group I. Unsolicited symptoms requiring a medically-attended visit were reported by 28% in both groups and the most common were hypertension (3) and sinusitis, depression and oropharyngeal pain (2 each), all in subjects in Group I.

Screening of the database for AEs with potential immune-mediated causation up to Day 182 identified reports in <3% of subjects overall, including 2.6% in the non-adjuvanted HA group, 2.0%, and 2.6% in the Q-Pan groups and 2.6% and 1.4% in the D-Pan groups. Many of these events seemed to be due to concurrent conditions or other environmental exposures, and essentially all proved to be transient. No vaccine-related SAEs were reported up to Day 182 and there were no deaths.

Q-Pan H5N1-010

In this extension study to Q-Pan H5N1-001 the percentage of subjects reporting any symptom (solicited or unsolicited, local or general) after booster doses was approximately twice as high in the groups boosted with adjuvanted vaccine (A, B1, C1, D1 and E1) vs. those boosted with unadjuvanted vaccine (B2, C2, D2 and E2). In particular, the incidence of local symptoms was much higher for adjuvanted booster vaccine recipients.

The overall incidence of Grade 3 symptoms ranged from 0 to 13.6%. A larger percentage of subjects in the adjuvanted booster groups experienced Grade 3 general and local symptoms compared with the unadjuvanted booster groups but only in the isolated case of group E1 was this difference substantial. Grade 3 local symptoms occurred in three subjects, all of whom received adjuvanted booster vaccine. In Group E1 there was a variety of Grade 3 solicited general symptoms, none of which required medical attention.

Table 30 Incidence and nature of symptoms (solicited and unsolicited) reported during the 7-day (Days 0-6) post-vaccination period following the booster dose (Total vaccinated cohort)

Group	Any symptom					General symptoms					Local Symptoms				
				95% CI					95% CI					95% CI	
	N	n	%	LL	UL	N	n	%	LL	UL	N	n	%	LL	UL
A	49	45	91.8	80.4	97.7	49	22	44.9	30.7	59.8	49	42	85.7	72.8	94.1
B1	72	60	83.3	72.7	91.1	72	40	55.6	43.4	67.3	72	59	81.9	71.1	90.0
B2	41	17	41.5	26.3	57.9	41	11	26.8	14.2	42.9	41	10	24.4	12.4	40.3
C1	60	55	91.7	81.6	97.2	60	37	61.7	48.2	73.9	60	53	88.3	77.4	95.2
C2	40	16	40.0	24.9	56.7	40	12	30.0	16.6	46.5	40	10	25.0	12.7	41.2
D1	61	52	85.2	73.8	93.0	61	36	59.0	45.7	71.4	61	48	78.7	66.3	88.1
D2	46	17	37.0	23.2	52.5	46	12	26.1	14.3	41.1	46	13	28.3	16.0	43.5
E1	59	51	86.4	75.0	94.0	59	39	66.1	52.6	77.9	59	46	78.0	65.3	87.7
E2	41	20	48.8	32.9	64.9	41	17	41.5	26.3	57.9	41	6	14.6	5.6	29.2

A = Q-Pan Indo 3.8 x 2 + Turkey 3.8, $AS03_A$
B1 = Q-Pan Indo 3.8, $AS03_A$ x 2 + Turkey 3.8, $AS03_A$
B2 = Q-Pan Indo 3.8, $AS03_A$ x 2 + Turkey 3.8
C1 = Q-Pan Indo 3.8, $AS03_B$ x 2 + Turkey 3.8, $AS03_A$
C2 = Q-Pan Indo 3.8, $AS03_B$ x 2 + Turkey 3.8
D1 = D-Pan Indo 3.8, $AS03_A$ x 2 + Turkey 3.8, $AS03_A$
D2 = D-Pan Indo 3.8, $AS03_A$ x 2 + Turkey 3.8
E1 = D-Pan Indo 3.8, AS03e x 2 + Turkey 3.8, $AS03_A$
E2 = D-Pan Indo 3.8, $AS03_B$ x 2 + Turkey 3.8

Pain at the injection site was reported by 83.7%, 81.9%, 88.3%, 80.0% and 79.3% in the adjuvanted booster vaccine groups (Groups A, B1, C1, D1, and E1) and 22.5%, 25.0%, 28.3% and 14.6% in the unadjuvanted booster vaccine groups (Groups B2, C2, D2, and E2), respectively. However, only 3 subjects reported Grade 3 pain (one in each of Groups B1, C1, and D1). Grade 2 pain was reported by 104 subjects, most of whom had received adjuvanted booster vaccination. Redness and swelling were much less common than pain in all treatment groups. Overall, subjects 41 to 64 years of age had slightly lower incidence rates of local solicited symptoms.

Muscle ache was the most commonly reported solicited general symptom and was reported at a higher rate (up to 48%) by recipients of adjuvanted booster vaccine. Only 0-7% per group reported severe muscle ache (Grade 3). Headache, fatigue and joint pain were very common (15.5-25.3%) with generally greater rates among the adjuvanted booster vaccine groups. Grade 3 fatigue, headache or joint pain was reported by 0-8.6% per group with highest rates among the adjuvanted booster vaccine recipients. Shivering, sweating and temperature elevation were reported by < 10% across all treatment groups. The incidence of temperature elevation was low, with 0-7% per group reporting this symptom. Severe shivering, sweating and elevated temperature were reported by 0-3.4% per group. Oral temperature ≥ 38.5º C occurred in one subject in Group D1.

At least one unsolicited AE was reported by 150 subjects with a higher rate (up to 43%) in those who received adjuvanted vaccine for priming and boosting. None showed a clear trend between groups or association with adjuvant. Vaccine-related unsolicited AEs were reported by 2.5 - 13.3% per group. The only vaccine-related unsolicited AEs reported by more than 3% in a treatment group were diarrhoea, lymphadenopathy, injection site haematoma, nausea and pain in extremity. Unsolicited symptoms requiring a medically attended visit were reported by 54 subjects (from 5.6 - 17.1% per group). No AEs that qualified as potential immune-mediated diseases occurred during the study (through Day 42). The two SAEs reported were considered by the investigators to be unrelated to study vaccine. No subject died through Day 42 and no subject experienced an AE or SAE that led to premature discontinuation.

Q-Pan H5N1-002

Pain in subjects aged ≤ 64 years was the most commonly reported solicited local symptom in both the Q-Pan group and the placebo group while redness and swelling were much less common. Local reactogenicity did not worsen after the second dose relative to the first dose. In those aged > 64 years pain was the most commonly reported solicited local symptom in both groups but was reported at slightly lower rates than in younger subjects. Grade 3 pain rates were low (0.8% with Q-Pan versus 0.3% in the placebo group) and redness and swelling were much less common than pain in both treatment groups. One Q-Pan vaccine dose was followed by swelling >100 mm.

Solicited local symptoms (per dose) in study Q-Pan-002 (Total vaccinated cohort)

Study (schedule) Group	N	Intensity	Pain %	Pain 95%CI LL	Pain 95%CI UL	Redness %	Redness 95%CI LL	Redness 95%CI UL	Swelling %	Swelling 95%CI LL	Swelling 95%CI UL
H5N1 Quebec	4453	Total	80.5	79.3	81.6	4.9	4.3	5.6	7.1	6.3	7.8
(HA 3.8μg) + AS03	4453	Grade 3	3.6	3.1	4.2	0.1	0.0	0.2	0.1	0.0	0.2
Placebo	1482	Total	14.0	12.3	15.9	0.5	0.2	1.0	0.5	0.2	1.0
	1482	Grade 3	0.4	0.1	0.9	0.0	0.0	0.2	0.0	0.0	0.2

In the 18-64 years stratum, muscle ache was the most commonly reported solicited general symptom and was reported at a higher rate for the Q-Pan group (39.3% of doses) than the placebo group (13% of doses). Grade 3 muscle ache was reported after 2.3% of doses in the Q-Pan group and 1.1% in the placebo group. Incidences of general symptoms were lower in the older age group but muscle ache was the most commonly reported solicited general symptom and grade 3 muscle ache was reported by (0.6-0.7%).

Up to D182 at least one unsolicited AE was reported in the younger cohort following 27.8% of Q-Pan doses and 26.7% of placebo doses but none was reported with more than 2.7% of doses. Most AEs seemed to be due to incidental URTI. Lymph node pain and/or lymphadenopathy AEs were reported following <1.5% of doses in both treatment groups. Reporting rates in the older cohort were 25.7% of Q-Pan doses and 21.4% of placebo doses but no AE was reported with more than 1.8% of doses.

Six subjects died in study Q-Pan-002 up to D182 including one death within D42 and five between Day 42 and Day 182. Four had received Q-Pan but all were considered unrelated to vaccination.

Safety follow-up through Day 364 showed that 160 subjects reported a total of 210 SAEs at any time during the study, including 111 (3.2%) in the Q-Pan group and 45 (4.0%) in the placebo group. No SAE preferred term was reported by more than 7 subjects. None of these SAEs showed a clear association with receipt of the study vaccine products and none was considered treatment-related by the investigators. At least one unsolicited MAE was reported by 1027 (30%) subjects in the Q-Pan group and 346 (30.4%) subjects in the placebo group, with no substantive differences between treatment groups or age strata. No MAE preferred term was reported by > 2.1% of subjects in either treatment group.

Thirteen subjects reported AESIs / pIMDs, including 12 subjects in the Q-Pan group and 1 subject in the placebo group. Two subjects each in the Q-Pan group reported psoriasis and polymyalgia rheumatica and one subject each in the Q-Pan group reported coeliac disease, Crohn's disease, autoimmune hepatitis, rheumatoid arthritis, facial palsy, erythema nodosum, radiculitis, temporal arteritis and fourth cranial nerve palsy. One subject in the placebo group reported psoriasis. An additional Q-Pan recipient was reported to have rheumatoid lung, a preferred term not captured in the initial AESI/pIMD query. None of these events was considered vaccine-related by the investigators and only the case of autoimmune hepatitis was retrospectively classified as a SAE although the case did not actually meet the criteria. Review of these cases suggested that a substantial proportion may have had alternative non-immunologic causes or triggering events other than the vaccine or may have represented conditions which antedated vaccination.

Q-Pan H5N1-009

The overall incidence of symptoms was comparable among all groups for both doses, although the proportion of subjects reporting either local or general symptoms declined modestly after the second dose relative to the first in each group. Pain was the most commonly reported solicited local symptom in all treatment groups and was reported at similar rates for all groups (around 80%). The incidence of severe pain (Grade 3) was low (per subject rates of 1.3%, 7.8%, 7.7% and 2.6%). Redness and swelling were much less common than pain in all treatment groups. No subjects reported redness or swelling > 100 mm in any treatment group.

Muscle ache was the most commonly reported solicited general symptom overall (64.5%, 55.8%, 51.3% and 51.9%) while severe muscle aches (Grade 3) showed overall per subject rates of 2.6%, 7.8%, 6.4% and 2.6%. Fatigue, headache and joint pain were fairly common (14.3-39.0% per subject) but Grade 3 events were reported in 1.3-5.3%. The remaining solicited general symptoms were reported by < 20% of subjects overall across all treatment groups.

Temperature elevation was reported by 2.6%, 5.2%, 1.3% and 1.3% and temperature ≥ 38.5º C occurred in 1.3%, 2.6%, 1.3% and 0%. Temperatures ≥ 39º C were reported by 2.6% of subjects in Group B and by no subjects in all other treatment groups.

At least one unsolicited AE was reported by 139 subjects overall (rates were 43.6%, 47.4%, 52.6% and 34.6%). No AE was reported by more than 6.4% of subjects in a treatment group. Lymphadenopathy occurred in 3.8%, 6.4%, 5.1% and 2.6% with no consistent pattern across treatment groups and no cumulative rates with doses. All lymphadenopathy occurred at the axillary or supraclavicular lymph nodes.

No subject experienced an AE that led to premature discontinuation from the study. There were six SAEs reported by three subjects up to Day 51 but all were considered by the investigators to be unrelated to study vaccine and were non-fatal.

Q-Pan H5N1-011

In the Japanese subjects included in the study the overall incidence of symptoms was high and comparable between age strata and all subjects reported at least one symptom. Grade 3 local and general symptoms were reported with low frequencies (≤ 16%). Subjects from the 20-40 years age stratum reported more Grade 3 general symptoms (10%, 5 subjects) compared to the older stratum but there was no clear difference between age strata in terms of Grade 3 local symptoms including those considered to be related to vaccination.

Local symptoms, predominantly driven by the incidence of injection site pain, were reported with high and similar frequencies in both age strata. Grade 3 local solicited symptoms were reported with low frequencies with Grade 3 pain at injection site reported in only one subject in the 20-40 years stratum. The overall per dose frequencies in both age strata ranged from 2% to 3% in the 20-40 years stratum and the 41-64 years stratum for redness and swelling/induration, respectively. In general, there was no increase in the incidence of local solicited symptoms of any type or grade between Dose 1 and Dose 2.

There were no differences observed in terms of reported frequency of joint pain and shivering between the age strata. In contrast, trends for higher frequencies were observed for the following solicited general symptoms in the 20-40 years stratum: fatigue, headache, muscle aches, shivering, increased sweating and fever. The reported frequencies of Grade 3 general symptoms were very low. Fatigue was the most frequently reported general symptom at 71% overall, 78% in the 20-40 years and 64% in the 41-64 years groups. Grade 3 and grade 3-related fatigue was reported only in the 20-40 years stratum (3 subjects, 6%). Fever of any grade (≥□38°C) was reported by 12% in the 20-40 years stratum compared to 10% of subjects in the 41-64 years stratum. Grade 3 and Grade 3-related fever was reported by one subject in the 20-40 years stratum with no reports of Grade 4 fever.

Unsolicited adverse events were reported by 51 subjects, including injection site pruritus, injection site warmth and nasopharyngitis. Three subjects in the younger cohort reported at least one Grade 3 unsolicited AE (joint sprain, urticaria and asthma) and 28 reported at least one unsolicited AE considered to be causally related to vaccination (including urticaria one day after dose 2 that occurred after a first episode of urticaria worsening two days after dose 1). AEs prompting medically-attended visits were reported by 16 subjects. Of these, 13 subjects were in the 20-40 years stratum. However, no specific clinical pattern could be identified.

Additional safety data taken into consideration:

The data from study D-Pan H1N1-009 (a phase II, randomised, open-label, multicentre study to evaluate the safety and immunogenicity of Pandemrix H1N1 following a homologous prime-boost schedule in children aged 6 to 35 months, assessed in Pandemrix variation II-0028) show that the reactogenicity of the second half adult dose in children 6 months to 35 months of age is greater than the first within the initial cohort and also when comparing the total 104 who have received a first half adult dose with the 51 who have received a second half adult dose. The patterns of reporting by age stratum indicate that local and general reactogenicity is higher after the second half adult dose for most individual symptoms and in all age strata.

The overall per-dose frequency of the following adverse reactions was as follows:

Adverse reactions	Post dose 1	Post dose 2
Pain	31.4%	41.2%
Redness	19.6%	29.4%
Swelling	15.7%	23.5%
Fever (≥38°C) axillary	5.9%	43.1%
Fever (≥39°C) axillary	0.0%	3.9%
Drowsiness	7.8%	35.3%
Irritability	21.6%	37.3%
Loss of appetite	9.8%	39.2%

The major concern is the rate of fever after the second half adult dose in children aged 6-35 months, which would not be predicted from the prior experience with H5N1/AS03 vaccine in older children aged 3-5 years. Nevertheless, fever may also occur after the first half adult dose and febrile convulsions have been reported in association.
The additional data from D-Pan H1N1-010 (assessed in Pandemrix II-0028) also show higher rates of fever after the second full adult dose in children aged from 3 years upwards but with decreasing rates with increasing age. It would be expected that rates after a second half adult dose in this age group would be lower.

Overall discussion on clinical safety

Based on the limited data from the direct comparison made within study Q-Pan-001 the safety profile of Q-Pan H5N1 appeared essentially the same as that previously described for D-Pan H5N1. The data from this study and all the other studies from which safety data were reported during the procedure did not raise any new issues for the vaccine construct as a whole. The issue of the potential for the AS03 adjuvant to trigger onset of auto-immune diseases in predisposed individuals remain under close scrutiny but so far without any definitive conclusion possible. The safety database available for Q-Pan H5N1 is sufficient to support its use in adults. The data from D-Pan H5N1-009 was considered sufficient to support the use in children from 3 years of age. as the data in adults strongly support a conclusion that the safety of D-Pan H5N1 in children would also apply to Q-Pan H5N1. The CHMP however noted the increased rate of fever after the second dose following the use of D-Pan H1N1 in previous studies, which was especially observed in children 6 – 35 months.

Assessment of paediatric data on clinical safety

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

Summary of main studies

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table: Summary of Efficacy for trial Q-PAN H5N1-001

Title: A phase I/II, observer-blind, randomized, active-controlled trial to evaluate the safety and immunogenicity of a two-dose series of monovalent H5N1 vaccine antigens without adjuvant and with two different strengths of AS03.		
Study identifier	**Q-PAN H5N1-001**	
Design	A randomised, observer-blind, multi-centre, active-controlled study that explored the effect of adjuvant (full strength and half strength AS03) administered with each of Q-Pan and D-Pan HA and compared to Q-Pan HA alone (all using 3.8 µg HA).	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	Approximately 6 months for each subject, from enrolment through the last study follow-up. not applicable not applicable
Hypothesis	Superiority	
Treatments groups and numbers enrolled	Group A:	Quebec-manufactured A/Indonesia/5/05 H5N1 antigen containing 3.8 µg of haemagglutinin (HA) without adjuvant, intramuscularly (IM) on Days 0 and 21 , (n=75)
	Group B	Quebec-manufactured A/Indonesia/5/05 H5N1 antigen containing 3.8 µg of HA with full strength adjuvant, intramuscularly on Days 0 and 21, (n=152)
	Group C	Quebec-manufactured A/Indonesia/5/05 H5N1 antigen containing 3.8 µg of HA with half strength AS03, intramuscularly on Days 0 and 21 (n=150),
	Group D	Dresden-manufactured A/Indonesia/5/05 (H5N1) antigen containing 3.8 µg of HA with full strength AS03, intramuscularly on Days 0 and 21 (n= 149)
	Group E:	A/Indonesia/5/05 (H5N1) antigen containing 3.8 µg of HA with half strength AS03, intramuscularly on Days 0 and 21 (n=150)
Endpoints and definitions	Primary endpoints	o Vaccine-homologous virus antibody response in subjects receiving two doses of vaccine, as demonstrated by the HI antibody titer at Day 42.
	Secondary endpoints	o Vaccine-homologous virus antibody response at 21 days following receipt of a first dose of vaccine, o Persistence of this response through approximately 6 months (182 days), as demonstrated by the vaccine-homologous virus HI antibody titer at Day 182.
Database lock	Study initiation date: 28 July 2007 Study completion date: 21 March 2008 Data lock date: 04 June 2008	
Results and Analysis		
Analysis population and time point description	According to Protocol (ATP) Until D182 (6 months)	

Descriptive statistics and estimate variability	Key Immunogenicity results

Anti-haemagglutinin (anti-HA) antibody responses in subjects aged 18-64 years (Q-Pan H5N1/Pumarix)

anti-HA antibody	Immune response to A/Indonesia/5/2005		
	Day 21 N=145	Day 42 N=145	Day 180 N=141
Seroprotection rate[1]	42.1%	97.2%	54.6%
Seroconversion rate[2]	42.1%	97.2%	54.6%
Seroconversion factor[3]	4.5	92.9	5.6

[1]seroprotection rate (i.e. proportion of subjects with HI titre ≥ 1:40);
[2]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);
[3]seroconversion factor (i.e. ratio of the post-vaccination GMT and the pre-vaccination GMT)

Vaccine Response Rates for A/Indonesia/5/05 Neutralizing Antibody at Days 21, 42, and 182			
Treatment Group	% of Vaccine Responders Day 21 (95% CI)	% of Vaccine Responders Day 42 (95% CI)	% of Vaccine Responders Day 182 (95% CI)
A (Q000AS03)	42.9 (28.8, 57.8)	73.5 (58.9, 85.1)	53.1 (38.3, 67.5)
B (Q100AS03)	76.6 (62.0, 87.7)	97.9 (88.7, 99.9)	91.5 (79.6, 97.6)
C (Q50AS03)	68.1 (52.9, 80.9)	91.3 (79.2, 97.6)	78.3 (63.6, 89.1)
D (D100AS03)	79.2 (65.0, 89.5)	95.8 (85.7, 99.5)	87.5 (74.8, 95.3)
E (D50AS03)	71.4 (56.7, 83.4)	96.0 (86.3, 99.5)	87.5 (74.8, 95.3)

A/Indonesia/5/05 Neutralizing Antibody GMTs Pre-vaccination and at Days 21, 42, and 182 Post First Dose				
Treatment Group	GMT Value PRE (95% CI)	GMT Value Day 21 (95% CI)	GMT Value Day 42 (95% CI)	GMT Value Day 182 (95% CI)
A (Q000AS03)	25.7 (19.9, 33.1)	78.6 (57.7, 107.1)	183.8 (138.4, 244.2)	108.9 (81.9, 144.7)
B (Q100AS03)	22.3 (17.4, 28.5)	199.0 (148.9, 266.1)	1566.8 (1227.3, 2000.2)	414.0 (346.2, 495.1)
C (Q50AS03)	31.8 (23.0, 43.8)	240.1 (191.4, 301.3)	1242.1 (902.2, 1710.0)	417.1 (335.0, 519.3)
D (D100AS03)	23.7 (18.3, 30.8)	260.5 (208.1, 326.0)	1497.2 (1192.0, 1880.5)	456.2 (387.4, 537.3)
E (D50AS03)	25.7 (19.6, 33.7)	248.7 (202.0, 306.3)	1352.8 (1075.5, 1701.6)	450.1 (384.7, 526.5)

Table: Summary of Efficacy for trial Q-PAN H5N1-002

Title: A Phase III, observer-blind, randomized, placebo-controlled, multi-centre trial to evaluate the safety and immunogenicity of a two-dose series of monovalent A/Indonesia/5/05 (H5N1) vaccine antigen in association with AS03 adjuvant in adults aged ≥18 years.		
Study identifier	**Q-PAN H5N1-002**	
Design	A randomized, observer-blind, multi-centred, placebo-controlled eight-arm trial. Subjects were to be randomized in a 3:1 ratio to treatment with active product, comprising 1 of 3 lots of study vaccine (GSK 1557484A), or placebo. Subjects were randomized at a 1:1:1:1 ratio to receive 1 of 4 treatments (3 lots of study vaccine and placebo). Within each treatment, the randomization was stratified by age to target age interval ratios of 1.5 (18 to 30 years): 1.5 (31 to 49 years): 1 (50 to 64 years): 1.5 (65 to 75 years): 0.5 (> 75 years).	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	6 months not applicable not applicable
Hypothesis	Non-inferiority	
Treatments groups	Group A (18-49 years)	Quebec-manufactured A/Indonesia/5/05 H5N1 antigen (lot A) containing 3.8 µg of haemagglutinin (HA) with adjuvant (lot 1), intramuscularly (IM) on Days 0 and 21 (N ≈ 555)
	Group B (18-49 years)	Quebec-manufactured A/Indonesia/5/05 H5N1 antigen (lot B) containing 3.8 µg of HA with adjuvant (lot 2), IM on Days 0 and 21 (N ≈ 555)
	Group C (18-49 years)	Quebec-manufactured A/Indonesia/5/05 H5N1 antigen (lot C) containing 3.8 µg of HA with adjuvant (lot 3), IM on Days 0 and 21 (N ≈ 555)
	Group D (18-49 years)	Placebo, IM on Days 0 and 21 (N ≈ 555)
	Group E (50-64 years)	Quebec-manufactured A/Indonesia/5/05 H5N1 antigen (lot A, B, or C) containing 3.8 µg of HA with adjuvant (lot 1, 2, or 3), IM on Days 0 and 21 (N ≈ 555; 185 per lot)
	Group F (50-64 years)	Placebo, IM on Days 0 and 21 (N ≈ 185)
	Group G (> 64 years)	Quebec-manufactured A/Indonesia/5/05 H5N1 antigen (lot A, B, or C) containing 3.8 µg of HA with adjuvant (lot 1, 2, or 3), IM on Days 0 and 21 (N ≈ 1110; 370 per lot)
	Group H (> 64 years)	Placebo, IM on Days 0 and 21 (N ≈ 370).

Endpoints and definitions	Primary endpoint	Vaccine-homologous virus antibody response in subjects receiving 2 doses of study vaccine, as demonstrated by the HI antibody titer at 21 days after the second dose of H5N1 vaccine for younger adults age 18 to 64 years and older adults age > 64 years (CBER analysis strata for age) to evaluate the following o H5N1 SCR; defined as the percentage of subjects who had either a pre-vaccination (Day 0) reciprocal HI titer < 10 and a post-vaccination (Day 42) reciprocal titer ≥ 40, or a pre-vaccination reciprocal HI titer ≥ 10 and at least a 4-fold increase in post vaccination reciprocal titer against A/Indonesia/5/05 virus 21 days after the second dose of H5N1 vaccine in both age strata. If the lower limit of the 95% confidence interval (CI) for SCR was ≥ 40% in subjects 18 to 64 years of age, and ≥ 30% in subjects > 64 years of age, then it was to be concluded that H5N1 antigen in association with AS03 elicited an immune response, measured by postimmunization vaccine-homologous virus HI titres, that met or exceeded CBER guidance targets for SCR. o H5N1 SPR, defined as the proportion of subjects with reciprocal HI titres ≥ 40 against A/Indonesia/5/05 virus 21 days after the second dose of H5N1 vaccine (abbreviated SPR) in both age strata. If the lower limit of the 95% CI for SPR was ≥ 70% in subjects 18 to 64 years of age, and ≥ 60% in subjects > 64 years of age, then it was to be concluded that H5N1 antigen in association with AS03 elicited an immune response, measured by post-immunization vaccine-homologous virus HI titres, that met or exceeded CBER guidance targets for proportion of subjects with reciprocal HI titres ≥ 40.
	Secondary endpoint	Vaccine-homologous virus antibody response in subjects receiving 2 doses of study vaccine, as demonstrated by the HI antibody titer at 21 days after the second dose of H5N1 vaccine for younger adults age 18 to 60 years and older adults age > 60 years (EMEA/CHMP analysis strata for age); vaccine-homologous virus antibody response in subjects receiving 2 doses of study vaccine, as demonstrated by the HI antibody titer at 6 months after the first dose of H5N1 vaccine for younger adults age 18 to 64 years and older adults age > 64 years;

	Secondary endpoint	Vaccine homologous virus and drift variant H5N1 virus antibody responses, as assessed by microneutralisation assays, in subjects receiving 2 doses of study vaccine.
Database lock	07 August 2008	
Results and Analysis		
Analysis population and time point description	According-to-protocol	
Descriptive statistics and estimate variability	**Key A/Indonesia/5/05 HI Antibody Response Parameters at Day 42 (21 days post dose 2) According to CBER-mandated Age Strata . ATP Immunogenicity Cohort**	

Treatment Group	% of Subjects Seroconverted (95% CI)	% of Subjects with Reciprocal HI Titer ≥ 40 (95% CI)
Q-Pan, 18-64 years	90.8 (89.3, 92.2)	90.8 (89.3, 92.2)
Placebo, 18-64 years	1.3 (0.0, 7.1)	1.3 (0.0, 7.1)
Q-Pan, > 64 years	74.0 (69.4, 78.2)	74.5 (69.9, 78.7)
Placebo, > 64 years	2.5 (0.1, 13.2)	2.5 (0.1, 13.2)

Key A/Indonesia/5/05 HI Antibody Response Parameters at Day 42 (21 days post dose 2) According to EMEA/CHMP-mandated Age Strata ATP Immunogenicity Cohort

Treatment Group	% of Subjects Seroconverted (95% CI)	% of Subjects with Reciprocal HI Titer ≥ 40 (95% CI)	GMFR (95% CI)
Q-Pan, 18-60 years	91.0 (89.4, 92.4)	91.0 (89.4, 92.4)	51.4 (47.8, 55.3)
Placebo, 18-60 years	1.5 (0.0, 7.9)	1.5 (0.0, 7.9)	1.0 (1.0, 1.1)
Q-Pan, > 60 years	76.4 (72.3, 80.1)	76.8 (72.8, 80.5)	17.2 (14.9, 19.9)
Placebo, > 60 years	2.1 (0.1, 11.1)	2.1 (0.1, 11.1)	1.1 (0.9, 1.3)

2.6.1. Conclusions on the clinical efficacy

The applicant has conducted a full clinical development programme with Pumarix containing Quebec manufactured H5N1 antigens. D-Pan and Q-Pan containing H5N1 strains have been shown to have comparable immunogenicity in adults in study Q-Pan H5N1-001 and there are also data in the elderly in study Q-Pan H5N1-002 that indicate comparable responses between Dresden and Quebec-manufactured vaccines.

There are currently no clinical data with Pumarix (i.e. Q-Pan H5N1) in children.

While awaiting paediatric data generated with Q-Pan in children, the CHMP agreed that the presentation of the clinical paediatric data obtained so far with D-Pan (H5N1) in the Pumarix SmPC is justified. This is based on the immunological equivalence that was previously shown between Q-Pan (H5N1) and D-Pan (H5N1) in adults.

The available data indicate that the safety profiles of H5N1 D-Pan and H5N1 Q-Pan vaccines are comparable. Taking this into consideration, along with the comparable immune responses observed in adults to D-Pan (H5N1) and Q-Pan (H5N1), the CHMP concluded that the dose recommendations for Pumarix in children can be based on the data presented from study D-Pan H5N1-009 carried out in children 3-9 years of age.

Overall the immune responses to Pumarix and Pandemrix/Prepandrix (as H5N1 vaccine) can be considered to be broadly comparable.

2.6.2. Conclusions on the clinical safety

The clinical safety data generated with Pumarix in adults indicate no safety concerns.

Across the studies pain at the injection site was the most frequently reported local symptom. There was also a trend for higher incidences of swelling and redness in groups with adjuvanted vaccines and the rate of induration was significantly higher in adjuvanted groups. However, severe swelling, redness and induration were all reported at low rates. Regional lymphadenopathy may also occur.
The most frequently reported general symptoms were fatigue and headache.
All SAEs besides one case of autoimmune hepatitis in study Q-Pan H5N1-002, which however did not meet the case definition criteria were considered as not related to vaccination by the investigator . Review of these cases suggested that a substantial proportion may have had alternative non-immunologic causes or triggering events other than the vaccine or may have represented conditions which antedated vaccination.

Six subjects died in study Q-Pan-002 up to D182 including one death within D42 and five between Day 42 and Day 182. Four had received Q-Pan but all were considered unrelated to vaccination.

Safety data generated with D-Pan H5N1 in children also did not reveal any safety concerns, and the CHMP considered that the safety in children can be extrapolated from D-Pan H5N1 to Q-Pan H5N1.

Safety data generated from FluLaval, a seasonal vaccine which is manufactured in the same way like Pumarix, and from Arepanrix, a pandemic H1N1v vaccine manufactured with the same process as Pumarix and which was widely used outside the EU during the 2009-2010 pandemic, indicate also no safety concerns.

The safety of Pumarix will be further assessed in a prospective non-interventional cohort safety study with the formulation of the vaccine to be used in the event of a pandemic in addition to the existing commitments for Pumarix as outlined in the RMP and Letter of Undertaking.

The data from studies carried out with Q-Pan H5N1 vaccine (i.e. Pumarix) is in keeping with the previous observations of comparable safety and immunogenicity between with D-Pan vaccines (i.e. Pandemic Influenza Vaccine (H5N1) (split virion, inactivated, adjuvanted) GlaxoSmithKline Biologicals and Prepandrix) containing HA derived from H5N1 strains.

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.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics. No post marketing data is currently available from the use of Q-Pan H5N1 vaccines.

2.7. Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system (version 3.06) as described by the applicant fulfils the legislative requirements.

Standard Pharmacovigilance activities will be conducted in pre-pandemic phases. However, in a pandemic situation the MAH agreed to adapt pharmacovigilance activities according to the CHMP recommendations for pharmacovigilance plans for pandemic vaccines (EMEA/359381/2009) and following updates. Modified pharmacovigilance activities in the pandemic period are described in the Risk Management Plan.

Risk Management Plan

The MAA submitted a risk management plan (version 2 June 2010) which included a risk minimisation plan.

Additional risk minimization activities are proposed to address the following potential risks:

- medical errors/misidentification of vaccine,
- contamination of the multiple-dose vials,
- coring of the rubber stopper on the antigen vial

which have not been properly reported in the summary table of the RMP activities. An updated EU-RMP will be submitted at the time of the next submission of PSUR, including the above potential risks in the summary table of the RMP activities and taking into account the classification of safety concerns as important identified risk, important potential risks and missing information (see section 2.7).

Summary Table of the Risk Management Plan

Potential theoretical safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
Anaphylaxis	• Enhanced pharmacovigilance o Weekly signal detection o Use of targeted follow-up questionnaires o Individual reports expedited to regulators o Included in Table 3 of simplified PSURs† o Cumulative analysis included in full PSUR following end of pandemic period o Ad hoc analyses if reporting rate exceeds 1/100,000 doses distributed • Incidence will be estimated in participants of the post-authorisation safety study	• Contraindication in the proposed labelling • Precaution in the proposed labelling regarding use in persons with known hypersensitivity, other than anaphylaxis, to vaccine components
Autoimmune hepatitis	• Enhanced pharmacovigilance o Weekly signal detection o Use of targeted follow-up questionnaires o Individual reports expedited to regulators o Cumulative analysis included in full PSUR following end of pandemic period o Ad hoc analyses if reporting rate exceeds 20/100,000 doses distributed	NA*
Bell's palsy	• Enhanced pharmacovigilance o Weekly signal detection o Use of targeted follow-up questionnaires o Individual reports expedited to regulators o Included in Table 3 of simplified PSURs o Cumulative analysis included in full PSUR following end of pandemic period o Ad hoc analyses if reporting rate exceeds 24/100,000 doses distributed • Incidence will be estimated in participants of the post-authorisation safety study	NA
Convulsion	• Enhanced pharmacovigilance o Weekly signal detection o Use of targeted follow-up questionnaires o Individual reports expedited to regulators o Included in Table 3 of simplified PSURs o Cumulative analysis included in full PSUR following end of pandemic period o Ad hoc analyses if reporting rate exceeds 3,000/100,000 doses distributed • Incidence will be estimated in participants of the post-authorisation safety study	NA

Potential theoretical safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
Demyelinating disorders	• Enhanced pharmacovigilance o Weekly signal detection o Use of targeted follow-up questionnaires o Individual reports expedited to regulators o Included in Table 3 of simplified PSURs o Cumulative analysis included in full PSUR following end of pandemic period o Ad hoc analyses if reporting rate exceeds published incidence rate • Incidence will be estimated in participants of the post-authorisation safety study	NA
Encephalitis	• Enhanced pharmacovigilance o Weekly signal detection o Use of targeted follow-up questionnaires o Individual reports expedited to regulators o Included in Table 3 of simplified PSURs o Cumulative analysis included in full PSUR following end of pandemic period o Ad hoc analyses if reporting rate exceeds 7/100,000 doses distributed • Incidence will be estimated in participants of the post-authorisation safety study	NA
Guillain-Barré syndrome	• Enhanced pharmacovigilance o Weekly signal detection o Use of targeted follow-up questionnaires o Individual reports expedited to regulators o Included in Table 3 of simplified PSURs o Cumulative analysis included in full PSUR following end of pandemic period o Ad hoc analyses if reporting rate exceeds 2/100,000 doses distributed o Active monitoring in collaboration with national groups/agencies • Incidence will be estimated in participants of the post-authorisation safety study	NA
Increased concentrations of hepatic enzymes	• Enhanced pharmacovigilance o Weekly signal detection o Use of targeted follow-up questionnaires o Individual reports expedited to regulators o Cumulative analysis included in full PSUR following end of pandemic period o Ad hoc analyses if signal detected	NA

Potential theoretical safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
Neuritis	• Enhanced pharmacovigilance o Weekly signal detection o Use of targeted follow-up questionnaires o Individual reports expedited to regulators o Included in Table 3 of simplified PSURs o Cumulative analysis included in full PSUR following end of pandemic period o Ad hoc analyses if reporting rate exceeds published incidence rate • Incidence will be estimated in participants of the post-authorisation safety study	NA
Vasculitis	• Enhanced pharmacovigilance o Weekly signal detection o Use of targeted follow-up questionnaires o Individual reports expedited to regulators o Included in Table 3 of simplified PSURs o Cumulative analysis included in full PSUR following end of pandemic period o Ad hoc analyses if reporting rate exceeds 2/100,000 doses distributed • Incidence will be estimated in participants of the post-authorisation safety study	NA
Vaccination failure	• Enhanced pharmacovigilance o Weekly signal detection o Use of targeted follow-up questionnaires o Individual reports expedited to regulators o Included in Table 3 of simplified PSURs o Cumulative analysis included in full PSUR following end of pandemic period • Incidence will be estimated in participants of the post-authorisation safety study	NA
Vaccine effectiveness	GSK Biologicals will obtain data from ECDC vaccine effectiveness studies	NA
Fever in children	• Additional clinical trial (Q-Pan-021) • Routine pharmacovigilance • Cumulative analysis in full PSUR prepared after the pandemic period	Safety data indicating higher incidence of fever in the undesirable effects section of the proposed labelling
Missing data in pregnant women	Routine pharmacovigilance, including follow-up of cases of pregnancy: • spontaneously reported by patients and HCPs • enrolled/observed during post-authorisation safety study • observed during clinical trials • reported via Pregnancy Registry	NA

Potential theoretical safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
Missing data in children	• Clinical trial Q-Pan-021 • Post-authorisation safety study (depending on vaccination policy)	Statement in proposed labelling that there is limited experience in children
Limited data in subjects with compensated underlying conditions; No data in subjects with severe underlying medical conditions and immunocompromised	• Routine pharmacovigilance • Post-authorisation cohort study: individuals will be included based on national recommendations, underlying medical conditions will be documented for *post hoc* analyses	NA

† PSUR = periodic safety update report; * NA = not applicable

User consultation

A justification for not performing a full user consultation with target patient groups on the package leaflet (PL) has been submitted by the applicant and has been found acceptable for the following reasons:

The applicant submitted:

- A full report on User Testing (UT) for the Arepanrix PL (i.e. Q-Pan H1N1v) and
- A "bridging report" for Pumarix PL.

The applicant justifies the lack of specific User Testing with Pumarix PL based on the fact that EU guidance states that where sufficient user testing has already been performed on products identified as being similar or related the results of UT can be applied across related leaflets to fulfil Council Directive 2004/27/EC, Article 59 (3). Thus EU guidance on bridging permits the use of successful User Tests carried out for one or more leaflets (parent leaflets) as justification for not testing similar leaflets (daughter leaflets) provided that the daughter leaflets are of sufficiently similar content, design, layout and writing style as the parent leaflet.

2.8. Benefit-Risk Balance

Benefits

- Beneficial effects

The manufacture of the H5N1 antigen, the H5N1 formulated vial and the ASO3 (adjuvant) vial are appropriately controlled. Adequate in-process controls, release and shelf life specifications have been set in line with relevant requirements and satisfactorily demonstrate the quality of the vaccine. Commitments are made by the applicant to update some information when available, which does not impact on the risk/benefit assessment of this vaccine.

The proposed formulation of Pumarix has been shown to elicit HI and NA responses to the homologous vaccine strain and to related (clade 2) virus. The data suggest that two doses of vaccine administered at least 21 days apart is likely to be a satisfactory regimen in adults.

- Uncertainty in the knowledge about the beneficial effects.

The actual protection that Pumarix might provide for a future pandemic influenza strain cannot be predicted from these HI and NA data. In addition, it cannot be ruled out that a lower dose of HA and/or a lower dose of AS03 might be just as satisfactory at least in some sub-groups (e.g. healthy adults aged < 40 years). The large difference in pre-vaccination seropositivity rates between HI and NA assays has been noted previously in H5N1 vaccine studies when testing against A/Vietnam and A/Indonesia but the explanation remains unclear and most likely reflects a combination of factors, e.g. assay variability, - sensitivity and - standardisation.

There are no data with Pumarix in children or in immunosuppressed subjects. The D-Pan H5N1 data available in children aged 3-9 years should be applicable to use of Q-Pan H5N1 in this age group and it is reasonable to suppose that the adult dose regimen would be applicable to those aged 10-17 years. However, it is not possible to predict whether half the adult dose would suffice in children aged < 3 years.

Risks

- Unfavourable effects

The effect of combining H5N1 HA with AS03 on rates of reported local and general symptoms is now very well documented. The substantial safety database available with Pumarix in adults together with the substantial experience now available with AS03-adjuvanted influenza vaccines demonstrates the reactogenicity related to the adjuvant but this is not of a degree that would preclude its use. Halving the AS03 per dose gave lower rates of pain but effects on general symptoms were variable with no consistent trend to lower rates with half the adjuvant.

- Uncertainty in the knowledge about the unfavourable effects.

The safety of D-Pan H5N1 in children aged 3-9 years may be extrapolated to use of Pumarix in this same age range and safety in children aged 10-17 years is likely to be comparable to that in adults. However, safety of full or half adult doses in children aged < 3 years cannot be predicted. Based on the recent experience with second doses of AS03-adjuvanted H1N1v vaccine in this age group there is a potential concern especially regarding the rates of fevers that might be observed.

Benefit-Risk Balance

- Importance of favourable and unfavourable effects

This core dossier is based on a vaccine containing a strain of influenza A/H5N1 with low pre-vaccination HI seropositivity but higher pre-vaccination NA seropositivity. The immune responses vary between baseline seropositive and seronegative subsets. However, there will be no pre-vaccination testing in a pandemic situation and it is important to provide a regimen that will trigger high immune responses even in those who are seronegative. In addition, to use a formulation and regimen that provide some degree of persisting and cross-reacting immune responses without eliciting unacceptable reactogenicity. For the paediatric population, data based on Pumarix (i.e. Q-Pan H5N1 vaccine) are awaited. However as the extrapolation of paediatric efficacy and safety data between D-Pan H5N1 and Q-Pan H5N1 vaccines was considered acceptable the data from the paediatric study D-Pan H5N1-009 can be taken into account for the benefit-risk balance in this population.

2.8.1. Conclusion on the benefit-risk balance

The overall benefit-risk of Pumarix is considered to be positive.

Risk management plan

A risk management plan was submitted, version 2 June 2010. The CHMP, having considered the data submitted, was of the opinion that:
- Standard Pharmacovigilance activities will be conducted in pre-pandemic phases. However, in a pandemic situation the MAH agreed to adapt pharmacovigilance activities according to the CHMP recommendations for pharmacovigilance plans for pandemic vaccines (EMEA/359381/2009) and following updates. Modified pharmacovigilance activities in the pandemic period are described in the Risk Management Plan.
- Additional risk minimization activities are proposed to address the following potential risks:
    - medical errors/misidentification of vaccine,
    - contamination of multiple-dose vials,
    - coring of the rubber stopper on the antigen vial

which have not been properly reported in the summary table of the RMP activities. An updated EU-RMP will be submitted at the time of the next submission of PSUR, including the above potential risks in the summary table of the RMP activities and taking into account the classification of safety concerns as important identified risk, important potential risks and missing information (see section 2.7).

2.9. Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Pumarix in the prophylaxis of influenza in an officially declared pandemic situation, in accordance with official guidance, was favourable and therefore recommended the granting of the marketing authorisation under exceptional circumstances.