European Medicines Agency

London, 01 October 2009
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ASSESSMENT REPORT

FOR

Celvapan

Common Name:
Pandemic influenza vaccine (H1N1)[1] (whole virion, Vero cell derived, inactivated)
A/California/07/2009 (H1N1)v

Procedure No. EMEA/H/C/982/PU/02

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

[1] This vaccine was initially developed as a Pandemic Mock-up file using H5N1 as the Pandemic strain.

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PRODUCT INFORMATION

Name of the medicinal product:	Celvapan
Applicant:	Baxter AG Industriestrasse 67 AT-1221 Vienna Austria
Active substance:	Whole virion, inactivated containing antigen*: A/California/07/2009 (H1N1)v * produced in Vero cells
Common Name:	Pandemic influenza vaccine (H1N1) (whole virion, Vero cell derived, inactivated) A/California/07/2009 (H1N1)v
Pharmaco-therapeutic group (ATC Code):	Influenza vaccines (J07BB01)
Therapeutic indication(s):	Prophylaxis of influenza in an officially declared pandemic situation. Pandemic influenza vaccine should be used in accordance with official guidance.
Pharmaceutical form(s):	Suspension for injection
Strength(s):	7.5 microgram haemagglutinin /0.5 ml
Route(s) of administration:	Intramuscular use
Packaging:	vial (glass)
Package size(s):	pack of 20

TABLE OF CONTENTS

1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The Applicant Baxter AG submitted on 30 January 2008 an application for Marketing Authorisation to the European Medicines Agency (EMEA) for Celvapan as an H5N1 mock-up vaccine, through the centralised procedure under Article 3 (2) (a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMEA/CHMP on 20 September 2007.

The legal basis for that application refers to:

A – Centralised / Article 8.3 /New active substance

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

The application submitted is a complete dossier: composed of administrative information, complete quality data, non-clinical and clinical data based on Applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

The CHMP, issued a positive opinion for granting a Marketing Authorisation under exceptional circumstances to Celvapan on 18 December 2008. The commission decision was issued on 4 March 2009.
The Applicant applied for the following indications: Prophylaxis of influenza in an officially declared pandemic situation. Pandemic influenza vaccine should be used in accordance with official guidance.

On 22 September 2009 the Marketing Authorisation Holder applied for a variation according to Article 8 of the Commission Regulation (EC) No. 1085/2003 in order to update to the composition of the strain of Celvapan to those officially recommended by WHO and CHMP for the Pandemic Influenza A (H1N1), and this is the following:

A/California/07/2009 (H1N1)v

Scientific Advice:
The Applicant received Scientific Advice from the CHMP on 19 July 2007. The Scientific Advice pertained to quality and clinical aspects of the dossier.

Licensing status:
The product was not licensed in any country at the time of submission of the initial application.

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

Rapporteur : **Christian K. Schneider** Co-Rapporteur : **Andrea Laslop**

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 30 January 2008.
- The procedure started on 27 February 2008.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 22 May 2008 . The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 16 May 2008. In accordance with Article 6(3) of Regulation (RC) 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 26 June 2008, 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 26 June 2008

- The Applicant submitted the responses to the CHMP consolidated List of Questions on 21 August 2008.
- The GCP inspection, requested by the CHMP, was carried out at two investigator sites in Austria (inspected 9-13 Jun and 30 Jun - 4th Jul 2008) and at the sponsor site in Austria (inspected 1-3 Sep 2008). The final Integrated Inspection report was issued on 17 October 2008.
- The Rapporteurs circulated the Joint Assessment Report on the Applicant's responses to the List of Questions to all CHMP members on 14 October 2008.
- During the CHMP meeting on 23 October 2008, 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 19 November 2008.
- The Rapporteurs circulated the Joint Assessment Report on the Applicant's responses to the List of Outstanding Issue to all CHMP members on 1 December 2008.
- During the meeting on 15-18 December 2008, 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 Celvapan on 18 December 2008. The Applicant provided the letter of undertaking on the specific obligations and follow-up measures to be fulfilled post-authorisation on 17 December 2008.
- On 23 July 2009, the CHMP adopted a positive Opinion on a type II variation (II-01) to update section 4.6 and 5.3 of the summary of product characteristics (SPC) to include results of two reproductive and developmental toxicology studies in the rat. The wording in annex II, labelling and package leaflet (PL) was also update to introduce corrections The European Commission adopted a positive Commission Decision for variation II-01 on 27 August 2009.
- On 1 September 2009 an interim Opinion on a rolling review (RR/01) was adopted by the ETF/CHMP to include a revision of the Pharmacovigilance System, Risk Management Plan, Module 3 (drug substance and drug product) and to revise the Product Information in support of a change on the pandemic strain vaccine composition to A/California/7/2009 (H1N1)v.
- On 1 September 2009 an interim Opinion on a rolling review (RR/02) was adopted by the ETF/CHMP to include a comparability exercise between H1N1 and H5N1 vaccine product in support of a change on the pandemic strain vaccine composition to A/California/7/2009 (H1N1)v.
- On 22 September 2009 an interim Opinion on a rolling review (RR/03) has been adopted by the ETF/CHMP to include additional information on the drug substance and drug product to support a change on the pandemic strain vaccine composition to A/California/7/2009 (H1N1)v.
- On 22 September the MAH submitted a variation to introduce the Pandemic strain A/California/7/2009 (H1N1)v (PU-02)
- On 24 September 2009, the CHMP adopted a Request for Supplementary Information (RSI) to be addressed in writing by the MAH, The RSI included also an inspection request.
- The MAH submitted his responses to the RSI on 26 September 2009.
- A product related inspection focused on the issues raised in the RSI of 24 September 2009 took place at the manufacturing site Baxter BioScience s.r.o. - Jevany Bohumil 138 - Kostelec nad Cernymi lesy - Czech Republic on 28 and 29 September 2009.
- The Rapporteur's Assessment Report on the MAH's responses to the RSI was circulated to CHMP on 29 September 2009.
- On 01 October 2009, the CHMP adopted a positive Opinion by written procedure on a variation (PU-02) to change the pandemic strain vaccine composition to A/California/7/2009 (H1N1)v.

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.
In April 2009, a new strain of human influenza A(H1N1)v was identified and characterised. On 11 June 2009 the WHO declared Phase 6 of the influenza pandemic. The declaration reflected sustained transmission of the virus from person to person in several WHO regions. WHO and other international agencies are calling the disease **pandemic (H1N1) 2009**. For the virus the nomenclature **influenza A(H1N1)v** (where v indicates variant) has been chosen.

The attack rate for the (H1N1)v virus strain is expected to be higher than for recently circulating seasonal strains because of the lower levels of pre-existing immunity in the population. Current estimates for the attack rate associated with the influenza A(H1N1)v virus over the first major wave of infection vary from approximately 10-30 % in different geographical areas. As a result, the actual numbers of clinically apparent infections, cases that require hospitalisation and deaths in the pandemic period is expected to be higher than in recent years for seasonal influenza. These estimates may change (upwards or downwards) during the course of the pandemic.

So far in this pandemic there has been a marked under-representation of infections in people over 65 years of age. In Europe, the median age has been 25 years in those who acquired the infection during travel and 13 years in those infected within the EU. Nearly 80% of cases have been in individuals under 30 years of age. Deaths have occurred in previously healthy subjects as well as in those with underlying conditions or pregnancy that would predispose them to complications of influenza. For more information about the known clinical features of the disease caused by influenza A(H1N1)v virus please see the updated Risk Assessment report from ECDC under:
http://ecdc.europa.eu/en/healthtopics/Documents/0908_influenza_AH1N1_Risk_Assessment.pdf

Specific guidance has been developed for the fast track assessment procedure for pandemic influenza vaccines[2], which can only be used once WHO/EU have officially declared the pandemic (WHO Phase 6). 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.

The approval of a core dossier followed by a strain change variation is based on a *Proof of Principle* approach by which safety and immunogenicity data are generated with mock-up vaccines containing subtypes of influenza A to which the majority of the population is naïve. These principles are based on:

- The immune responses to a specific mock-up vaccine containing a strain to which subjects within a specific age range were immunologically naïve is expected to predict responses to the same vaccine construct containing an alternative strain of the same subtype or an alternative subtype of influenza A in a comparable population.
- The safety data generated with a specific mock-up vaccine in clinical studies is expected to predict the safety profile observed with the same vaccine construct containing an alternative strain of the same subtype or an alternative subtype of influenza A in a comparable population.

[2] *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).

On the basis of these assumptions the mock-up/core dossier construct allows for insertion of the pandemic strain into a vaccine construct based on all the data obtained with the corresponding mock-up vaccine together with specific data relating to the pandemic strain. This approach rests on the premise that the final pandemic vaccine is produced in the same way (i.e. with regards to the formulation, manufacturing process and control methods) as approved for the mock-up vaccine. Therefore the strain change variation contains mainly the quality data that are new and relevant for the pandemic influenza vaccine virus.

Baxter AG received a Marketing Authorisation Application (core pandemic dossier) for the mock-up vaccine Celvapan in line with the above mentioned guidelines. The mock-up vaccine is a whole virion inactivated influenza vaccine, which is produced in Vero cells and employing a wild type virus H5N1 strain. The final vaccine comprises 7.5µg of HA antigen of strain A/Vietnam/1203/2004 (or A/Indonesia/05/2005) per 0.5 ml dose and is presented in a 10-dose vial with no preservative added.

On 22 September 2009 Baxter applied for a variation to change the strain used for manufacture of Celvapan to A/California/07/2009 (H1N1)v. The strain used has been officially recommended by WHO and CHMP for the manufacture of vaccines during the current pandemic.

Celvapan is based on a wild type strain and the proposed new strain, A/California/7/2009 (H1N1)v, complies with the WHO[3] and CHMP[4] recommendations for the emergent novel H1N1 influenza vaccine composition. In support of the strain change to A/California/07/2009 (H1N1)v, Baxter submitted quality data in accordance with the quality requirements for a novel influenza H1N1 vaccine, the *Guideline On Dossier Structure And Content For Pandemic Influenza Vaccine Marketing Authorisation* (CPMP/VEG/4717/03 Rev. 1) and *EMEA fast track procedure for community human influenza inactivated vaccines annual strain(s)* (CHMP/BWP/99698/07). The same manufacturing process, with the exception of strain-dependent parameter and safety precautions were applied to the production of H5N1 and H1N1, which includes the release and shelf-life specifications. The MAH provided quality data in support of this variation to demonstrate that the vaccine containing A/California/07/2009 (H1N1)v is comparable from the Quality point of view to the Mock-up containing H5N1 A/Vietnam/1203/2004 (or A/Indonesia/05/2005).

Data from ongoing and planned clinical trials and as specified in the agreed pharmacovigilance/risk management plan using the Celvapan vaccine construct with the influenza A(H1N1)v strain are reviewed on an ongoing basis. These studies will allow to obtain safety, immunogenicity and efficacy data for the influenza A(H1N1)v pandemic vaccine. The first clinical data from the vaccine including the (H1N1)v strain become available end September 2009 as regards safety and immunogenicity will become available in October 2009. The Celvapan SPC summarises the existing clinical data. The Clinical Particulars will be updated as new data are submitted and reviewed..

2.2 Quality aspects

The quality section is divided into two parts of which chapter 3.2.1 describes quality characteristics pertaining to the initial Mock-up vaccine (developed with A/Vietnam/1203/2004 (H5N1) with supporting data from A/Indonesia/05/2005 (H5N1)) and chapter 3.2.2. describes quality characteristics submitted in support of the strain change variation to introduce the new pandemic strain A/California/07/2009 (H1N1)v.

[3] http://www.who.int/csr/resources/publications/swineflu/vaccine_recommendations/en/index.html

[4] EU recommendation for the emergent novel H1N1 influenza vaccine composition (EMEA/CHMP/BWP/3408312009 Rev 1). http://www.emea.europa.eu/pdfs/human/bwp/34083109enrev1.pdf

2.2.1 Mock-up vaccine (A/Vietnam/1203/2004 (H5N1) with supporting data from A/Indonesia/05/2005 (H5N1))

Celvapan is a Vero cell-derived, monovalent, whole virion, inactivated vaccine containing 7.5 µg/dose of Haemagglutinin (HA). The whole virions of Influenza type A as the active ingredient is inactivated both by formaldehyde and UV-irradiation and purified on a sucrose density gradient. The present core pandemic dossier describes a mock-up vaccine derived from the reference virus A/Vietnam/1203/2004 (H5N1) with supporting data from A/Indonesia/05/2005 (H5N1).

The production process of the pandemic influenza vaccine is based on previous experience with Baxter's interpandemic influenza process. The Active Substance is the Vero cell-derived, formaldehyde- and UV-inactivated and sucrose gradient purified whole virions of influenza virus. The finished product is a suspension for injection presented in a multidose formulation with no preservative added.

For details on the composition of Celvapan please refer to Table 1.

Table 1. Composition of Celvapan

	Name of Ingredients		Content (per 0.5 mL dose)	Function	Monograph
Active Ingredient	Whole virion, inactivated containing antigen*: A/Vietnam/1203/2004 (H5N1) * produced in Vero cells		7.5 µg Haemagglutinin (HA), lower limit of confidence interval (μ=95) ≥ 6µg HA	Active Antigen Substance	Ph. Eur. 2308
Excipients	Tween 80		0.10-0.15 % (target 0.125 % i.e. 0.63 mg/dose)	Prevention of micro-aggregation	Ph.Eur. 0428, USP
	Tris-buffered Saline	NaCl	4.4 mg	Electrolyte	Ph.Eur. 0193, USP
		Tris (Trometamol)	1.2 mg	Buffer Substance	Ph.Eur. 1053, USP
	Water for Injection		filled to 0.5 mL	Solvent	Ph.Eur. 0169, USP

Active Substance

The Active Substance is an aqueous solution containing Vero cell-derived, formaldehyde- and UV-inactivated, and sucrose gradient purified whole virions of influenza virus. Additional components of the Active Substance are Tween 80, Sodium Chloride and Tris-buffer (TBS, containing Trometamol). The strain change variation to replace H5N1 with H1N1 virus antigen does not affect the quality of the active substance. For further information on the quality data submitted in support of the strain change variation see the section on Pandemic Strain Variation below.

- Manufacturer

All manufacturing steps of Celvapan are performed in Baxter facilities under Good Manufacturing Practice (GMP) conditions. The involved facilities Baxter AG in Orth/Donau- Austria and Baxter BioScience s.r.o. in Kostelec nad Cernymi lesy - Czech Republic hold current GMP certificates and valid Manufacturing Authorisations. The specific development work was performed with H5N1 strain A/Vietnam/1203/2004 and A/Indonesia/05/2005.

The production process using the Vero cell technology can be divided into four main stages:

- Vero Cell Propagation
- Virus Propagation and Harvesting
- Inactivation
- Purification and sterile Filtration

In the upstream processing, cells are produced and then infected with the respective influenza virus (i.e. H5N1). Then the virus is harvested and inactivated by sequential formaldehyde and Ultraviolet Irradiation (UV) inactivation steps. Two separate inactivation steps were designed for two separate targets i.e. primarily protein for formaldehyde and nucleic acid as a target for UV irradiation. In Purification I, the product is concentrated and purified using ultra-centrifugation with a sucrose gradient. During Purification II, the product is homogenized and sucrose and further impurities are removed by ultrafiltration. The final stage of Active Substance manufacture is the sterile filtration of the Monovalent Bulk.

- Control of Materials

The following starting materials used in the production of monovalent bulk are of biological origin: Vero cell line used in the production of viral antigens and Influenza virus seed. The H5N1 working seed is derived from the Strain A/Vietnam/1203/2004 and Strain A/Indonesia/05/2005.

The different Vero cell populations Master Cell Bank (MCB), Working Cell Bank (WCB) and Post Production Cell Bank (PPCB) were tested for characterisation and safety according to Ph. Eur. 5.2.3. including DNA fingerprinting on MCB, WCB, and PPCB. Mycoplasma testing by indicator DNA fluorochrome test or by cultivation assay. Morphology examination, extraneous agents testing and tests for bacterial and fungal contamination and retroviruses. In conclusion the testing panel on the cell bank system provide assurance that the cell banks can be considered free of extraneous agents according to Ph. Eur. 5.2.3.

Extraneous Agents were evaluated *in vitro* and *in vivo*. *In vitro* testing of the neutralized Vietnam strain Production Virus Banks, both from the Orth and Bohumil facility, confirmed the absence of extraneous agents in the Production Virus Banks.

Additionally the Applicant studied the evaluation of feasibility to completely neutralize H5N1 for the purpose of extraneous agents *in vivo* testing on the Production Virus Banks of the Vietnam strain, as sufficient neutralisation of the virus banks is a prerequisite for the performance of the *in vivo* testing. The neutralized samples were inoculated into appropriate numbers of adult mice suckling and guinea pigs as per Ph. Eur. 2.6.16. Animals were observed for the requested time period for sign of disease or death. The studies in suckling mice, guinea pigs and adult mice were considered to have been completed successfully in compliance with Ph.Eur. 2.6.16. In addition the extraneous agents test program for virus banks of a future pandemic strain will be revised to be fully in line with Ph. Eur 2308. In conclusion sufficient data on extraneous agents testing *in vitro* and *in vivo* as well as by polymerase chain reaction (PCR) have been generated to demonstrate absence of extraneous agents.

The excipients of animal origin, Trypsin and Cytodex, are used in the production of the Active Substance.
The two animal components and the manufacturing process itself (including media used in equipment with direct contact with the product) have been evaluated according to the relevant guidelines and found to present no risk of TSE transmission. Biological reagents involved in routine manufacture of the active substance do not contain components of bovine origin.

- Process validation

Production of the Active Substance starts with the Vero Cell Inoculum and the Production Virus Bank. Quality control testing is performed on intermediate products at the following steps:

- Vero cell culture in Fermenter step 3 prior to infection
- Fermentation Broth
- Formaldehyde Treated Virus Harvest
- Purified Monovalent Virus Harvest (PMVH) as the result of Purification I

Critical steps in the production of the Active Substance are those associated with viral safety and sterility. These include tests for inactivation with formaldehyde, inactivation by UV light, control of total inactivation process and sterile filtration, which has been tested through filtration contact time, filter integrity and sterility according to Ph.Eur.

Validation studies for Celvapan were based on the H5N1 Influenza strains A/Vietnam/1203/2004 and A/Indonesia/05/2005. The validation of Active Substance manufacture has been carried out with the Vietnam/1203/2004 strain. The occurrence of human infections with Clade 2 H5N1 influenza strains in Indonesia, and the high mortality rate (56 %) associated with these infections, has prompted Baxter to also produce a whole virus H5N1 influenza candidate vaccine based on the Clade 2 A/Indonesia/05/2005 strain for a clinical Phase 1 study, which was used to validate the formulation and filling process steps.

The validation of WCB production was performed retrospectively on all relevant WCBs produced in the last years at the Orth/Austria facility. The WCB lots listed in the dossier were used for production of material for clinical trials of several investigational products, e.g. pandemic and interpandemic influenza, SARS and Ross River vaccine. In conclusion, sufficient information has been provided regarding the specific WCB(s) used for production of Celvapan clinical trial material and conformance lots. All tests according to Ph. Eur. 2308 and 5.2.3 have been conducted and were included in the specification for production of future Working Cell Banks.

Process validation of the Vero Cell Inoculum in Bohumil included twelve consecutive lots. The conformity of the cell propagation from 120 L up to 6000 L bioreactors was tested on three consecutive lots for the purpose of the Process Validation of the Cell Propagation at different stages of Fermentation. These results demonstrated that different lots used for both the vero cell inoculation and fermentation process were found to be comparable.

The strain used for process validation covering virus propagation, harvest and inactivation was A/Vietnam/1203/2004 (Clade 1). Three conformance lots were produced in the Bohumil facility and the results confirmed the consistency of the manufacturing process. During the process validation for Celvapan production, it was verified that the manufacturing process of the virus propagation, harvest and inactivation, purification and transport conforms to the process validation protocols.

In conclusion, the data generated during process validation at both facilities Orth/Austria and Bohumil/Czech Republic demonstrated a consistent manufacturing process.

- Characterisation and Specification

The biological, immunological, genetic and physicochemical characterisation included a comparison between egg-derived and vero-cell derived influenza virus seeds.

The biological characterisation of the inactivated whole virus vaccine Active Substance was carried out by determining the haemagglutination (HA) titre and the infectious titre. For this purpose the egg infectious dose 50 (EID50/mL) as well as the plaque forming units (pfu/mL) were determined. Additionally the Applicant also detected the neuraminidase (NA) activity. The Applicant tested whether egg-derived influenza virus vaccine strains would differ from the vero cell derived ones with respect to their biological characterisation, however, no significant differences could be detected.

The genetic stability of the influenza virus grown in Vero cells versus egg derived virus was evaluated by comparing the genetic sequence of the Haemagglutinin gene sequence of an egg-derived Seed Virus Bank to that of a Post Production Virus developed in Vero cells. The egg-derived Seed Virus Bank and the Vero-derived post production virus preparations were identical on the DNA and on the amino acid level, demonstrating that once a recommended vaccine strain has been adapted to sufficient growth in eggs, no re-adaptation during the passages in serum free Vero cells occurs.

Immunological characterization was carried out on the egg derived and vero derived by haemagglutination inhibition (HI) assay, neuraminidase inhibition (NAI) assay and Western blot analysis. Further immunological characterization was done by infection and immunization studies in mice with egg-derived and Vero-derived viruses and vaccines. Additionally, a challenge experiment was carried out in ferrets. There were generally no significant differences in hemagglutination inhibition assay (HI) titres between any of the samples from any season, egg-derived or Vero cell-derived. These results demonstrate that passages of egg-derived influenza virus on Vero cells do not change their antigenicity.

The physicochemical characterization was carried out by Coomassie staining of the viral proteins, separated by polyacrylamide gel electrophoresis (PAGE). The protein compositions of the Vero cell-derived influenza virus MVBs were comparable to those of the egg-derived MVBs or standard antigen reagents.

The following product- and process-related impurities have been identified during the Active Substance manufacturing process and are routinely tested for during the process: Vero Cell DNA during Manufacturing of Monovalent Bulk (MVB); Residual Vero Cell DNA in the Monovalent Bulk; Vero Host Cell Protein; residuals of formaldehyde, sucrose, trypsin and benzonase.

The agreed specifications for the monovalent bulk include a test for vero cell protein via ELISA, the Haemagglutinin assay and single radial immunodiffusion (SRD) test for HA protein, the Bradford Method for total protein, the Haemagglutination inhibition (HI) test, H5N1 identity test using RT PCR, a safety test for preparative influenza virus on Vero cells, a test for Tween 80 concentration via photometric detection, the LAL test for bacterial endotoxin and a sterility test.

The specifications of the monovalent bulk have been sufficiently justified and are considered adequate.

- Stability

Stability test results of up to 12 month on 4 lots of Purified Monovalent Virus Harvest and 5 lots of monovalent bulk have been provided. An apparent decrease in protein concentration measured by the Bradford method was observed after 9 month with all MVBs produced to date. Therefore the shelf life of the monovalent bulk has been set at 6 month. The Applicant committed to provide the outcome of the his investigations regarding the decrease of total protein in the MVB and further results of stability studies on Monovalent Bulk as a follow up measure as soon as they become available.

Medicinal Product

The strain change variation to replace H5N1 with H1N1 virus antigen does not affect the quality of the finished product. For further information on the quality data submitted in support of the strain change variation see the section on Pandemic Strain Variation below.

- Pharmaceutical Development

Celvapan finished product contains the formalin- and UV-inactivated, purified whole virion in a formulation of 7.5 µg HA/0.5 mL dose without adjuvant. The product is presented in a 10 mL glass vial of hydrolytic type I. The filling volume corresponds to a content of 10 doses with 0.5 mL. The stopper consists of latex-free halogen-butyl rubber and is qualified by the supplier to be penetrated up to ten times. Overfilling of the vials by 0.85 mL minimum ensures that the nominal amount of product

doses (10 doses per vial) can be drawn from the vial. Therefore, the 10 dose vial contains at least 5.85 mL of Medicinal product solution.

The Applicant's pharmaceutical development was based on experience with various influenza strains, which have shown that individual strains exhibit different aggregation behaviour which results in losses during sterile filtration. Therefore, prior to sterile filtration a homogenization step is performed in the course of the Purification process. No additives or preservatives are added, except for Tween 80, which prevents re-aggregation of the virions. The excipients Tris-buffered saline (TBS containing Tris (Trometamol) and Sodium Chloride, Tris (Trometamol, 20 mM) as buffer, NaCl (137 mM) as electrolyte and Tween 80 detergent are used for the finished product (see Table 1).

The most critical aspect of formulation and filling is to maintain sterility of the Medicinal product as the sterile filtration is performed at the final stages of Active Substance preparation. All added buffer solutions are sterile filtered directly prior to introduction into the formulation system. Primary container components are sterilized and the vials depyrogenized before filling. The second critical aspect is the homogeneity of the product throughout the filling process. This is guaranteed by continuous stirring of the formulation vessel.

Formulation and filling steps are performed according to established and validated procedures. The Bulk Medicinal product is prepared in a closed production system that assures aseptic working conditions. The Bulk Medicinal product is filled clean room Class A conditions according to EU cGMP Guide, in multi dose vials and the vials are stoppered and crimped under class A conditions to give the Final Container Product. All components of the final container that come into contact with the product comply with the respective requirements in USP, Ph. Eur. and ISO standard specifications concerning containers for injectables.

The components of the Medicinal product have been adequately described and justified. No novel or unusual excipients are used and the formulation development is supported by clinical development. The manufacturing process complies with standard formulation and filling procedures used for inactivated viral vaccines.

- Adventitious Agents

No materials of animal origin are added to the Active Substance in the manufacture of the finished product. Only the excipients Tris-buffered saline and Sodium Chloride and Tween 80 are used for the finished product. The excipients used are tested for sterility using membrane filtration, bacterial endotoxins using the LAL test, pH, conductivity and Tween 80 content. The analytical methods are performed according to Ph. Eur. where applicable and are validated according to ICH guidelines.

The two excipients of animal origin, Trypsin and Cytodex, used in the production of the Active Substance have been evaluated and found to present no risk of TSE transmission. No biological reagents involved in routine manufacture of the active substance contain any components of bovine origin. Overall, sufficient data is provided to exclude a risk of TSE transmission through Celvapan. The risk of transmitting TSE by Celvapan is thus considered very remote.

- Manufacture of the Product

Sterile Monovalent Bulks (MVB) are transported at +2 to +8 °C from the Bohumil facility in the Czech Republic to Vienna/Austria for formulation. Tris-buffer and Tween 80 solution are delivered from the Orth/Austria facility to Lange Allee 51. The Bulk Medicinal product is prepared in a closed production system, which has been validated by media runs. The calculated amount of Tween 80 solution and Tris-Buffer are sterile filtered into the formulation tank. No preservatives are added. The mobile tank is stored in a cold storage at 2-8 °C until filling. The Bulk Medicinal product is filled under clean room Class A conditions (EU cGMP Guide) in multi dose vials and the vials are stoppered and crimped under class A conditions to give the Final Container Product. All components of the final container that come into contact with the product comply with the respective requirements in Ph. Eur., USP, and ISO standard specifications concerning containers for injectables. Visual inspection is

generally performed together in one step with labelling and packaging. No reprocessing is performed or foreseen in the course of the production of the Medicinal product.

- Product Specification

The quality control program performed on the Bulk Medicinal product for Celvapan include the single radial haemolysis (SRH) Assay for quantification of haemagglutinin (HA), the Bradford assay to determine total protein, a PCR test for detection of residual Vero cell DNA, an ELISA test for residual benzonase as well as tests for Tween 80 concentration, sucrose, formaldehyde, ph and sterility. Quality control testing performed on Final Container Product consists of SRH Assay for quantification of haemagglutinin (HA), extractable volume, ph, bacterial endotoxin using the LAL test and sterility. All analytical methods are performed according to Ph. Eur. where applicable and are validated according to ICH guidelines.

To overcome a possible limitations of availability of SRD reagents during a pandemic situation, the Applicant developed an alternative haemagglutinin (HA) quantification method based on HPLC determination of the HA-1 subunit of the HA protein. The value determined with this HPLC testing is compared to results of Influenza strains where SRD reagents are available. The acceptability of the alternative HPLC method was subject of a Scientific Advice and was assessed to be acceptable. The Applicant has committed to complete the validation and implementation of this method in follow-up measures.

Compliance with the product specifications has been shown on three conformance lots each, the A/Vietnam/1203/2004 and the A/Indonesia/05/2005 strain. The provided data is considered acceptable.

- Stability of the Product

The stability indicating parameters cover identity, potency and purity as well as general quality and safety parameters. The specifications used in the stability studies and the end of shelf life specifications, are identical with the acceptance criteria defined in the release specification for the respective production stage. Stability studies are performed using the actual final container (10 dose vials), except for the studies performed on clinical Phase 1/2 material, which was filled in single-dose syringes of the same glass material.

Based on the data currently available on the Pandemic Influenza Vaccine for Clinical Phase 1/2, Phase 3 and Conformance Batches and taking the experience with several inter-pandemic Vero cell derived Influenza Vaccine lots into consideration a shelf life of 12 months for the Medicinal product was accepted. To investigate the source of an apparent upward trend of the HA content detected in the SRD assay stability of the H5N1 vaccine will be further addressed in a follow up measure.

The open shelf life following the first withdrawal of a dose is the following: “vial to be used within one vaccination session or within 3 hours, whichever is less”

2.2.2 Pandemic Strain Variation (A/California/7/2009 (H1N1)v)

With regard to the quality requirements for a novel influenza H1N1 vaccine, the *Guideline On Dossier Structure And Content For Pandemic Influenza Vaccine Marketing Authorisation* (CPMP/VEG/4717/03 Rev. 1) and *EMEA fast track procedure for community human influenza inactivated vaccines annual strain(s)* (CHMP/BWP/99698/07) are applicable. The same quality requirements and safety precautions apply to production of H5N1 and H1N1.

The proposed influenza strain for Celvapan is: A/California/7/2009 (H1N1)v. This vaccine strain is a wild type strain and complies with the WHO[5] and CHMP[6] recommendations for the emergent novel H1N1 influenza vaccine composition and therefore is accepted.

[5] http://www.who.int/csr/resources/publications/swineflu/vaccine_recommendations/en/index.html

The MAH provided quality data in support of this variation to ensure that the manufacture of the drug substance and drug product are appropriately controlled. Adequate release and shelf-life specifications have been set.

Drug Substance

Information is presented on the source and passage level history of the primary seed virus as well as on the preparation and qualification of the working seed virus lots for the strain.

Process validation data on virus inactivation have been submitted for three consecutive pilot scale batches. The absence of validation data from commercial scale production batches on virus inactivation by formaldehyde was raised as a major objection, which remained unresolved until the last round of responses. The major objection has eventually been resolved following the assessment of the MAH's responses on 15 September 2009. The scientific justification a well as the chronological events that led to resolving this major objection is detailed in the discussion below. Overall, the validation data demonstrated the high capacity of the process in place to effectively inactivate H1N1 virus within a short time frame. Hence it can be concluded that a sufficient safety margin exists for vaccine produced at industrial scale. In addition the MAH is committed to provide additional inactivation data on three industrial scale batches to confirm these results.
An overview of commercial batches of drug substance produced until 23 September 2009 revealed that bacterial contamination of cell culture medium was detected in 3 out of 16 produced fermentation batches. The three contaminations occurred in a row due to the fact that the root cause could not be identified immediately and because of the fact that nonetheless production was continued to meet urgent product requests. Contaminated batches have not been released from quarantine. Major concerns were raised in a Request for Supplementary Information in this respect on 24 September 2009. In his responses, Baxter provided data to demonstrate that the root cause for these bacterial contaminations had been identified and corrective measures were put into place preventing future contaminations.. This was further demonstrated by raw data from an additional 11 batches of manufactured drug substance, which was contamination free. Following the assessment of these data by the Rapporteur, the CHMP considered that the major objections and other concerns raised in the Request of Supplementary Information were resolved. A product related inspection on the issues was raised in the RSI of 24 September 2009, which took place at the Baxter BioScience manufacturing site (Baxter BioScience s.r.o. - Jevanská Bohumil 138 - Kostelec nad Cernymi lesy - Czech Republic), confirmed the conclusions that the root cause for bacterial contamination during fermentation was identified and corrective measures were effective. The manufacturing site was confirmed to be compliant with GMP.

Due to the general growth characteristics of the A/California/07/2009 (H1N1)v virus strain the specification for HA content of the drug substance has been adjusted to be not less than 40 µg/mL. Other approved specifications for the drug substance have not been changed.

Qualification studies for the SRD and HPLC assay were performed for the new virus strain. Sufficient assay qualification data is provided to assure acceptable performance of SRD assay to quantify HA content in the monovalent bulk and as such also of the drug product. The MAH commits to provide SRD validation data as follow-up measure. A comparability analysis on the physico-chemical level confirms the equivalence of the H1N1 based vaccine to the H5N1 based vaccine.

Batch analysis results of monovalent bulk were provided. All batches have been tested according to the approved specifications. The results of the production batch analysis demonstrate the reproducibility of the manufacturing process for the drug substance.

[6] EU recommendation for the emergent novel H1N1 influenza vaccine composition (EMEA/CHMP/BWP/3408312009 Rev 1). http://www.emea.europa.eu/pdfs/human/bwp/34083109enrev1.pdf

Stability of drug substance

The following maximum storage durations are set for the H1N1 Pandemic Vaccine in line with the Celvapan license based on the data currently available on the H5N1 Pandemic Influenza Vaccine for Clinical Batches and Conformance Batches and taking into consideration the experience with seasonal Vero cell derived influenza vaccine:

- PMVH: 2 years at ≤ -60 °C
- MVB: 6 months at 2 – 8 °C

Stability studies on the PMVH and Drug Substance (MVB) of the H1N1 Influenza Vaccine conformance/clinical lots have been initiated in July/August 2009 according to the stability program approved for Celvapan.

The company commits to report any unexpected results generated during the ongoing stabilities studies, in case of a confirmed out-of-specification or unexpected trend not supporting the registered shelf-life.

Drug Product

Each 0.5 ml dose of vaccine has the following composition:

Active Ingredient:
The quantitative composition of the drug product remains unchanged.

Whole virion, inactivated containing antigen*:

A/California/07/2009 (H1N1)v 7.5 micrograms** per 0.5 ml dose

* propagated in Vero cells (continuous cell line of mammalian origin)
** expressed in micrograms haemagglutinin

Other Ingredients:
Tween 80
Tris-buffered saline
Water for injections

The formulation contains no adjuvant.

Neither the approved specifications nor the analytical methodology for final bulk and final container drug product have been changed. Quality control release data for Celvapan A/California/7/2009 (H1N1)v final bulk and finished product presented conform to the specifications approved for the antigen component of Celvapan H5N1.

Batch analysis results for four final bulk and four final containers lots produced at manufacturing scale were provided to confirm consistent production. All batches have been tested according to the approved specifications.

Stability of drug product

The shelf life of 12 months when stored at 2-8°C for the final container product as claimed by the Applicant is considered acceptable. Stability evaluation for final lot container material is currently ongoing and the company has committed to provide these additional stability results when available.

Kinetics of the viral inactivation

The viral inactivation processes were reviewed by the CHMP.

With regard to whole H1N1v inactivation data which have been provided to date including

- o virus inactivation validation data by formaldehyde treatment and UV irradiation on 100 L scale including formaldehyde kinetics
- o virus inactivation data by UV irradiation only on research scale including UV kinetics
- o virus validation report (validation of technical parameters during inactivation (i.e. temperature, formaldehyde, titre) on 10 consecutive commercial batches including safety data (virus inactivation test in Vero cells and chicken embryo fibroblasts as part of the release specifications) from the PMVH. Virus inactivation kinetics, however, were not preformed on commercial batches.
- o supportive information including an inactivation study using inactivation conditions applied for seasonal Influenza strains and preliminary day 7 safety data on 100 subjects

it is sufficiently ensured that an acceptable safety margin is obtained after formaldehyde treatment and UV irradiation for the vaccine produced at industrial scale. Nevertheless, the MAH has committed to provide additional inactivation data in order to verify the results from small scale validation runs as done for H5N1 previously.

Conclusion

Overall, the information presented in Modules 2.3 and 3 was considered in accordance with the above-mentioned guidelines and was therefore acceptable.

Minor issues have been identified and remain unresolved, which do not impact on the overall benefit-risk of the product. The Applicant has committed to resolve these issues through follow-up measures.

2.3 Non-clinical aspects

Introduction

A preliminary challenge experiment without testing the vaccine has been conducted, and only limited non-clinical data with Celvapan has been generated with vaccine materials that contain the pandemic Influenza A (H1N1) strain, whereas most of the non-clinical data was generated with vaccine made of an influenza A (H5N1) strain.
Pharmacology studies evaluated both the immunogenicity and protective efficacy of the vaccines in small animals. Mice subcutaneously (s.c.) immunized with the A/Vietnam/1203/2004 candidate vaccines developed anti-H5 HA-specific antibodies as well as functional antibodies (HI and/or microneutralization (MN) titers), and survived the challenge with homologous or heterologous (clade 2.1 A/Indonesia/05/2005 or clade 3 A/HongKong/156/1997) strains. The vaccines were also demonstrated to be immunogenic in rats and guinea pigs in terms of all three serological tests (H5 specific binding ELISA, HI and MN assay). Immune antisera raised against non-GMP research material in guinea pigs cross-neutralized an array of heterologous H5N1 strains (3x Clade 1, 1x Clade 2.2, 2x Clade 2.2, 1x Clade 3, and H5N3) in vitro. Further supportive data on the immunogenicity and (cross-)protective efficacy were generated in small animals (mice, guinea pigs: s.c., preclinical materials) with the A/Indonesia/05/2005 H5N1 candidate vaccines.

Pharmacology

- Primary Pharmacodynamics

Non-clinical studies with H5N1 antigen:

Two ferret challenge studies demonstrated protective efficacy against a homologous challenge with 2.1 x 10^6 $TCID_{50}$ in the ferrets previously immunised using a clinical lot of H5N1 vaccine prepared from strain A/Vietnam (Lot VNV1G001A, 7.5µg HA) and using the intended route and time interval. Whereas all animals in the control group receiving buffer died 4 to 7 days after administration of the challenge dose, 100% of ferrets in the vaccine group survived challenge. Data on virus recovery from post-mortem tissues confirmed that every animal in the control and vaccine group demonstrated some level of virus replication either in nasal wash or in one or more tissues. At moribund sacrifice, all animals of the control cohort except one had high titres of virus in the lungs (between 3.8 to 6.4 logs $TCID_{50}$ per gram of tissue), liver (4.3 to 5.9 logs $TCID_{50}$ per gram), brain (2.9 to 4.9 logs $TCID_{50}$ per gram) and olfactory bulb (5.4 to 7.1 logs $TCID_{50}$ per gram). One animal only had virus recovered from the nasal wash and the liver (4.3 logs $TCID_{50}$ per gram) and was found to have an atypical course of infection. The animals of the vaccinated cohort, having all survived to day 14, had for the most part cleared virus from every tissue examined except the liver. There was an absence of detectable virus in the lungs of all but one animal and in the brain of all but two animals. All olfactory bulbs taken from the vaccinated ferrets were negative for virus. The viral titres in the livers of the vaccinated ferrets were lower (between 3.5 to 4.4 logs $TCID_{50}$ per gram) than for the control cohort (4.3 to 5.9 logs $TCID_{50}$ per gram). In general disease symptoms were mitigated in the vaccinated ferrets compared with the control group, i.e. reduced weight loss, a less pronounced and shorter increase in temperature, a less marked reduction in lymphocyte counts and in reduction of necrosis in the brain and olfactory bulb.

Protection against homologous or heterologous challenge was investigated using ferrets immunised with a Clade 2 strain A/Indonesia/05/2005 vaccine. Sixty-six animals were divided into 6 cohorts and received either a dose of 7.5µg HA, 3.75µg HA or buffer on days 0 and 21. Animals were challenged intranasally with either A/Indonesia/05/2005 ($1.0x10^5$ $TCID_{50}$, 1 log lower as targeted) or A/Vietnam/1203/2004 ($1.5x10^6$ $TCID_{50}$) on day 35. Both the high and low doses of A/Indonesia/05/2005 vaccine were shown to be efficacious with 100% survival, reduced incidence of fever, reduced weight loss, reduced virus burden, and reduced haematological changes in the vaccinated cohorts following homologous challenge. However, due to the low challenge dose, 2 out of 8 animals in the control group survived the homologous challenge.
Cross-protection against a heterologous challenge indicated a vaccine dose-dependent survival as compared to the control cohort. All control animals infected with A/Vietnam/1203/04 died between days 3 and 7 following heterologous challenge, while 38% of animals vaccinated with 2 doses of 7.5µg HA and 63% of animals vaccinated with 2 doses of 3.75µg HA died between days 6 and 10. Similarly to the homologous challenge, vaccination reduced virus burden, and reduced haematological changes against a heterologous challenge. Moreover, there is some evidence that survival correlates with absence of viraemia since hepatic inflammatory necrosis was not found in any of the ferrets which survived 14 days post challenge.

Non-clinical studies with (H1N1)v antigen:

In support of the strain change two studies were submitted, PAN0080E01 and PAN0090E01.

PAN0080E01 was a challenge study (non-GLP condition) with the aim to establish a challenge model.

Design:
Three animal species, mice (outbred CD1 and inbred Balb/c), hamsters, and guinea pigs were challenged either intranasally (i.n.), intraperitoneally (i.p.) or intravenously (i.v.) with the wild-type A/California/07/2009 H1N1 strain, at doses ranging from 10^1 to 10^6 TCID50. Challenging experimental conditions were:

- Female mice (6-9 weeks old), anesthetized, i.n. – 20 µl ($2.9x10^4$/dose); i.p. – 100 µl ($1.45x10^5$/dose; i.v. – 100 µl ($1.45x10^5$/dose); control mice received buffer;
- Female hamsters (20 weeks old), anesthetized, i.n. – 50 µl ($4.4x10^4$/dose); i.p. – 1 ml ($8.7x10^5$/dose); control hamsters received buffer;
- Female guinea pigs (6-9 weeks old), anesthetized, i.n. – 50 µl ($4.4x10^4$/dose); i.p. – 1 ml ($8.7x10^5$/dose); control animals received buffer.

Challenged and control animals were observed for disease signs and death over a period of 14 days. On day 14 (+/- 1 day) post inoculation, sera were also collected from mice and guinea pigs to investigate whether A/H1N1 challenge provoked an antibody response.

Results and Discussions:
Irrespective of the route of challenge (i.n., i.p., or i.v.), infection with A/California/07/2009 H1N1 wild-type influenza virus was not lethal for adult CD1 or Balb/c mice.
At a dose of $\geq 10^4$ TCID50, CD1 mice inoculated i.n. showed clinical symptoms. Symptom severity and the number of animals affected appeared to be dependent on challenge dose; In addition, high titer of infectious A/California/07/2009 H1N1 virus were detected on day 4 post inoculation in lungs of all CD1 mice examined which received $\geq 10^3$ TCID50 i.n. challenge. In contrast to the i.n. route, however, the i.p. challenge ($\geq 10^4$ TCID50/dose) only caused mild disease symptoms (i.e. ruffled fur) in some but not all mice. In contrast to outbred CD1 mice, however, inbred Balb/c mice inoculated i.n. with a dose of $\geq 10^4$ TCID50 showed only mild disease symptoms (i.e. ruffled fur) within 7 days after challenge. Moreover, whereas CD1 mice suffered on average for around 7 days, most Balb/c mice had recovered the following day. As expected, all control mice remained healthy and displayed no clinical symptoms during in-life phase of studies.

Irrespective of inoculation route, CD1 mice elicited detectable HI antibodies against A/California/07/2009 H1N1 virus 14 days after challenge. In contrast, i.n. challenge in Balb/c mice failed to provoke a consistent antibody response and HI antibodies undetectable in 4/5 challenged Balb/c mice.

None of the hamsters or guinea pigs died or showed any disease symptoms throughout the 14 day observation period. i.n. inoculation of guinea pigs could elicit relatively low but detectable HI antibodies against A/California/07/2009 H1N1 virus 14 days after challenge, however, i.p. inoculation failed to do so.

In conclusion, CD1 mouse is susceptible to A/California/07/2009 H1N1 influenza virus infection and therefore is a suitable small animal challenge model, whereas Balb/c mice, hamsters and guinea pigs are not suitable as a challenge model for A/California/07/2009 H1N1 influenza virus.
Overall, the preliminary challenge study without testing vaccine demonstrated that the CD1 mice are a suitable model for investigating the protective efficacy of Celvapan in against H1N1 in animal models.

PAN0090E01 was an immunogenicity, dose-finding and adjuvant study (non-GLP), with the aim to evaluate the immunogenicity of Baxter's 100 L GMP monovalent bulk material A/H1N1 vaccine in CD1 mice.

Design:
10 groups of 10 female CD1 mice (6-9 weeks old) were immunized s.c. twice, 3 weeks apart, with one of 6 doses of the vaccine (dose ranges from 0.0012 µg to 3.75 µg HA in 5-fold increments): 6 groups received non-adjuvanted vaccine and the remaining 6 groups received the 0.3% alum-adjuvanted vaccine. For each animal the 1st and 2nd immunizations comprised the same antigen dose and formulation. Negative controls received buffer alone or buffer containing 0.3% alum hydroxide.

Blood samples were taken for serological analyses prior to and 3 weeks after the primary immunization, and both 2 and 3 weeks after the secondary immunization.

Results and Discussions:

Three weeks after the primary, two and three weeks after the secondary immunization, the immunogenicity of Baxter's 100 L GMP monovalent bulk material A/H1N1 candidate vaccine was determined using the HI assay. The results are presented in the tables below.

Immunogenicity of Baxter's 100 l scale non-adjuvanted GMP research monovalent bulk material A/California/07/2009 candidate vaccine in CD1 mice.
HI antibody titres were determined three weeks after the first and two and three weeks after the second immunization.

Vaccine Dose (µg HA)	Adjuvant	HI titre (Week 3) (GMT) *1,2*	HI titre (Week 5) (GMT) *1,2*	HI titre (Week 6) (GMT) *1,2*
3.75	None	98	788	1154
0.750	None	23	299	640
0.150	None	28	260	557
0.030	None	10	53	139
0.006	None	6	12	26
0.0012	None	5	7	7
Buffer	None	5	5	5

1 serum from individual mice was tested and the geometric mean titre (GMT) calculated.
2 for the HI assay, chicken erythrocytes were used.

Immunogenicity of Baxter's 100 l scale alum-adjuvanted GMP research monovalent bulk material A/California/07/2009 candidate vaccine in CD1 mice.
HI antibody titres were determined three weeks after the first and two and three weeks after the second immunization.

Vaccine Dose (µg HA)	Adjuvant	HI titre (Week 3) (GMT) *1,2*	HI titre (Week 5) (GMT) *1,2*	HI titre (Week 6) (GMT) *1,2*
3.75	0.3% alum	35	640	1576
0.750	0.3% alum	197	597	1576
0.150	0.3% alum	8	61	597
0.030	0.3% alum	7	21	80
0.006	0.3% alum	5	9	11
0.0012	0.3% alum	5	5	5
Buffer	0.3% alum	5	5	5

1 serum from individual mice was tested and the geometric mean titre (GMT) calculated.
2 for the HI assay, chicken erythrocytes were used.

It was found that a single immunization (Week 3 data) with the 100 L GMP monovalent bulk material A/H1N1 candidate vaccine provoked antibody responses against A/California/07/2009 influenza virus as measured by the HI assay. A dose-dependent antibody response was provoked by both the non-adjuvanted and adjuvanted candidate vaccines. Furthermore, HI antibodies against A/California/07/2009 influenza virus increased when measured two weeks after the secondary immunization, the effective dose 50% (that is, the dose inducing an HI titre of at least 1:40 in half of the immunized mice) decreasing from 300ng for a single immunization to 13ng for sera collected two weeks after a second immunization (Week 5 data). Interestingly, the HI titre continued to increase when measured one week later (Week 6 data). The effective dose 50% was calculated to be 7ng for sera collected three weeks after a second immunization. These results demonstrate that the 100 L GMP monovalent bulk material A/H1N1 candidate vaccine is immunogenic in CD1 mice.

In conclusion, the MAH's 100 L GMP monovalent bulk material A/California/07/2009 H1N1 candidate vaccine, was demonstrated to be immunogenic in mice both with non-adjuvanted formulation and with adjuvanted formulation containing 0.3% aluminum hydroxide. Furthermore,

based on the ED50 values, it appears that the non-adjuvanted formulation is superior to the adjuvanted formulation.

The initial immunogenicity dose-finding study demonstrated that the MAH's 100 L GMP monovalent bulk material A/California/07/2009 (H1N1)v candidate vaccine, adjuvanted or not with 0.3% aluminum hydroxide, was immunogenic in CD1 mice. A clear dose-dependent antibody response was demonstrated. However, these initial data presented with research material should be regarded as preliminary, and the results generated with the final container product of the A/California/07/2009 H1N1 vaccine are awaited.

- Secondary pharmacodynamics

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

No studies were conducted as no specific concerns in physiological functions are raised.

- Pharmacodynamic drug interactions

No studies were performed.

Pharmacokinetics

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

Toxicology

The non-clinical toxicological testing program comprises a literature-based risk assessment of Tween 80 (Polysorbate 80), a non-GLP rabbit pyrogenicity study, a GLP single-dose toxicity study and a GLP pivotal repeat-dose toxicity study in which local tolerance assessment was included.

- Single-dose toxicity

The GLP single-dose toxicity study assessed the acute toxicity and local tolerance of the candidate vaccine after single intramuscular injection in Wistar rats. In this study, the vaccine used was Pre-clinical 100L GMP material, and both adjuvanted (0.2% alum, 30 µg HA) and non-adjuvanted (45 µg HA) formulations were tested. No treatment-related systemic and local reactions (except the expected microscopical findings at the injection sites) were noted. However, the potency of these preparations in the tested rat strain is not known and the magnitude of immune responses to vaccines after single intramuscular injection was not shown.

- Repeat-dose toxicity (with toxicokinetics)

The repeat-dose toxicity study performed in CD rats was a pivotal GLP study and is considered appropriate for toxicity evaluation (local and systemic). In this study, an appropriate number of animals per sex per group was included and relevant vaccine exposure (clinical lot, intramuscular route, 3x injections at a dose of either 24 µg HA with alum or 36 µg HA without adjuvant) given. The study consisted of a main study arm (32 days) and a 2-week recovery arm (46 days). The induction of relevant, functional immune response was provided by the induction of functional immune response (HI titers, on day 32 and 46). Overall, no treatment-related effects were observed on general conditions, clinical signs (including injection sites), body weight, food consumption, ophthalmology,

urine analysis, haematology, clinical chemistry, bone marrow, gross macroscopical pathology, or organ weight. However, dose-dependent or treatment-related abnormalities in two clinical pathology parameters were noted: one was a slight but statistically significant increase in the liver enzymes (ALT, AST, ALP) and the other is slight but statistically significant decrease in plasma calcium, both occurring in male animals. These changes are small at group mean levels, however, some individual ALT values reached 2-fold increase relative to concurrent controls and many individual plasma calcium values were found out of the range of control values. Whether these variations are within the limits of biological variability of these clinical parameters in the tested animal strain is unknown. Histology analysis (in this study, that is liver on day 46, and parathyroid gland and bone on days 32 and 46) has not been performed.

Also in this pivotal toxicity study it was found that the mean weights of lungs and bronchi (absolute change) were lower and of the thyroids (adapted change) were higher in females treated with non-adjuvanted vaccine in comparison with concurrent control. A relationship of this change with treatment is difficult to determine, because the finding was only observed on one occasion (day 46). The MAH considered the finding to be of doubtful toxicological importance, and justified the statement by providing new histological data for thyroids/parathyroids and lungs and bronchi in the recovery group animals (Day 46). There were no abnormal findings or treatment-related changes in the concerned organ/tissues, and therefore it is considered that the slight changes seen in the weights of these organs were of less toxicological importance.

- Genotoxicity and Carcinogenicity

No studies on genotoxicity and carcinogenicity were conducted with the candidate vaccines.

- Reproduction Toxicity

Two reproductive and developmental toxicity studies have been submitted post authorisation of the initial H5N1 mock-up vaccine. Data was not available at the time of the evaluation of the initial marketing authorisation. This was accepted taking into account the relevant guidelines as there is not a requirement for mock up vaccines to have such data before authorisation. The results of these studies have been reflected in the SPC in variation IB/01.

The same study design was used for both studies.

Two groups of 44 females rats were allocated to each study and treated with a series of 3x i.m. injections (400 µl/occasion) of either inactivated wild type H5N1 influenza vaccine or the Vehicle control (buffer) on Day -42 and Day -14 before pairing and Day 7 after mating. Treated female rats were paired with stock males of the same strain and for each group, 22 females were killed on Day 20 after mating (embryo-fetal phase) and 22 females were allowed to rear their young to Day 21 of age (littering phase). Unselected offspring were killed on Day 21 of lactation and the selected F1 offspring were raised to sexual maturity and killed at seven weeks of age. The F1 offspring received no direct administration of the test substance; any exposure was *in utero* or via the milk.
During the study, clinical condition, dosing observations, bodyweight, food consumption, gestation length, parturition observations and macroscopic pathology investigations were undertaken on the F0 females. Fetuses on the embryo-fetal phase of the study were examined macroscopically at necropsy and subsequently by detailed internal visceral examination or skeletal examination. For offspring on the littering phase of the study, clinical condition, survival, sex ratio, bodyweight, pre-weaning reflex development and macropathology findings were assessed. For selected F1 offspring clinical condition, bodyweight, sensory examinations, sexual maturation, organ weights and macroscopic pathology investigations were undertaken.

In each study, the vaccine dose used was equivalent to 80% of human dose (i.e. 6 µg HA compared to 7.5 µg HA chosen for human use).

Serum samples were obtained from all F0 females on Days -49 and -7 before pairing, from embryo-fetal phase animals at Day 20 of gestation, from fetuses at Day 20 of gestation, from littering phase

females at Day 21 of lactation, from up to 1 male and 1 female offspring in all litters at Day 21 of age and from all selected F1 offspring at either week 6 (BAX0012) or week 7 (EWA0013) of age.

Based on the results of the studies with A/Indonesia/05/2005 and the A/Vietnam/1203/2004 candidate vaccines, it was concluded that treatment of female CD rats with these Influenza vaccines on Days -42 and -14 before pairing and on Day 7 of gestation did not affect mating performance or fertility, embryo-fetal survival or growth or, pre- and post-natal survival and growth of the offspring or, adversely affect the pre- and post-natal development of the offspring up to 7 weeks of age.

The design of the studies was considered adequate with the endpoints appropriately selected and evaluated. The serological responses to the vaccine and exposure of fetuses to specific antibodies were demonstrated. According to the data presented, no vaccine-related harmful effects were seen on mating performance or female fertility, embryo-foetal survival and pre- and post-natal development.

- Local tolerance

See single-dose studies.

- Other toxicity studies

A non-GLP rabbit pyrogenicity study investigated the pyrogenicity characteristics of the H5N1 whole viral candidate vaccine in comparison with a licensed seasonal influenza vaccine, Vaxigrip, as a Standard Reference. In this study, the vaccine formulation used (final container sample) and the vaccine exposure (i.v., 5 human doses) were relevant. Two separate tests (12 rabbits in total) suggested that the candidate vaccine is non-pyrogenic.

Ecotoxicity/environmental risk assessment

No environmental risk assessment is 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.

2.4 Clinical aspects

Introduction

The avian influenza strain H5N1 strain was initially considered as a possible candidate to cause the next influenza pandemic. Therefore the MAH decided to base the mock-up dossier on clinical studies performed (immunogenicity and safety) with vaccine containing the A(H5N1) strain.

Since then, Phase 6 of the influenza pandemic has been declared and the strain A/California/07/2009 (H1N1)v was officially recommended. Clinical data on Celvapan A(H1N1)v are expected in accordance with agreed timelines.

GCP Inspection performed

The clinical trial 810601 was performed in accordance with the quality standards of the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP) and reflected the requirements of the EMEA guidance. Study 810601 was performed in Europe. Written informed consent was obtained from each subject prior to entry into the study.

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).

Pharmacodynamics

The pharmacodynamic principle of vaccines generally could be regarded as the induction of an immune response sufficient to protect from infection with or disease arising from the specific pathogen, the vaccination is directed against. In the context of influenza, surrogate parameters are defined (CPMP/VEG/4717/03) that allow conclusion on the efficacy of the vaccine. Clinical studies performed on Celvapan were designed to obtain information on these specific surrogate parameters and further characteristics of the immune response, i.e. the level and type of specific antibodies elicited the persistence of antibody titres and the investigation of a dose response relationship to define the appropriate dosing recommendation. Thus the immunological response to Celvapan is covered as part of the evaluation of efficacy.

Clinical efficacy

The initial mock-up application was based on two clinical studies 810501 and 810601 that are summarised below. Both studies were multicentre uncontrolled studies. Whereas in study 810501 different vaccines formulations containing H5N1 whole virion inactivated antigen derived from Vero cells were investigated in adults aged 18-45 years study 810601 employed the final formulation in two age groups - healthy adults (18-59 years) and elderly (60 years and older).
For the primary vaccination series H5N1 strain A/Vietnam/1203/2004 was used to prepare the investigational vaccine, whereas for the booster immunizations strain A/Vietnam/1203/2004 (clade1, Month 6 booster), and strain A/Indonesia/05/2005 (clade 2; Month 6, M12, M24 booster) were used to prepare the prototype vaccine. In study 810601 vaccine derived from both strains were administered for the booster immunisations, whereas in study 810703 – the follow-up of subjects enrolled in study 810501 - 7.5µg HA of vaccine prepared from strain A/Indonesia was given as booster immunisation.

Table 1: Summary of Clinical Studies

	810501	**810601**
Design	Phase I/II, randomised, partially blinded, multicentre, dose escalating uncontrolled	Phase III, open-label, multicentre, randomised only for booster vaccination, uncontrolled
Countries and No of study sites	Austria (1 site) and Singapore (2 sites)	Germany (3 sites) and Austria (5 sites)
Sample size and study posology	284 healthy subjects aged 18 to 45 years divided in 6 vaccine groups receiving H5N1 strain A/Vietnam/1203/2004 for primary vaccination series: 7.5µg HA, N = 45 15µg HA, N=45 3.75µg HA+ alum, N = 45 7.5µg HA+ alum, N = 45 15µg HA+ alum, N = 46 30µg HA+ alum, N = 49 2 doses, i.m., 0, 21 days	561 healthy adults (18-59 years; N=280) and elderly subjects (>60 years; N=281) 7.5 µg HA of H5N1 strain A/Vietnam/1203/2004 2 doses i.m., 0, 21 days Booster immunisation at month 6 with either 3.75µg HA or 7.5µg HA prepared from H5N1 strains A/Vietnam/1203/2004 or A/Indonesia/05/2005, respectively Booster immunisation at month 12 to 15 with 3.75µg or 7.5µg HA prepared from H5N1 strain A/Indonesia/05/2005

	810501	810601
		Booster immunisation at month 24 with 3.75µg HA prepared from H5N1 strain A/Indonesia/05/2005
Study Objectives	To assess the immunogenicity and safety of different doses of adjuvanted and non-adjuvanted mock-up pandemic influenza vaccine (whole virion, Vero cell derived, inactivated)	To assess the immunogenicity and safety in adults and elderly To assess the need of a booster dose To evaluate the cellular immune response in a subset of subjects
Immune Response Assessments	<u>All subjects:</u> anti-HA antibodies by HI; SRH; neutralizing antibodies by MN <u>Subset of subjects:</u> Cell mediated immune response	<u>All subjects:</u> anti-HA antibodies by HI; SRH; neutralizing antibodies by MN <u>Subset of subjects</u>: Cell mediated immune response
Study Duration	Date of first enrollment:12.06.2006 Part A (through day 42): 05.10.2006 Part B (through Day 180): 16.02.2007 Part C (through Day 250): 07.03.2007 For each subject: • 42 days (Part A) • 180 days (Parts A and B combined) • Up to 250 days for subgroup of subjects continuing participation through Part C (Austrian site only) Interim reports on Part A and B available	First subject enrolled: 10.04.2007 Last subject completed Part A (through Day 42): 02.08.2007 For each subject • through 42 days (primary immunisation series; Part A) For subset of subjects • 21 days following 6-months booster (Part B) • 21 days following 12-months booster (Part C) • 21 days following 24-months booster (Part D) • evaluation of cell mediated immunity (Part E) Study ongoing

Interim clinical reports were planned for study 810501 following the primary immunisation series and at 6 months after first vaccination in order to get information on antibody persistence. For 810501 two clinical study reports (Part A alone, and Part A and B combined) were submitted containing the analyses after completion of the primary series and analyses for antibody persistence up to 6 months after primary vaccination. The 6-months safety analysis and analysis of cellular immune responses were available during the procedure (Part C).

For study 810601 an interim report after completion of the primary immunisation series (Part A) was submitted in the initial marketing authorisation application. Results on antibody persistence derived from study 810601 and the 6-months booster immunisations of study 810601 (Part B) and 12-15 month booster immunisation of study 810703 have been provided during the initial marketing authorisation procedure. A clinical study report (CSR) for Part C of study 810601 was made available post-authorisation in Q2/2009 and has been assessed as a Follow-Up Measure (FUM). Parts D and E of the study 810601 are ongoing and the anticipated completion of the CSR is given as Q2 2010.

Post-authorisation the final CSR of Study 810701 became available. Study 810701 was an open-label Phase I/II study to assess the safety and immunogenicity of two doses (3.75µg or 7.5µg HA) of a Vero cell-derived, whole virion Clade 2 H5N1 Influenza vaccine (strain A/Indonesia/05/2005) in healthy volunteers aged 21 to 45 years. The study was conducted in Hong Kong and Singapore and an interim CSR was available during the initial procedure for marketing authorisation of the mock-up vaccine.

Immunogenicity results

The immunogenicity of Celvapan was investigated in two clinical trials using haemagglutination inhibition (HI) assays, microneutralisation (MN) assays and single radial haemolysis (SRH) assays. For both studies the interpretation of the HI and SRH results for each H5N1 vaccine formulation after each injection was linked to the immunogenicity requirements defined by the Note for Guidance on Harmonisation for Influenza vaccines (CPMP/BWP/214/96).

Table 2: Parameters of the Note for Guidance (CPMP/BWP/214/96)

Defined from D0 to D21 and D0 to D42	**Age**	
	18 to 60 years	**> 60 years**
Seroconversion*or significant increase† rate of titer	>40%	>30%
Mean Geometric fold increase‡	>2.5	>2.0
Seroprotection rate (HI titer ≥1:40, SRH area ≥25mm^2)	>70%	>60%

* Proportion of subjects with a pre-vaccination HI titer <1:10 to a post-vaccination HI titer ≥1:40
Proportion of subjects with a baseline haemolysis area of ≤4 mm² and an area of ≥25 mm² post vaccination

† Proportion of subjects with HI titres ≥1:10 before vaccination and ≥4-fold increase of the titer.
Proportion of subjects with a ≥ 50% increase in haemolysis area if the pre-vaccination area is >4 mm²

‡ Geometric mean of individual ratios (post-/pre-vaccination titres: D21/D0 or D42/D0)

With regards to the MN assay similar requirements were defined for the calculation of seroneutralisation rates using a cut-off of ≥1:20. Further as proposed in guideline EMEA/CHMP/VWP/263499/2006 the proportions of achieving at least a fourfold increase in the neutralising antibody titer (criterion for seroconversion) and GMTs were reported along with reverse cumulative distribution curves.

To allow the use of the immunogenicity criteria it should be demonstrated that the Vero-cell derived pandemic influenza vaccine is antigenically similar to the egg-cultured vaccine, as requested in the NfG on influenza vaccines (CPMP/BWP/214/96). The MAH provided data on the characterization of egg-derived and Vero cell-derived influenza virus vaccine strains of previous influenza seasons. No significant differences in their infectivity, antigenicity and immunogenicity in mice were demonstrated. Moreover the egg-derived seed virus remains genetically stable during five passages in Vero cells. Hence it can be anticipated that the production system has no influence on the antigenicity of the vaccine.

HI assay

The evaluation of human sera by HI assays revealed a high variability in the test results, although varying designs of the assay were applied: HI titres were assessed using horse or turkey erythrocytes as well as utilising antigen from homologous or heterologous wild type or RG reassortant strains from different sources (egg-derived or MDCK-derived). Surprisingly the highest immune responses across all vaccine groups were found with antigen of the RG reassortants regardless whether it was egg or MDCK derived or represent a homologous or heterologous strain. In general, a low responsiveness was observed throughout all analyses of human sera most probably due to a low sensitivity of the assay in clinical studies – in contrast to pre-clinical studies. Similar findings were reported for some other H5N1 vaccines.

The high variability and low sensitivity of the HI assay was also subject of the EMEA Scientific Advice (EMEA/CHMP/SAWP/310862/2007) and the company was encouraged to provide further immunogenicity data based on the SRH assay and challenge studies using the ferret model to confirm proof-of-concept.

MN assay

The MN assay is based on ability of neutralising antibodies to inhibit the attachment of virus to cells as well as intracellular penetration and propagation. Such assays are commonly used to detect

protective antibodies in human reconvalescent sera or sera from vaccinees. However, at present it is not known which neutralising antibody titer confers protection against a potential pandemic strain. Moreover there is a high variability in test results depending on the laboratory and the specific neutralisation assay employed. Several studies have indicated that a cut-off of 1:20 is appropriate whereas others have used a cut-off of 1:40. The interpretation of results based on different neutralisation assays is further hampered because no international reference material is available for standardisation.

The MAH performed passive immune transfer studies in mice to evaluate whether the chosen cut-off titer of 1:20 is appropriately defined. A MN titer of 1:5 (mouse immune sera) or 1:7 (guinea pig immune sera), respectively, was demonstrated to correlate with 50% protection against a lethal challenge. In addition two independent passive immune transfer experiments using pooled human immune sera from vaccinees enrolled in study 810601 were conducted. One day after intravenous injection of different dilutions of the human antibodies mice were challenged with a lethal dose of wild type virus strain A/Vietnam/1203/2004 of 133 LD_{50} units. Two hours before challenge the animals were bled and the neutralising antibody titres were determined before and after administration. The calculated MN titre of 1:10 was found to protect 50% of animals, whereas those calculated MN titres were not measurable after administration. However, these data suggest that the cut-off titer of 1:20 is appropriately defined for the MN assay and that the neutralising antibody response as measured in cell culture corresponds to a functional immune response in vivo.

With regard to assay validation an initial validation report was presented. In addition upon request during the procedure and following a GCP inspection revalidation of the assay was conducted. In summary, the new validation data were found to be satisfactory.

SRH assay
As requested per EMEA Scientific Advice standard SRH assays were conducted to confirm the results obtained with the MN assay. A detailed description of the assay and the validation report was provided in the Applicant's response to the day120 LoQ. The performance of the assay was found to be satisfactorily validated.

Cellular immunity
Preliminary data on cellular immunity were provided and demonstrate a strong bias towards a humoral immune response.

- Dose response studies

Dose response study 810501
In the dose-response study 810501 four vaccine formulations adjuvanted with alum (3.5µg, 7.5µg, 15µg and 30µg) and 2 non-adjuvanted vaccine formulations (7.5µg and 15µg) were evaluated in healthy adults of 18-45 years of age. Vaccines were administered intramuscularly on day 0 and day 21
.
Based on the MN and SRH assay using the homologous vaccine strain (A/Vietnam) the highest immune responses were achieved following two immunisations with the non-adjuvanted vaccine formulations. Moreover after the first vaccination significantly higher seroprotection rates by SRH assay and seroneutralisation rates (percentage of subjects with MN titre ≥ 1:20) by MN assay were observed in the non-adjuvanted vaccine groups compared to the adjuvanted vaccine groups indicating no adjuvanting but rather an inhibitory effect of alum throughout all antigen concentrations. These results are contrary to the experience with an already approved whole virion vaccine where an adjuvanting effect of alum could be demonstrated. The controversial effects might be explained by the fact that different manufacturing processes are used for the two vaccines. Celvapan is based on a wild type virus strain propagated in Vero cells whereas the other whole virion vaccine utilises a reassortant strain grown in embryonated hen eggs.

The seroprotection and seroneutralisation rates following the 2-dose vaccination schedule and 6 months later are summarised in Table 3 (MN assay) and Table 4 (SRH assay).

Table 3: Number of subjects with neutralising antibody responses (cut-off titer ≥1:20), 21 days after 1st/2nd vaccination and 180 days after the first vaccination measured by MN titer (ITT dataset)

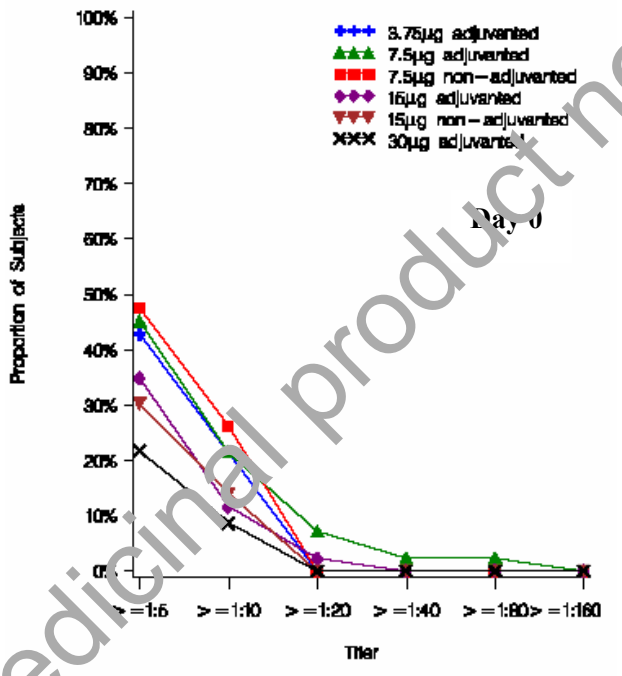
Day	Study Group											
	3.75µg + Al		7.5µg +Al		15µg +Al		30µg +Al		7.5µg		15µg	
	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.
A/Vietnam												
0	0/42 0.0%	0.0%; 8.4%	3/42 7.1%	1.5%; 19.5%	1/43 2.3%	0.1%; 12.3%	0/46 0.0%	0.0%; 7.7%	0/42 0.0%	0.0%; 8.4%	0/43 0.0%	0.0%; 8.2%
21	9/42 21.4%	10.3%; 36.8%	11/42 26.2%	13.9%; 42.0%	7/43 16.3%	6.8%; 30.7%	5/46 10.9%	3.6%; 23.6%	17/42 40.5%	25.6%; 56.7%	17/43 39.5%	25.0%; 55.6%
42	29/42 69.0%	52.9%; 82.4%	25/39 64.1%	47.2%; 78.8%	25/41 61.0%	44.5%; 75.8%	29/44 65.9%	50.1%; 79.5%	32/42 76.2%	60.5%; 87.9%	29/41 70.7%	54.5%; 83.9%
180	9/42 21.4%	10.3%; 36.8%	9/38 23.7%	11.4%; 40.2%	15/41 36.6%	22.1%; 53.1%	18/43 41.9%	27.0%; 57.9%	23/42 54.8%	38.7%; 70.2%	29/41 70.7%	54.5%; 83.9%
A/Indonesia												
0	1/42 2.4%	0.1%; 12.6%	1/42 2.4%	0.1%; 12.6%	1/43 2.3%	0.1%; 12.3%	0/46 0.0%	0.0%; 7.7%	0/42 0.0%	0.0%; 8.4%	0/43 0.0%	0.0%; 8.2%
21	5/42 11.9%	4.0%; 25.6%	5/42 11.9%	4.0%; 25.6%	1/43 2.3%	0.1%; 12.3%	3/46 6.5%	1.4%; 17.8%	10/42 23.8%	12.1%; 39.5%	7/43 16.3%	6.8%; 30.7%
42	12/42 28.6%	15.7%; 44.6%	14/39 35.9%	21.2%; 52.8%	3/41 7.3%	1.5%; 19.9%	13/44 29.5%	16.8%; 45.2%	19/42 45.2%	29.8%; 61.3%	15/41 36.6%	22.1%; 53.1%
180	5/42 11.9%	4.0%; 25.6%	5/38 13.2%	4.4%; 28.1%	1/41 2.4%	0.1%; 12.9%	13/41 31.7%	18.1%; 48.1%	14/42 33.3%	19.6%; 49.5%	2/43 4.7%	0.6%; 15.8%
A/Hongkong												
0	0/42 0.0%	0.0%; 8.4%	4/42 9.5%	2.7%; 22.6%	2/43 4.7%	0.6%; 15.8%	1/46 2.2%	0.1%; 11.5%	2/42 4.8%	0.6%; 16.2%	1/43 2.3%	0.1%; 12.3%
21	9/42 21.4%	10.3%; 36.8%	13/42 31.0%	17.6%; 47.1%	9/43 20.9%	10.0%; 36.0%	7/46 15.2%	6.3%; 28.9%	20/42 47.6%	32.0%; 63.6%	18/43 41.9%	27.0%; 57.9%
42	28/42 66.7%	50.5%; 80.4%	25/39 64.1%	47.2%; 78.8%	26/41 63.4%	46.9%; 77.9%	34/44 77.3%	62.2%; 88.5%	32/42 76.2%	60.5%; 87.9%	32/41 78.0%	62.4%; 89.4%
180	18/42 42.9%	27.7%; 59.0%	22/38 57.9%	40.8%; 73.7%	25/41 61.0%	44.5%; 75.8%	25/43 58.1%	42.1%; 73.0%	30/42 71.4%	55.4%; 84.3%	35/41 85.4%	70.8%; 94.4%

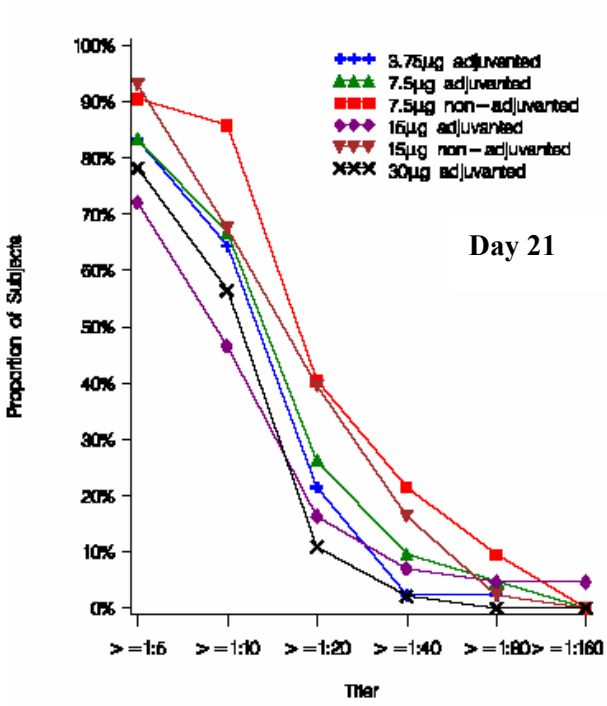
Table 4: Number of subjects with antibody response associated with protection as defined by SRH area >=25mm^2, 21 days after 1st/2nd vaccination and 180 days after the first vaccination (ITT dataset)

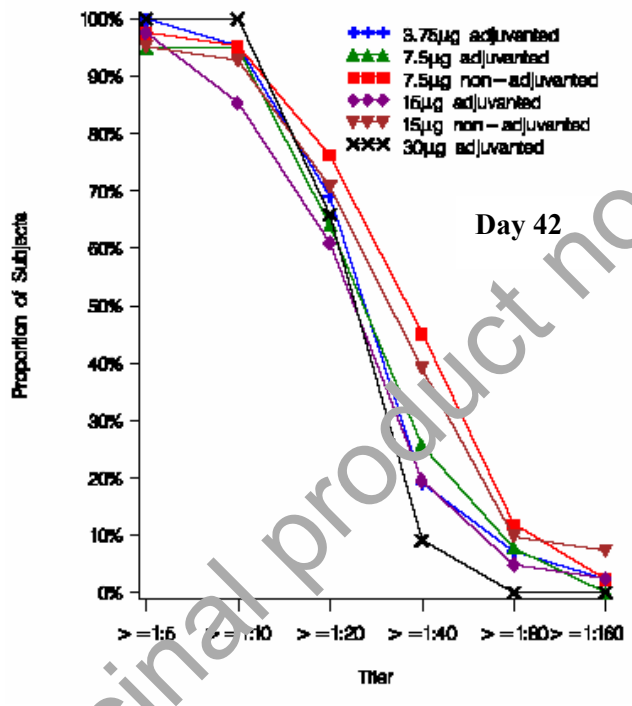
Day	Study Group											
	3.75µg + Al		7.5µg +Al		15µg +Al		30µg +Al		7.5µg		15µg	
	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.
A/Vietnam												
0	2/42 4.8%	0.6; 6.2	2/42 4.8%	0.6; 16.2	2/43 4.7%	0.6; 15.8	1/46 2.2%	0.1; 11.5	3/42 7.1%	1.5; 19.5	1/43 2.3%	0.1; 12.3
21	11/42 26.2%	13.9; 42.0	11/42 26.2%	13.9; 42.0	7/43 16.3%	6.8; 30.7	10/46 21.7%	10.9; 36.4	29/42 69.0%	52.9; 82.4	18/43 41.9%	27.0; 57.9
42	21/42 50.0%	34.2; 65.8	14/39 35.9%	21.2; 52.8	16/41 39.0%	24.2; 55.5	25/43 58.1%	42.1; 73.0	33/42 78.6%	63.2; 89.7	25/41 61.0%	44.5; 75.8
180	11/42 26.2%	13.9; 42.0	6/38 15.8%	6.0; 31.3	11/41 26.8%	14.2; 42.9	15/43 34.9%	21.0; 50.9	22/42 52.4%	36.4; 68.0	20/41 48.8%	32.9; 64.9

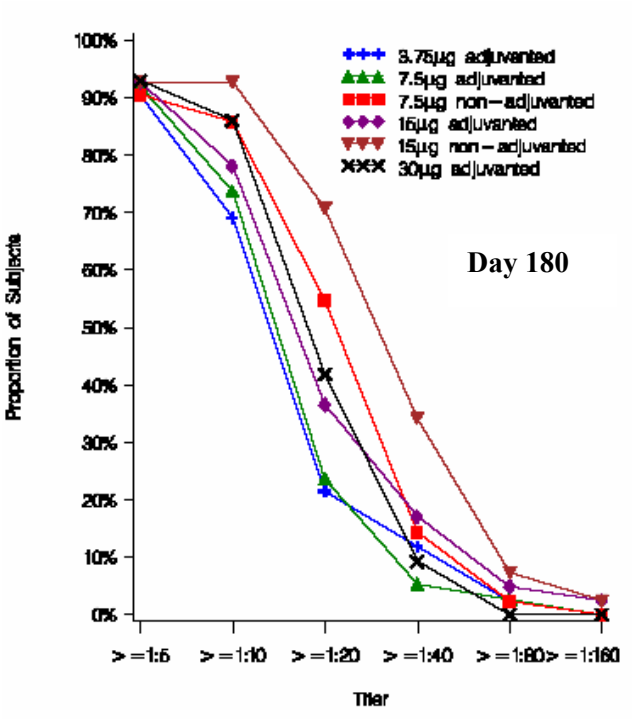
Reverse cumulative analyses on MN titre distributions post dose 1 and 2 provide additional evidence on the lack of an adjuvanting effect of alum and demonstrate that there is no impact of the antigen concentration on the immune response, i.e no dose-response is observed neither for the adjuvanted nor the non-adjuvanted vaccine formulations (Figure 1).

Figure 1: Reverse cumulative distributions of neutralising (MN) antibody responses (A/Vietnam)









With both the SRH and the MN assay all three requirements were fulfilled following two immunisations with the non-adjuvanted 7.5µg vaccine formulation with seroprotection rate of 78.6% by SRH assay and seroneutralisation rate of 76.2% by MN assay, seroconversion rates of 69.0% and 73.8% and a GM fold increase of 5.3 and 6.3, respectively. Moreover cross-neutralisation experiments indicate a high responsiveness for the original prototype A/Hongkong strain (76.2%) and a reasonable cross-neutralising response for the further evolved strain A/Indonesia (45.2%). The neutralising antibody responses against all three virus strains persist over 6 months with low to moderate decline rates (A/Vietnam: 54.8%; A/Indonesia: 33.3%; A/Hongkong: 71.4%).
Thus, the choice of the non-adjuvanted 7.5µg formulation is justified for Celvapan.

- Main studies

Study 810601 immunogenicity of the 7.5µg vaccine in healthy adults and elderly

METHODS (The methods for study 810501 and 810601 are described together in this section)

Study Participants

The inclusion and exclusion criteria for both studies 810501 and 810601 were in general identical except for the age at the time of first vaccination. In study 810501 healthy adults aged 18 to 45 years were enrolled, whereas in study 810601 persons 18-59 years of age and 60 years of age and older were included.

Treatments

Study 810501:
Four different alum adjuvanted (3.75µg, 7.5µg, 15µg, 30µg HA) and two non-adjuvanted (7.5µg, 15µg HA) vaccine formulations of the pandemic candidate influenza vaccine (single-dose presentation) were administered each on D0 and D21 as primary vaccinations. Each subject received two injections of 0.5ml of the same vaccine dose and formulation by intra-muscular injection into the musculus deltoideus. Blood samples were taken on day 0, day 21 and 41 as well as on day 180 (+14 days) for the immunogenicity assessment.

Study 810601:
One lot (Lot Number VNV1G001A) of the candidate vaccine was used for the first and second vaccinations in all subjects. The vaccine for the primary vaccination series was produced of strain A/Vietnam/1203/2003 according to the final manufacturing process. It was provided as multi-dose presentation containing no preservative

Objectives

Study 810501:
The primary objective of this study was to identify the immunogenicity and safety of different doses of an adjuvanted and non-adjuvanted mock-up pandemic influenza vaccine.

Study 810601:
The primary objectives of this study were:
To assess the immune response to an H5N1 influenza vaccine in an adult and elderly population;
To assess the safety and tolerability of an H5N1 influenza vaccine in an adult and elderly population;
To assess the need for and timing of a booster vaccination;
For a subset of subjects further objectives of the study included:
To evaluate the T-cell mediated immune response induced by an H5N1 influenza vaccine after the first, second and booster vaccination.

Outcomes/endpoints

Study 810501:
Primary endpoints
Number of subjects with antibody response to the vaccine strain (A/Vietnam/1203/04) associated with protection 21 days after the first and second vaccination defined as either Haemagglutination Inhibition (HI) titer ≥ 1:40 or titer measured by Microneutralisation (MN) test ≥1:20.

Secondary endpoints included the antibody response 21 days after the first and second vaccinations in terms of:
- Fold increase of antibody response 21 days after the first and second vaccinations as compared to baseline measured by HI and MN assays
- Number of subjects with seroconversion defined as a minimum four fold increase in titer measured by HI or MN assay 21 days after the first and second vaccinations as compared to baseline
- Antibody response 180 days after the first vaccination measured by HI and MN assays
- Fold increase of antibody response 180 days after the first vaccination as compared to baseline measured by HI and MN assays
- Number of subjects with antibody response associated with protection 180 days after the first vaccination defined as either HI titer ≥ 1:40 or titer measured by MN ≥ 1:20
- Number of subjects with antibody response associated with protection 21 days after the first and second vaccinations as well as 180 days after the first vaccination defined as Single Radial Haemolysis (SRH) area ≥ 25 mm²;

For a subset of subjects cellular immunity has been assessed.

Study 810601:
Primary endpoints
Number of subjects with antibody response to the vaccine strain (A/Vietnam/1203/2004) associated with protection 21 days after the second vaccination defined as titer measured by microneutralisation (MN) test ≥ 20

Secondary endpoints included the number of subjects with antibody response associated with protection 21 days after the first vaccination measured by MN assay, number of subjects with HI titer ≥ 40 and SRH area ≥ 25 mm^2 measured 21 days after the first and second vaccinations, antibody titer 21 days after the first and second vaccinations as measured by MN, SRH and HI assays, fold increase of antibody response as compared to baseline 21 days after the first and second vaccinations as measured by MN, SRH and HI assays, number of subjects with seroconversion (defined as a minimum

four fold titer increase) 21 days after the first and second vaccinations as measured by MN, SRH and HI assays and booster data measured with different assays.
For a subset of subjects cellular immunity has been assessed.

Sample size

Study 810501: The sample size was planned under the assumption that for a seroprotection rate of 80% and 40 subjects per group, the (half-) width of the two-sided 95% CI for this rate is at most 15.2%. To account for a drop-out rate of about 10% forty-five subjects had to be enrolled per group.
Study 810601: Anticipating an observed seroprotection rate of about 60%, with a sample size of 250 subjects, the (half-) width of the two-sided 95% CI for this rate is at most 6.4%. In order to account for a drop-out rate of 10% a total number of 275 subjects were to be included into each of the 2 age strata (18 to 59 years, ≥ 60 years).

Randomisation

In study 810501 patients were randomised in cohorts. In cohort 1 patients were randomised applying a randomisation ratio of 1:1:1 to receive 3.75µg adjuvanted, 7.5µg adjuvanted or 7.5µg non-adjuvanted H5N1, in cohort 2 patients were randomised in an 1:1 ratio to receive either 15µg adjuvanted or 15µg non-adjuvanted H5N1 while patients in cohort 3 were not randomised but received 30 µg adjuvanted H5N1.

In study 810601 initially all patients received 7.5µg non-adjuvanted H5N1. Subjects were randomised at visit 4 (day 180 +/- 14 days) in a ratio of 2:1:1 to receive either 6-months, 12-months or 24-months booster vaccinations.

Blinding (masking)

Study 810501 was blinded with respect to the individual treatment group within cohorts 1 and 2 respectively. The reported part of study 810601 was performed as a not controlled, open label trial.

Statistical methods

Seroprotection rates were the primary efficacy parameter in both trials. In study 810501 for each treatment group the seroprotection rates (defined as MN titer ≥ 1:20 and HI titer ≥ 1:40 respectively) 21 days after the first and second vaccination and their 95% CIs intervals were calculated separately for both, HI and MN assays. In study 810601 the seroprotection rates (defined as MN titer ≥ 1:20) 21 days after the second vaccination and their 95% confidence intervals calculated separately for both age strata.
All secondary immunogenicity endpoints were described by means of point estimates including their 95%-CIs stratified for the pre-defined strata.
In order to assess the effect of adjuvant, in study 810501 the antibody response to the two vaccine doses prepared with and without adjuvant (with 7.5 µg and 15 µg of antigen) was evaluated by an analysis of covariance. Dose, presence of adjuvant and the interaction between dose and adjuvant were the factors included into the analysis model; baseline values were considered as covariates. These analyses were done separately for the HI assay and the MN assay, as well as for the first and second vaccination. Logistic regression was used to perform similar analyses with respect to seroprotection rates and seroconversion rates.

Study population

Subjects are included in the Intent to treat Population (ITT) datasets if they received the 1st/2nd vaccination and have available serology data at Day 21 after the $1^{st}/2^{nd}$ vaccination.

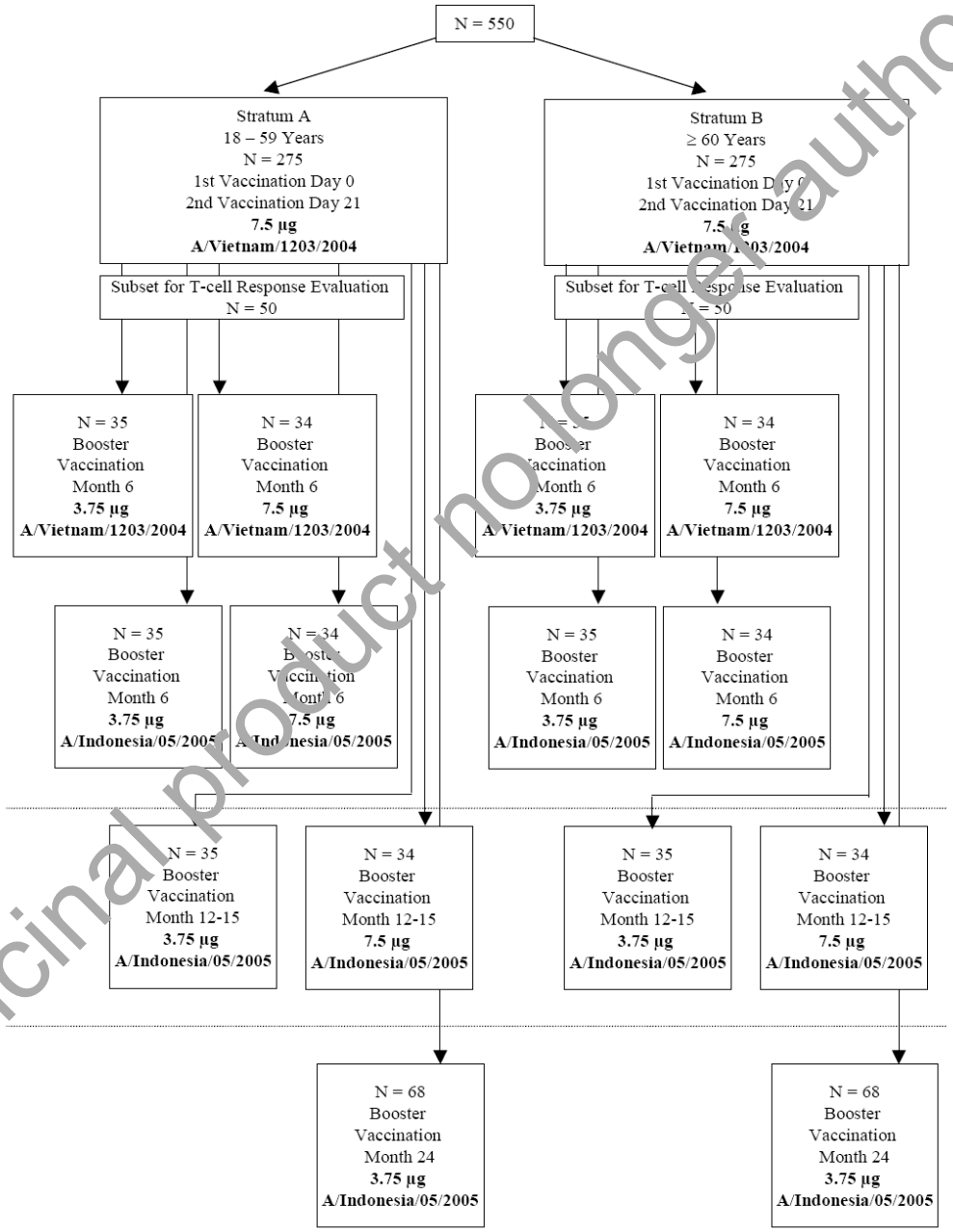
Subjects are included in the Per Protocol Population (PP) analysis if they fulfil inclusion/exclusion criteria, have no major protocol violations, received both vaccinations and have available serology data at Day 21 after the $1^{st}/2^{nd}$ vaccination.

RESULTS

Participant flow

Study 810601:
Each subject received two 0.5ml doses of the same vaccine intramuscularly in the primary vaccination series (D0 and D21) and a booster dose of the vaccine containing either the homologous A/Vietnam strain or the heterologous A/Indonesia/05/2005 strain was administered to a subset of subjects on month 6, month 12 or month 24, respectively (see flow chart below).

Study Design for Baxter Clinical Study 810601:



For immunogenicity evaluation blood samples were drawn on day 0 pre-vaccination and 21 days after the first and second vaccinations. Further samples were drawn before and 21 days after each booster immunisation.

Recruitment

In study 810501 the date of first enrolment was 12.06.2006, for Part A (through day 42): 05.10.2006, for Part B (through Day 180): 16.02.2007 and the last subject completed Part C (through Day 250) on 07.03.2007.
In study 810601 the first subject has been enrolled 10.04.2007 and the last subject completed Part A (through Day 42) at 02.08.2007.

Conduct of the study

In **study 810501** a total of 284 subjects were enrolled of which 275 received the first vaccination and 257 subjects received the second vaccination. In total, 249 subjects were valuable for the immunogenicity analysis. Seventeen subjects did not come back after the first vaccination and eight subjects did not come back after the second vaccination at day 42.

Study **810601** had 8 amendments to the original protocol, but only 7 were ultimately implemented. For the German study centres, a blood draw to evaluate liver function 7 days after the first and second vaccination was introduced in response to elevated liver enzymes in a preclinical test in rats. The amended booster vaccination schedule includes a booster vaccination at 6-months, 12-months and 24-months using the H5N1 influenza vaccine containing alternatively the vaccine strain or the clade 2 A/Indonesia/05/2005 strain. In the amendment 5, the principal investigator of a study site in Austria was replaced because of GCP/GDP related irregularities at this site. Amendment 6 comprised of a revision of the 12 months booster to include both the 3.75 and 7.5 µg dose of A/Indonesia/05/2005 strain vaccine. In amendment 7 to the study protocol the 24 months booster was revised to include both the 3.75 and 7.5 µg dose of A/Indonesia/05/2005 strain vaccine.

Baseline data

In **study 80501** slightly more male subjects (143 for the first and 137 for the second vaccination) than female subjects (115 for the first vaccination and 112 for the second vaccination) were included in the immunogenicity dataset. On Day 180 slightly more male subjects (136) than female subjects (111) were included in the immunogenicity dataset. The largest number of subjects in both datasets was aged 18 to 25 years (23%-35% across groups), the second largest number of subjects was aged 26 to 30 years (19%-35% across groups).

Study 810601
Gender was evenly distributed in both strata. Age was well distributed in Stratum A, in Stratum B 51.1 % of subjects were between 60 and 65 and a further 32.5 % of subjects between 66 and 70 years old.
Seropositive antibody titres against the H5N1 vaccine strain (A/Vietnam/1203/2004) at baseline were shown in 4.1% and 16.9% of subjects for MN, and 4.5% and 5.3% for SRH in Stratum A and B, respectively.

Numbers analysed

In **study 810501 t**he immunogenicity dataset was used for the analysis of antibody response after the first and second vaccinations and on Day 180 and comprised the subjects who fulfilled the inclusion/exclusion criteria and had immunogenicity data available for the first (n=258) and second (n=249) vaccination, as well as for Day 180 (n=247). No subjects were excluded for major protocol violations.

In **study 810601** number of subjects planned were 550 (275 Stratum A, 275 Stratum B) and analyzed (Part A) were 561 (281 Stratum A, 280 Stratum B) in full analysis dataset for first vaccination,
542 (270 Stratum A, 272 Stratum B) in ITT dataset for first vaccination (ITT 1), 539 (269 Stratum A, 270 Stratum B) received second vaccination, 539 (269 Stratum A, 270 Stratum B) in full analysis dataset for second vaccination, 535 (265 Stratum A, 270 Stratum B) in ITT dataset for second vaccination (ITT 2) and 525 (257 Stratum A, 268 Stratum B) in PP dataset for second vaccination

Outcomes and estimation

Following two vaccinations and based on the MN assay all three requirements were fulfilled in the age group of adults and 2 out of 3 requirements were met in the elderly (Table 5). With regards to the group of adults a seroneutralisation rate of 72.5%, a seroconversion rate of 60.8% and a 4.7 fold GM increase was achieved. In the elderly a seroneutralisation rate of 74.1%, a seroconversion rate of 26.7% and a 2.8 fold increase was obtained (Table 5). In summary based on the MN assay 3 out of 3 CHMP requirements were met for the adults and 2 out of 3 requirements were fulfilled for the elderly subjects.

Table 5: Immunogenicity evaluation using the MN assay and wild type strain A/Vietnam (ITT dataset)

	Age groups					
	18-59 yrs			**≥60 yrs**		
Seroneutralisation rates (MN titer >=1:20) 21 days after 1st/2nd vaccination						
Day	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**
0	11/270	4.1	2.1; 7.2	46/272	16.9	12.7; 21.9
21	137/270	50.7	44.6; 56.9	148/272	54.4	48.3; 60.4
42	192/265	72.5	66.7; 77.7	200/270	74.1	68.4; 79.2
180	85/243	35.0	29.0; 41.3	104/257	40.5	34.4; 46.7
Seroconversion rates 21 days after the 1st and 2nd vaccination as compared to baseline						
Day	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**
21	107/270	39.6	33.8; 45.7	39/272	14.3	10.4; 19.1
42	161/265	60.8	54.6; 66.7	72/270	26.7	21.5; 32.4
Geometric Mean measured 21 days after 1st/2nd vaccination						
Day	**N**	**GMT**	**95% CI**	**N**	**GMT**	**95% CI**
0	270	5.7	5.3 ; 6.1	272	10.5	9.7 ; 11.4
21	270	19.5	17.9 ; 21.2	272	21.6	19.8 ; 23.6
42	265	26.5	24.4 ; 28.7	270	29.5	27.2 ; 31.9
180	243	16.0	14.7 ; 17.4	257	18.5	16.9 ; 20.1
Geometric Mean fold Increase measured 21 days after 1st/2nd vaccination as compared to baseline						
Day	**N**	**GM**	**95% CI**	**N**	**GM**	**95% CI**
21	270	3.4	3.1 ; 3.7	272	2.1	1.9 ; 2.2
42	265	4.7	4.2 ; 5.1	270	2.8	2.6 ; 3.0

The results of the MN assay were generally confirmed by the SRH assay (Table 6). Following two vaccinations 2 out of 3 three CHMP requirements were fulfilled in adults and all three 3 requirements were met in the elderly. In the group of the adults a seroprotection rate of 63.3%, a seroconversion rate

of 60.2% and a 4.6 fold GM increase was achieved. In the elderly a seroprotection rate of 67.7%, a seroconversion rate of 62.4% and a 4.6 fold increase was obtained.

Table 6: Immunogenicity evaluation using the SRH assay and wild type strain A/Vietnam (ITT dataset)

	Age groups					
	18-59 yrs			≥60 yrs		
Seroprotection rates (SRH area >=25 mm^2) 21 days after 1st/2nd vaccination						
Day	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**
0	12/268	4.5	2.3; 7.7	14/266	5.3	2.9; 8.7
21	142/266	53.4	47.2; 59.5	157/271	57.9	51.8; 63.9
42	164/259	63.3	57.1; 69.2	180/266	67.7	61.7; 73.3
180	58/243	23.9	18.7; 29.7	69/258	26.7	21.[illegible]; 32.9
Seroconversion rates 21 days after the 1st and 2nd vaccination as compared to baseline						
Day	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**
21	132/266	49.6	43.5; 55.8	142/271	52.4	46.3; 58.5
42	156/259	60.2	54.0; 66.2	166/266	62.4	56.3; 68.2
Geometric Mean measured 21 days after 1st/2nd vaccination						
Day	**N**	**GMT**	**95% CI**	**N**	**GMT**	**95% CI**
0	268	4.9	4.6 ; 5.3	266	5.4	5.0 ; 5.8
21	266	17.2	14.8 ; 20.0	271	19.6	17.0 ; 22.7
42	259	22.7	19.6 ; 26.4	266	25.0	21.7 ; 28.8
180	243	9.3	8.2 ; 10.6	258	9.8	8.6 ; 11.2
Geometric Mean fold increase measured 21 days after 1st/2nd vaccination as compared to baseline						
Day	**N**	**GM**	**95% CI**	**N**	**GM**	**95% CI**
21	264	3.5	3.0 ; 4.1	265	3.6	3.1 ; 4.2
42	257	4.6	4.0 ; 5.4	260	4.6	4.0 ; 5.3

Of note is the high rate of seropositivity in the MN assay prior to vaccination. Detectable pre-vaccination anti H5N1 neutralising antibodies were found in 4.1% of subjects in the group of adults (11 subjects) and 16.9% of subjects in the group of elderly (46 subjects). This finding is confirmed by the reverse distribution of MN titres where 60% of elderly subjects achieved MN titres of at least 1:10. Considering that elderly are routinely vaccinated with seasonal influenza vaccines, it can be assumed that an antibody response against N1 is at least partially responsible for the pre-existing immunity towards H5N1 viruses. The presence of cross-reactive antibodies especially at older ages is well documented and was also reported for other pandemic vaccines. It should be noted however, that cross-neutralisation experiments conducted in guinea pigs demonstrate that the immune response to Celvapan is predominantly directed against the H5 molecule and not the N1 protein. This implies that a pre-existing immunity against the N1 protein is probably not boostered by Celvapan. In order to

clarify, whether the baseline seropositivity is due to cross reactive anti NA antibodies cross-absorption analyses using different concentrations of NA and HA were requested and have been assessed as a Follow Up Measure.

These data confirmed the results of cross-neutralisation experiments conducted in guinea pigs where it was found that the immune response to Celvapan (H5N1) was predominantly directed against the H5 molecule and not the N1 protein.

Although a high proportion of the elderly were found to have pre-existing neutralising antibodies only a low seroconversion rate (defined as 4-fold increase) could be achieved post dose II indicating that there is a reduced ability to react to antigen or to boost the immune response. Moreover the comparison of the seroconversion rates measured by MN vs. SRH assay reveals significant differences for elderly subjects. Post dose I seroconversion rates of 14.3 % (MN assay) and 52.4 % (SRH assay) were obtained and reached 26.7 % and 62.4 % by MN assay and SRH assay, respectively following post dose II. In order to dispel the influence of baseline H5N1 antibody titres on the immunogenicity results, a detailed analysis of the serology endpoints according to baseline status was requested. The study population was divided into two groups by using a cut-off of <25mm² for the SRH and <1:20 for the MN assay. Therefore, one group consisted of those subjects who already had so-called "protective" titres at baseline and the other group was made up of subjects who where either seronegative or had low titres before the first immunization. This analysis predictably showed that those subjects who had a high titre at baseline still had high titres at day 42, but fold increase and seroconversion rates were lower for both assays. The subjects with low or negative baseline titres showed adequate SRH fold increase and seroconversion rates, but the rate of subjects with a titre ≥25mm² was 61.8% in the group of adults and therefore well below the acceptance limit. In the group of the elderly all 3 requirements for the SRH assay were met. Regarding the MN assay, if the CHMP guideline requirements are applied, all of them can be satisfied in both age strata. A further analysis of subjects negative for baseline neutralising antibodies is deemed to be of greater relevance to identify the responsiveness of immunologically naïve subjects.

Antibody persistence

Data on antibody persistence up to day 180 were also provided by the MAH (see table 5 (MN assay) and Table 6 (SRH assay) above). The data on antibody persistence reveal a decline in seroneutralisation/seroprotection rates of 35% to 40% for both age groups using either the MN or the SRH assay. The decline in the neutralizing antibody responses is however less pronounced than the decline in antibody responses determined by SRH assay. Whereas a substantial number of vaccinees have neutralizing antibody titres (of at least of 1:10) up to 180 days post vaccination (Figure 2), for only approximately 50% of adults and elderly subjects antibodies ≥4mm^2 are detectable in the SRH assay (Figure 3).

Figure 2: Reverse cumulative distributions of neutralizing (MN) antibody responses (A/Vietnam)

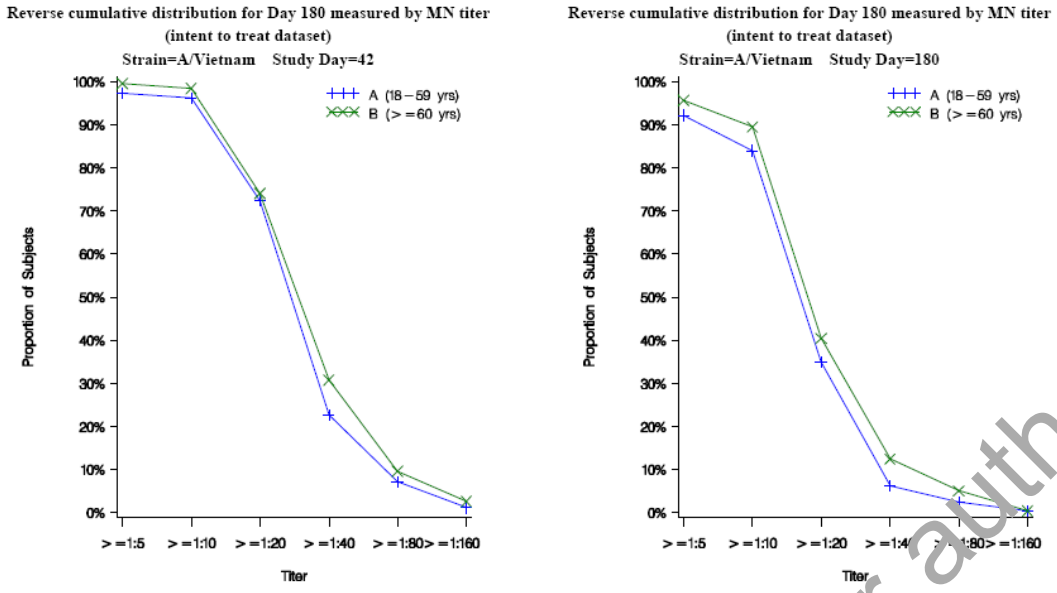
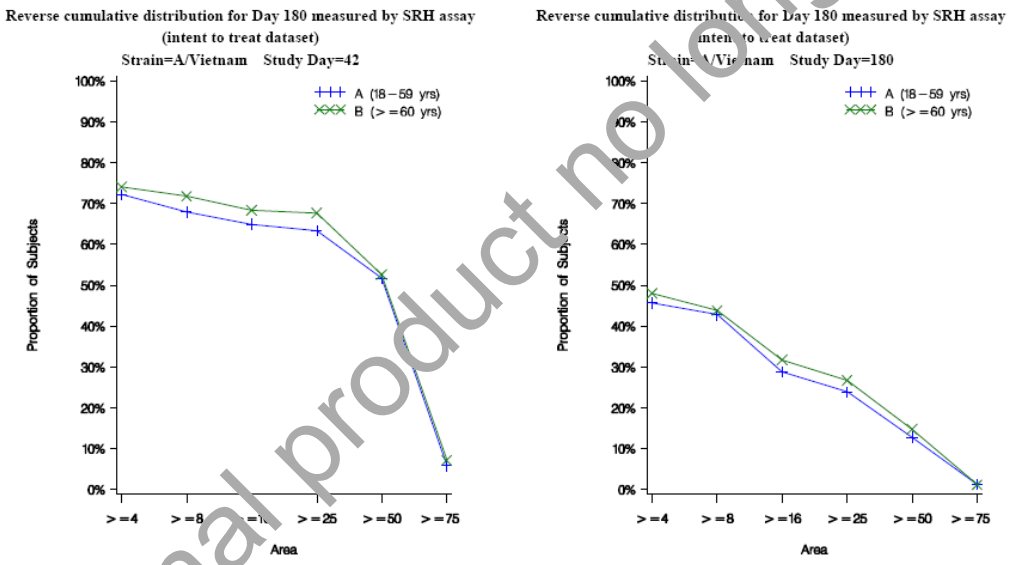


Figure 3: Reverse cumulative distributions of antibody responses as measured by SRH assay (A/Vietnam)



Results following booster immunisation

The effects of a homologous and heterologous booster immunisation were evaluated in study 810703 (follow-up to dose-finding study 810501) and in study 810601 (Parts B and C). The study reports were assessed in the initial MAA.

Study 810703 (follow-up to study 810501)

All subjects (N=141) who were vaccinated and completed the Day 42 visit at the Austrian study site in Study 810501 were invited to participate in this follow-up study. Only 77 of the 141 subjects who completed Study 810501 through Day 42 and were eligible for this follow-up agreed to participate.

Each subject received one dose of 7.5 µg A/H5N1/Indonesia/05/2005 HA antigen in a non-adjuvanted formulation as a heterologous booster vaccination 12 to 17 months (360 to 510 days) after the first vaccination with a two-dose regimen of the A/Vietnam/1203/2004 strain influenza vaccine administered in Study 810501. Blood samples were drawn on Day 0 before vaccination, as well as on Day 7 and 21 of the study.

The following serological assays were performed to assess the antibody response to the vaccine: MN, SRH and HI. The HI results were again consistently low with and highly inconsistent with the immune response detected with MN and SRH assays.

The seroneutralisation/seroprotection rates against strain A/Vietnam and strain A/Indonesia following a heterologous booster immunisation with 7.5µg HA strain A/Indonesia/05/2005 are summarised in Table 7 for the MN assay and in Table 8 for the SRH assay.

Table 7: Number of subjects with neutralising antibody response (MN titer ≥1:20) following a booster with non-adjuvanted 7.5µg A/Indonesia/05/2005 vaccine dose (ITT dataset)

	Study Group in Study 810501											
	3.75µg + Al		7.5µg +Al		15µg +Al		30µg +Al		7.5µg		15µg	
	n/N %	95% CI	n/N %	95% CI	n/N %	95% CI	n/N %	95% CI	n/N %	95% CI	n/N %	95% CI
	A/Vietnam											
D0	2/17 11.8%	1.5%; 36.4%	2/15 13.3%	1.7%; 40.5%	2/13 15.4%	1.9%; 45.4%	3/12 25.0%	5.5%; 57.2%	3/12 25.0%	5.5%; 57.2%	4/8 50.0%	15.7%; 84.3%
D7	13/16 81.3%	54.4% 96.0%	14/15 93.3%	68.1%; 99.8%	12/13 92.3%	64.0%; 99.8%	11/12 91.7%	61.5%; 99.8%	10/11 90.9%	58.7% 99.8%	8/8 100.0%	63.1%; 100.0%
D21	16/17 94.1%	71.3; 99.9%	14/15 93.3%	68.1%; 99.8%	13/13 100.0%	75.3%; 100.0%	12/12 100.0%	73.5%; 100.0%	11/12 91.7%	61.5% 99.8%	7/7 100.0%	59.0%; 100.0%
	A/Indonesia											
D0	0/17 0.0%	0.0%; 19.5%	1/15 6.7%	0.2%; 31.9%	0/13 0.0%	0.0%; 24.7%	1/12 8.3%	0.2%; 38.5%	0/12 0.0%	0.0%; 26.5%	0/8 0.0%	0.0%; 36.9%
D7	13/16 81.3%	54.4; 96.0%	14/15 93.3%	68.1%; 99.8%	12/13 92.3%	64.0%; 99.8%	12/12 100.0%	73.5%; 100.0%	10/11 90.9%	58.7% 99.8%	8/8 100.0%	63.1%; 100.0%
D21	16/17 94.1%	71.3% 99.9%	15/15 100.0%	78.2%; 100.0%	13/13 100.0%	75.3%; 100.0%	12/12 100.0%	73.5%; 100.0%	12/12 100.0%	73.5% 100.0%	6/7 85.7%	42.1%; 99.6%

Table 8: Number of subjects with antibody response associated with protection as defined by SRH area ≥25mm^2 following a booster with non-adjuvanted 7.5µg A/Indonesia/05/2005 vaccine dose (ITT dataset)

	Study Group in Study 810501											
	3.75µg + Al		7.5µg +Al		15µg +Al		30µg +Al		7.5µg		15µg	
	n/N %	95% CI	n/N %	95% CI	n/N %	95% CI	n/N %	95% CI	n/N %	95% CI	n/N %	95% CI
	A/Vietnam											
D0	0/17 0.0%	0.0%; 19.5%	0/15 0.0%	0.0%; 21.8%	1/13 7.7%	0.2%; 36.0%	0/12 0.0%	0.0%; 26.5%	0/12 0.0%	0.0%; 26.5%	2/8 25.0%	3.2%; 65.1%
D7	11/16 68.8%	41.3% 89.0%	10/15 66.7%	38.4%; 88.2%	9/13 69.2%	38.6%; 90.9%	11/12 91.7%	61.5%; 99.8%	10/11 90.9%	58.7% 99.8%	5/8 62.5%	24.5% 91.5%
D21	15/17 88.2%	63.6% 98.5%	13/15 86.7%	59.5%; 98.3%	13/13 100.0%	75.3%; 100.0%	12/12 100.0%	73.5%; 100.0%	10/12 83.3%	51.6% 97.9%	6/7 85.7%	42.1% 99.6%
	A/Indonesia											
D0	0/17 0.0%	0.0%; 19.5%	0/15 0.0%	0.0%; 21.8%	0/13 0.0%	0.0%; 24.7%	0/12 0.0%	0.0%; 26.5%	0/12 0.0%	0.0%; 26.5%	0/8 0.0%	0.0%; 36.9%
D7	9/16 56.3%	29.9% 80.2%	10/15 66.7%	38.4%; 88.2%	9/13 69.2%	38.6%; 90.9%	11/12 91.7%	61.5%; 99.8%	8/11 72.7%	39.0% 94.0%	3/8 37.5%	8.5%; 75.5%
D21	13/17 76.5%	50.1% 93.2%	11/15 73.3%	44.9%; 92.2%	12/13 92.3%	64.0%; 99.8%	12/12 100.0%	73.5%; 100.0%	8/12 66.7%	34.9% 90.1%	4/7 57.1%	18.4% 90.1%

The GM fold increase following the heterologous 7.5µg booster immunisation is given in Table 9 (MN assay) and Table 10 (SRH assay).

Table 9: Geometric Mean fold increase of MN titer measured 7 and 21 days after booster vaccination with 7.5µg HA strain A/Indonesia/05/2005

	Study Group in Study 810501											
	3.75µg + Al		7.5µg +Al		15µg +Al		30µg + Al		7.5µg		15µg	
	N	GMI 95% CI	N	GMI 95% CI	N	GMI 95% CI	N	GMI 95% CI	N	GMI 95% CI	N	GMI 95% CI
A/Vietnam/1203/2004												
D7	16	3.8 2.8; 5.1	15	6.9 3.9; 12.4	13	6.5 3.6 ; 11.8	12	6.6 4.0 ; 10.9	11	6.1 3.8 ; 9.7	8	3.2 1.7 ; 5.9
D21	17	6.1 3.7; 9.8	15	12.8 6.9; 23.5	13	11.6 6.9 ; 19.3	12	12.4 8.0 ; 19.2	12	7.0 4.1 ; 12.0	7	4.8 2.1 ; 11.2
A/Indonesia/05/2005												
D7	16	8.4 5.1;13.8	15	10.8 6.0; 19.4	13	11.8 6.3 ; 22.1	12	15.1 7.4 ; 30.8	11	11.8 7.0 ; 19.9	8	5.6 2.6 ; 11.9
D21	17	15.5 8.7; 27.6	15	24.0 13.7;42.0	13	25.6 15.8; 41.5	12	33.0 16.8; 64.8	12	14.3 8.4 ; 24.5	7	9.2 3.2 ; 27.1

Table 10: Geometric Mean of fold increase of antibody responses measured by SRH assay 7 and 21 days after booster vaccination with 7.5µg HA strain A/Indonesia/05/2005

	Study Group in Study 810501											
	3.75µg + Al		7.5µg +Al		15µg +Al		30µg + Al		7.5µg		15µg	
	N	GMI 95% CI	N	GMI 95% CI	N	GMI 95% CI	N	GMI 95% CI	N	GMI 95% CI	N	GMI 95% CI
A/Vietnam/1203/2004												
D7	16	5.6 3.0;10.3	15	5.7 3.0 ; 10.7	13	5.4 2.5 ; 11.5	12	10.0 6.1 ; 16.3	11	11.3 6.5 ; 19.6	8	2.6 0.9 ; 7.2
D21	17	10.2 6.8; 15.5	15	9.6 5.6 ; 16.5	13	11.9 7.4 ; 19.1	12	14.5 12.2; 17.1	12	10.0 5.0 ; 19.8	7	4.5 1.4 ; 14.5
A/Indonesia/05/2005												
D7	16	4.4 2.4; 8.0	15	6.5 3.8 ; 10.9	13	6.6 3.9 ; 11.1	12	10.9 6.6 ; 17.9	11	8.1 4.1 ; 16.0	8	3.0 1.0 ; 9.1
D21	17	7.6 4.6; 12.7	15	8.5 5.0 ; 14.5	13	12.2 9.2 ; 16.0	12	15.4 13.3; 17.8	12	7.4 3.4 ; 15.8	7	4.5 1.2 ; 16.7

Seroconversion rates as determined by MN assay (4-fold increase, Table 11) or SRH assay (50% increase in haemolysis, Table 12) at 7 and 21 days after heterologous 7.5µg booster immunisation are given below.

Table 11: Rate of subjects with >=4 fold increase measured by MN titer 7 and 21 days after booster vaccination with 7.5µg HA strain A/Indonesia/05/2005

	Study Group in Study 810501											
	3.75µg + Al		7.5µg +Al		15µg +Al		30µg +Al		7.5µg		15µg	
	n/N (%)	95% C.I.	n/N (%)	95% C.I.	n/N (%)	95% C.I.	n/N (%)	95% C.I.	n/N (%)	95% C.I.	n/N (%)	95% C.I.
	A/Vietnam											
D7	7/16 43.8%	19.8%; 70.1%	10/15 66.7%	38.4; 88.2	7/13 53.8%	25.1%; 80.8%	8/12 66.7%	34.9%; 90.1%	8/11 72.7%	39.0%; 94.0%	3/8 37.5%	8.5%; 75.5%
D21	11/17 64.7%	38.3%; 85.8%	11/15 73.3%	44.9; 92.2	12/13 92.3%	64.0%; 99.8%	12/12 100.0%	73.5%; 100.0%	8/12 66.7%	34.9%; 90.1%	4/7 57.1%	18.4%; 90.1%
	A/Indonesia											
D7	13/16 81.3%	54.4%; 96.0%	13/15 86.7%	59.5; 98.3%	11/13 84.6%	54.6%; 98.1%	12/12 100.0%	73.5%; 100.0%	10/11 90.9%	58.7%; 99.8%	5/8 62.5%	24.5%; 91.5%
D21	15/17 88.2%	63.6%; 98.5%	15/15 100.0%	78.2; 100.0%	13/13 100.0%	75.3%; 100.0%	12/12 100.0%	73.5%; 100.0%	11/12 91.7%	61.5%; 99.8%	5/7 71.4%	29.0%; 96.3%

Table 12: Number of subjects with seroconversion measured by SRH assay§ 7 and 21 days after booster vaccination with 7.5µg HA strain A/Indonesia/05/2005

	Study Group in Study 810501											
	3.75µg + Al		7.5µg +Al		15µg +Al		30µg +Al		7.5µg		15µg	
	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.	n/N %	95% C.I.
	A/Vietnam											
D7	11/16 68.8%	41.3%; 89.0%	10/15 66.7%	38.4; 88.2%	8/13 61.5%	31.6%; 86.1%	11/12 91.7%	61.5%; 99.8%	10/11 90.9%	58.7%; 99.8%	4/8 50.0%	15.7%; 84.3%
D21	16/17 94.1%	71.3%; 99.9%	13/15 86.7%	59.5; 98.3%	12/13 92.3%	64.0%; 99.8%	12/12 100.0%	73.5%; 100.0%	10/12 83.3%	51.6%; 97.9%	5/7 71.4%	29.0%; 96.3%
	A/Indonesia											
D7	9/16 56.3%	29.9%; 80.2%	10/15 66.7%	38.4; 88.2%	9/13 69.2%	38.6%; 90.9%	11/12 91.7%	61.5%; 99.8%	8/11 72.7%	39.0%; 94.0%	3/8 37.5%	8.5%; 75.5%
D21	13/17 76.5%	50.1%; 93.2%	11/15 73.3%	44.9; 92.2%	12/13 92.3%	64.0%; 99.8%	12/12 100.0%	73.5%; 100.0%	8/12 66.7%	34.9%; 90.1%	4/7 57.1%	18.4%; 90.1%

§ defined as either a >=25 mm^2 haemolysis area after vaccination if baseline sample is negative [<= 4mm^2] or a >=50% increase in haemolysis area if the baseline sample is > 4mm^2

With the MN assay a seroneutralisation rate of 100%, a GM fold increase of 14.0 and a seroconversion rate of 91.7% were achieved against the booster strain A/Indonesia. Based on the SRH assay all subjects were found to be seronegative (<25mm^2) for the heterologous strain A/Indonesia prior booster immunisation and 7 to 21 days after the heterologous booster SPR of ~70%, a GM increase of 7.4 and a SCR of ~70% were obtained. While the neutralising antibody response against the A/Vietnam strain was generally lower than against strain A/Indonesia after the heterologous booster immunisation it was significantly higher against strain A/Vietnam than against strain A/Indonesia by SRH analysis. These findings indicate that most likely different types of antibodies are measured by the two different assays. While for the SRH assay complement is used, it is not specifically added to the MN assay. Consequently antibodies not binding to and thereby activating complement will not be detected in the SRH assay but might be measured in the MN assay. It can be speculated that complement dependent antibodies are more specific in their epitope binding activity than complement independent neutralising antibodies. Another possible explanation for the different antibody responses to

homologous and heterologous antigens could be the presence of anti NP or M2 antibodies detectable in one assay but not the other.

Study 810601

For the 6-months booster immunisation half of the subjects were randomized into 4 groups to receive one of the following dosages:

- 3.75 µg HA antigen, strain A/Vietnam/1203/2004 per 0.25 mL
- 7.5 µg HA antigen, strain A/Vietnam/1203/2004 per 0.5 mL
- 3.75 µg HA antigen, strain A/Indonesia/05/2005 per 0.25 mL
- 7.5 µg HA antigen, strain A/Indonesia/05/2005 per 0.5 mL

Antibody response to the vaccine was assessed using the following assays:

- Microneutralisation (MN)
- Haemagglutination Inhibition (HI)
- Single Radial Haemolysis (SRH)

Immunogenicity endpoints determined by MN, HI and SRH assay were evaluated against the H5N1 influenza strain contained in the vaccine for the 6-months booster vaccination (either A/Vietnam/1203/2004 or A/Indonesia/05/2005). Currently no SRH analysis was provided using strain A/Indonesia/05/2005 as antigen.

Immunogenicity endpoints were analyzed for the ITT dataset only and comprised all subjects who had data available on Day 180 (± 14 days) and for the subjects randomized to receive the 6-months booster vaccination with available data on Day 201 (21 ± 3 days).
The ITT dataset for Day 180 (pre booster vaccination) comprises 501 subjects (243 in Stratum A - adults and 258 in Stratum B - elderly). The post 6-months booster vaccination ITT dataset comprises 243 subjects (116 adults and 127 elderly).

The Day 201 results of the HI assay reported (using horse erythrocytes) were consistently low with respect to all measures i.e. seroprotection rate, seroconversion rate, GMT and GM fold increase from baseline after the 6-months booster vaccination. These tests were inconclusive due to the apparent insensitivity of the HI assay.

Seroneutralisation/seroprotection

The rates of subjects who achieved an antibody titer ≥1:20 measured by MN against the vaccine strain A/Vietnam/1203/2004 or A/ Indonesia/05/2005 after the 6-months booster vaccination are presented in Table 13 (Adults) and Table 14 (Elderly). The rates of subjects with antibody response associated with protection as defined by area $\geq 25 mm^2$ is presented in Table 15.

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Table 13: Number of subjects with neutralising antibody titer ≥1:20, 21 days after the 6-months booster measured by MN assay (intent to treat dataset) - Adults 18-59 years

Strain used for analysis	Day	Booster immunisation with							
		A/Vietnam				A/Indonesia			
		3.75µg		7.5µg		3.75µg		7.5µg	
		n/N	% 95% CI	n/N	% 95% CI	n/N	% 95% CI	n/N	% 95% CI
A/Vietnam	0	1/30	3.3 0.1; 17.2	0/29	0.0 0.0; 11.9	2/30	6.7 0.8; 22.1	0/30	0.0 0.0; 11.6
	21	17/30	56.7 37.4; 74.5	19/29	65.5 45.7; 82.1	15/30	50.0 31.3; 68.7	17/30	56.7 37.4; 74.5
	42	24/30	80.0 61.4; 92.3	23/29	79.3 60.3; 92.0	22/30	73.3 54.1; 87.7	25/30	83.3 65.3; 94.4
	180	12/30	40.0 22.7; 59.4	8/29	27.6 12.7; 47.2	13/30	43.3 25.5; 62.6	11/30	36.7 19.9; 56.1
	201	20/29	69.0 49.2; 84.7	25/29	86.2 68.3; 96.1	21/29	72.4 52.8; 87.3	25/29	86.2 68.3; 96.1
A/Indonesia	0	1/30	3.3 0.1; 17.2	0/29	0.0 0.0; 11.9	0/30	0.0 0.0; 11.6	0/30	0.0 0.0; 11.6
	21	8/30	26.7 12.3; 45.9	7/29	24.1 10.3; 43.5	8/30	26.7 12.3; 45.9	9/30	30.0 14.7; 49.4
	42	14/30	46.7 28.3; 65.7	7/29	24.1 10.3; 43.5	14/30	46.7 28.3; 65.7	12/30	40.0 22.7; 59.4
	180	4/30	13.3 3.8; 30.7	2/29	6.9 0.8; 22.8	9/30	30.0 14.7; 49.4	7/30	23.3 9.9; 42.3
	201	14/29	48.3 29.4; 67.5	19/29	65.5 45.7; 82.1	21/29	72.4 52.8; 87.3	27/29	93.1 77.2; 99.2

Table 14: Number of subjects with neutralising antibody titer ≥1:20, 21 days after the 6-months booster measured by MN assay (ITT dataset) - Elderly ≥60 years

Strain used for analysis	Day	Booster immunisation with							
		A/Vietnam				A/Indonesia			
		3.75µg		7.5µg		3.75µg		7.5µg	
		n/N	% 95% CI	n/N	% 95% CI	n/N	% 95% CI	n/N	% 95% CI
A/Vietnam	0	4/31	12.9 3.6; 29.8	5/32	15.6 5.3; 32.8	8/32	25.0 11.5; 43.4	3/32	9.4 2.0; 25.0
	21	17/31	54.8 36.0; 72.7	17/32	53.1 34.7; 70.9	19/32	59.4 40.6; 76.3	20/32	62.5 43.7; 78.9
	42	24/31	77.4 58.9; 90.4	22/32	68.8 50.0; 83.9	23/32	71.9 53.3; 86.3	24/32	75.0 56.6; 88.5
	180	15/30	50.0 31.3; 68.7	11/30	36.7 19.9; 56.1	14/32	43.8 26.4; 62.3	14/32	43.8 26.4; 62.3
	201	20/31	64.5 45.4; 80.8	20/31	64.5 45.4; 80.8	19/32	59.4 40.6; 76.3	21/32	65.6 46.8; 81.4
A/Indonesia	0	2/30	6.7 0.8; 22.1	1/32	3.1 0.1; 16.2	3/32	9.4 2.0; 25.0	5/32	15.6 5.3; 32.8
	21	8/31	25.8 11.9; 44.6	11/32	34.4 18.6; 53.2	14/32	43.8 26.4; 62.3	17/32	53.1 34.7; 70.9
	42	15/31	48.4 30.2; 66.9	15/32	46.9 29.1; 65.3	20/32	62.5 43.7; 78.9	23/32	71.9 53.3; 86.3
	180	11/30	36.7 19.9; 56.1	7/30	23.3 9.9; 42.3	11/32	34.4 18.6; 53.2	9/32	28.1 13.7; 46.7
	201	17/31	54.8 36.0; 72.7	17/31	54.8 36.0; 72.7	24/32	75.0 56.6; 88.5	23/32	71.9 53.3; 86.3

Table 15: Number of subjects with antibody response associated with protection against A/Vietnam as defined by Single Radial Haemolysis (SRH) area ≥25mm^2 (ITT dataset)

Age group	Day	Booster immunisation with A/Vietnam 3.75µg n/N	A/Vietnam 3.75µg % 95% CI	A/Vietnam 7.5µg n/N	A/Vietnam 7.5µg % 95% CI	A/Indonesia 3.75µg n/N	A/Indonesia 3.75µg % 95% CI	A/Indonesia 7.5µg n/N	A/Indonesia 7.5µg % 95% CI
Adults 18-59 years	**0**	1/30	3.3 0.1; 17.2	2/28	7.1 0.9; 23.5	1/29	3.4 0.1; 17.8	1/30	0.0 0.1; 17.2
	21	20/30	66.7 47.2; 82.7	16/29	55.2 35.7; 73.6	15/29	51.7 32.5; 70.6	18/30	60.0 40.6; 77.3
	42	22/30	73.3 54.1; 87.7	18/29	62.1 42.3; 79.3	19/30	63.3 43.9; 80.1	21/30	70.0 50.6; 85.3
	180	10/30	33.3 17.3; 52.8	6/29	20.7 8.0; 39.7	8/30	26.7 12.3; 45.9	5/30	16.7 5.6; 34.7
	201	15/29	51.7 32.5; 70.6	19/29	65.5 45.7; 82.1	15/29	51.7 32.5; 70.6	20/29	69.0 49.2; 84.7
Elderly >=60 years	**0**	1/30	3.3 0.1; 17.2	3/32	9.4 2.0; 25.0	2/31	6.5 0.8; 21.4	1/31	3.2 0.1; 16.7
	21	16/31	51.6 33.1; 69.8	19/32	59.4 40.6; 76.3	20/32	62.5 43.7; 78.9	19/32	59.4 40.6; 76.3
	42	19/31	61.3 42.2; 78.2	22/32	68.8 50.0; 83.9	22/32	68.8 50.0; 83.9	20/32	62.5 43.7; 78.9
	180	10/30	33.3 17.3; 52.8	7/30	23.3 9.9; 42.3	14/32	43.8 26.4; 62.3	5/32	15.6 5.3; 32.8
	201	18/31	58.1 39.1; 75.5	19/32	59.4 40.6; 76.3	17/32	53.1 34.7; 70.9	13/32	40.6 23.7; 59.4

GM of fold increase

The GMs of fold increase of MN titer post booster vaccination are presented in Table 16 (Adults) and Table 17 (Elderly). The GM of fold increase as measured by SRH assay is shown in Table 18.

In adults aged 18 to 59 years, the highest GM fold increase of MN titer (3.3) was observed in the 7.5µg A/Indonesia/05/2005 booster vaccine group when tested against the A/Indonesia/1205/05 strain. The GM fold increase in SRH area was 2.6 in the 7.5 µg A/Vietnam/1203/2004 dose group and 3.8 in the 7.5 µg A/Indonesia/05/2005 dose group. In elderly subjects, the GM fold increase in MN titer was lower compared to adults. The GM of fold increase in SRH area was only slightly lower than the defined CPMP criterion (>2.0) in the 7.5 µg A/Indonesia/05/2005 dose group (2.0).

Table 16: Geometric Mean fold increase of MN titer measured 21 days after the 6-months booster as compared to baseline (intent to treat dataset) – Adults 18-59 years

Strain used for analysis	Day	Booster immunisation with A/Vietnam 3.75µg N	A/Vietnam 3.75µg GMI 95% CI	A/Vietnam 7.5µg N	A/Vietnam 7.5µg GMI 95% CI	A/Indonesia 3.75µg N	A/Indonesia 3.75µg GMI 95% CI	A/Indonesia 7.5µg N	A/Indonesia 7.5µg GMI 95% CI
A/Vietnam	21[a]	30	3.4 2.5 ; 4.7	29	4.5 3.3 ; 6.2	30	3.1 2.4 ; 3.9	30	3.3 2.5 ; 4.3
	42[a]	30	4.4 3.2 ; 6.1	29	5.6 4.1 ; 7.5	30	4.1 3.1 ; 5.5	30	5.1 4.0 ; 6.5
	201[b]	29	1.6 1.3 ; 2.1	29	1.9 1.6 ; 2.4	29	1.7 1.4 ; 2.1	29	2.1 1.6 ; 2.6
A/Indonesia	21[a]	30	2.1 1.7 ; 2.6	29	2.6 2.0 ; 3.3	30	2.4 1.8 ; 3.2	30	2.3 1.8 ; 2.9
	42[a]	30	2.7 2.2 ; 3.3	29	3.2 2.6 ; 3.9	30	3.2 2.4 ; 4.1	30	3.4 2.7 ; 4.2
	201[b]	29	1.9 1.5 ; 2.4	29	2.5 1.9 ; 3.2	29	2.4 1.9 ; 2.9	29	3.3 2.4 ; 4.6

a Fold increase as compared to Day 0.
b Fold increase as compared to Day 180.

Table 17: Geometric Mean fold increase of MN titer measured 21 days after the 6-months booster as compared to baseline (intent to treat dataset) – Elderly ≥60 years

Strain used for analysis	Day	Booster immunisation with A/Vietnam 3.75µg N	A/Vietnam 3.75µg GMI 95% CI	A/Vietnam 7.5µg N	A/Vietnam 7.5µg GMI 95% CI	A/Indonesia 3.75µg N	A/Indonesia 3.75µg GMI 95% CI	A/Indonesia 7.5µg N	A/Indonesia 7.5µg GMI 95% CI
A/Vietnam	21[a]	31	2.6 2.0 ; 3.4	32	2.0 1.6 ; 2.5	32	1.8 1.5 ; 2.1	32	2.3 1.8 ; 3.0
	42[a]	31	3.4 2.7 ; 4.3	32	2.9 2.2 ; 3.6	32	2.2 1.9 ; 2.7	32	2.8 2.2 ; 3.7
	201[b]	30	1.4 1.2 ; 1.6	29	1.7 1.2 ; 2.4	32	1.5 1.2 ; 1.8	32	1.7 1.4 ; 2.2
A/Indonesia	21[a]	30	1.6 1.4 ; 1.9	32	1.5 1.3 ; 1.8	32	1.5 1.4 ; 1.7	32	1.8 1.5 ; 2.3
	42[a]	30	2.0 1.6 ; 2.5	32	2.0 1.7 ; 2.4	32	1.9 1.6 ; 2.2	32	2.2 1.7 ; 2.8
	201[b]	30	1.4 1.2 ; 1.7	29	1.9 1.5 ; 2.4	32	1.8 1.4 ; 2.3	32	2.3 1.7 ; 3.0

a Fold increase as compared to Day 0.
b Fold increase as compared to Day 180.

Table 18: Geometric Mean fold increase of antibody response against strain A/Vietnam measured by SRH assay as compared to baseline (intent to treat dataset)

Age group	Day	Booster immunisation with A/Vietnam 3.75µg N	A/Vietnam 3.75µg GMI 95% CI	A/Vietnam 7.5µg N	A/Vietnam 7.5µg GMI 95% CI	A/Indonesia 3.75µg N	A/Indonesia 3.75µg GMI 95% CI	A/Indonesia 7.5µg N	A/Indonesia 7.5µg GMI 95% CI
Adults 18-59 years	**21**[a]	30	5.1 3.2 ; 8.3	28	3.2 2.0 ; 5.3	28	4.4 2.6 ; 7.4	30	4.0 2.5 ; 6.4
	42[a]	30	6.4 4.1 ; 10.1	28	4.3 2.6 ; 7.0	29	5.6 3.4 ; 9.1	30	5.3 3.4 ; 8.3
	201[b]	29	1.7 1.2 ; 2.4	29	2.6 1.6 ; 4.2	29	1.7 1.2 ; 2.5	29	3.8 2.4 ; 5.9
Elderly >=60 years	**21**[a]	30	3.6 2.3 ; 5.6	32	3.1 2.1 ; 4.7	31	3.5 2.2 ; 5.6	31	4.3 2.6 ; 7.0
	42[a]	30	4.5 2.8 ; 7.1	32	4.3 2.8 ; 6.5	31	4.1 2.6 ; 6.4	31	4.7 2.9 ; 7.7
	201[b]	30	1.9 1.3 ; 2.7	30	2.8 1.8 ; 4.3	32	1.3 1.0 ; 1.7	32	2.0 1.4 ; 2.9

a Fold increase as compared to Day 0.
b Fold increase as compared to Day 180.

Seroconversion

The number of subjects with cross-strain seroconversion (defined as a >4 fold increase in MN titer/50% increase in haemolysis area 21 days after booster vaccination) was low across both dose groups strains. This is most likely due to the higher percentage of subjects with pre-existing antibodies elicited by the primary vaccination series with A/Vietnam/1203/2004 vaccine 6 months prior to the booster (Table 19, Table 20 and Table 21).

Table 19: Number of subjects with seroconversion (defined as a >=4 fold increase after vacc.) measured by MN titer 21 days after the 6-months booster as compared to baseline (intent to treat dataset) – Adults 18-59 years

Strain used for analysis	Day	Booster immunisation with A/Vietnam 3.75µg n/N	A/Vietnam 3.75µg % 95% CI	A/Vietnam 7.5µg n/N	A/Vietnam 7.5µg % 95% CI	A/Indonesia 3.75µg n/N	A/Indonesia 3.75µg % 95% CI	A/Indonesia 7.5µg n/N	A/Indonesia 7.5µg % 95% CI
A/Vietnam	**21**[a]	10/30	33.3 17.9; 52.8	17/29	58.6 38.9; 76.5	8/30	26.7 12.3; 45.9	12/30	40.0 22.7; 59.4
	42[a]	15/30	50.0 31.3; 68.7	21/29	72.4 52.8; 87.3	17/30	56.7 37.4; 74.5	22/30	73.3 54.1; 87.7
	201[b]	2/29	6.9 0.8; 22.8	4/29	13.8 3.9; 31.7	1/29	3.4 0.1; 17.8	3/29	10.3 2.2; 27.4
A/Indonesia	**21**[a]	3/30	10.0 2.1; 26.5	7/29	24.1 10.3; 43.5	8/30	26.7 12.3; 45.9	4/30	13.3 3.8; 30.7
	42[a]	8/30	26.7 12.3; 45.9	10/29	34.5 17.9; 54.3	11/30	36.7 19.9; 56.1	7/30	23.3 9.9; 42.3
	201[b]	3/29	10.3 2.2; 27.4	7/29	24.1 10.3; 43.5	5/29	17.2 5.8; 35.8	10/29	34.5 17.9; 54.3

a Fold increase as compared to Day 0.
b Fold increase as compared to Day 180.

Table 20: Number of subjects with seroconversion (defined as a >=4 fold increase after vacc.) measured by MN titer 21 days after the 6-months booster as compared to baseline (intent to treat dataset) – Elderly ≥60 years

Strain used for analysis	Day	Booster immunisation with A/Vietnam 3.75µg n/N	A/Vietnam 3.75µg % 95% CI	A/Vietnam 7.5µg n/N	A/Vietnam 7.5µg % 95% CI	A/Indonesia 3.75µg n/N	A/Indonesia 3.75µg % 95% CI	A/Indonesia 7.5µg n/N	A/Indonesia 7.5µg % 95% CI
A/Vietnam	21[a]	6/31	19.4 7.5; 37.5	5/32	15.6 5.3; 32.8	1/32	3.1 0.1; 16.2	6/32	18.8 7.2; 36.4
	42[a]	13/31	41.9 24.5; 60.9	8/32	25.0 11.5; 43.4	5/32	15.6 5.3; 32.8	10/32	31.3 16.1; 50.[illegible]
	201[b]	1/30	3.3 0.1; 17.2	3/29	10.3 2.2; 27.4	1/32	3.1 0.1; 16.2	3/32	9.4 2.0; 25.0
A/Indonesia	21[a]	1/30	3.3 0.1; 17.2	0/32	0.0 0.0; 10.9	0/32	0.0 0.0; 10.9	6/32	18.8 7.2; 36.4
	42[a]	4/30	13.3 3.8; 30.7	2/32	6.3 0.8; 20.8	1/32	3.1 0.1; 16.2	7/32	21.9 9.3; 40.0
	201[b]	2/30	6.7 0.8; 22.1	3/29	10.3 2.2; 27.4	2/32	6.3 0.8; 20.8	6/32	18.8 7.2; 36.4
a	Fold increase as compared to Day 0.								
b	Fold increase as compared to Day 180.								

Table 21: Number of subjects with seroconversion measured by SRH assay using strain A/Vietnam 21 days after the 6-months booster vaccinations (intent to treat dataset)

Age group	Day	Booster immunisation with A/Vietnam 3.75µg n/N	A/Vietnam 3.75µg % 95% CI	A/Vietnam 7.5µg n/N	A/Vietnam 7.5µg % 95% CI	A/Indonesia 3.75µg n/N	A/Indonesia 3.75µg % 95% CI	A/Indonesia 7.5µg n/N	A/Indonesia 7.5µg % 95% CI
Adults 18-59 years	21[a]	19/30	63.3 43.9; 80.1	13/28	46.4 27.5; 66.1	15/28	53.6 33.9; 72.5	17/30	56.7 37.4; 74.5
	42[a]	21/30	70.0 50.6; 85.3	16/28	57.1 37.2; 75.5	18/29	62.1 42.3; 79.3	21/30	70.0 50.6; 85.3
	201[b]	6/29	20.7 8.0; 39.7	14/29	48.3 29.4; 67.5	7/29	24.1 10.3; 43.5	17/29	58.6 38.9; 76.5
Elderly >=60 years	21[a]	16/30	53.3 34.3; 71.7	17/32	53.1 34.7; 70.9	17/31	54.8 36.0; 72.7	17/31	54.8 36.0; 72.7
	42[a]	18/30	60.0 40.6; 77.3	20/32	62.5 43.7; 78.9	19/31	61.3 42.2; 78.2	18/31	58.1 39.1; 75.5
	201[b]	7/30	23.3 9.9; 42.3	14/30	46.7 28.3; 65.7	5/32	15.6 5.3; 32.8	8/32	25.0 11.5; 43.4
a	Fold increase as compared to Day 0.								
b	Fold increase as compared to Day 180.								

Antibody responses following the 12 to 15 months booster against strain A/Indonesia are shown in Table 22. , The data showed that booster vaccinations of either 3.75 µg or 7.5 µg HA antigen given 12 to 15 months apart elicit an anamnestic immune responses. The heterologous booster using strain A/Indonesia/05/2005 elicited a good immune response against both strains. These results were consistent with those of previously submitted studies.

Table 22: Number of subjects with seroconversion measured by MN assay using strain A/Vietnam and A/Indonesia 21 days after the 12-15 months booster vaccinations (intent to treat dataset)

Strain	Day	Booster immunisation with A/Indonesia A(18-59 years) 3.75µg n/N	3.75µg % 95% CI	7.5µg n/N	7.5µg % 95% CI	A/Indonesia B(≥60 years) 3.75µg n/N	3.75µg % 95% CI	7.5µg n/N	7.5µg % 95% CI
A/Vietnam	21[a]	13/29	44.8 26.4;64.3	11/29	37.9 20.7; 75.7	19/31	61.3 42.2;78.2	15/31	48.4 30.2;66.9
	42[a]	22/29	75.9 56.5;89.7	18/29	62.1 42.3; 79.3	21/31	67.7 48.6;83.3	23/31	74.2 55.4;88.
	381-471[b]	21/28	75 55.1;89.3	28/29	96.6 82.2;99.9	15/31	48.4 30.2;66.9	26/31	83.9 66.3;94.5
A/Indonesia	21[a]	5/29	17.2 5.8;35.8	5/28	17.9 6.1;36.9	14/31	45.2 27.3;64.0	10/31	32.3 16.7;51.4
	42[a]	10/29	34.5 17.9;54.3	11/29	37.9 20.7;57.7	20/31	64.5 45.4;80.8	16/31	51.6 33.1;69.8
	201[b]	22/28	78.6 59.0;91.7	27/29	93.1 77.2;99.2	25/31	80.6 62.5;92.5	26/31	83.9 66.3;94.5

a Fold increase as compared to Day 0.
b Fold increase as compared to Day 360-450.

Based on these data it can be concluded that a homologous or heterologous booster immunisation has no added value as regards higher seroconversion rates but might elicit stronger cross-reactive antibody responses. Generally the antibody responses following the homologous and heterologous booster are however less pronounced compared to study 810703 indicating a moderate anamnestic response. In summary the responses are comparable to what is expected for seasonal revaccination.

Ancillary analyses

- Supportive studies

Study 810701

Study 810701 is an open-label Phase I/II study to assess the safety and immunogenicity of two doses of a Vero cell-derived, whole virion clade 2 H5N1 Influenza vaccine (strain A/Indonesia, 3.75µg and 7.5µg) in 110 healthy adult males and females aged 21 to 45 years. This multi-centre study is conducted in 4 centres in Hong Kong and Singapore.

Subjects were randomized 1:1 to receive 2 intramuscular injections of the whole virion, Vero cell-derived influenza vaccine containing either 3.75µg or 7.5µg H5N1 haemagglutinin (HA) antigen, strain A/Indonesia/05/2005, in a non-adjuvanted formulation on Day 0 and Day 21.
The study is being conducted in 2 parts:

- Part A was concluded 21(± 2) days after the second vaccination (Day 42 visit). These data are provided in the response document at day 121.
- All subjects will be monitored until Day 180 (±14 days) after the first vaccination. After the last subject has completed the Day 180 visit, a final clinical study report including all safety and immunogenicity data collected will be written.

The primary endpoints for evaluation were:

- Frequency and severity of systemic reactions after the first and second vaccinations
- Number of subjects with antibody response to the vaccine strain (A/Indonesia/05/2005) associated with protection 21 days after the second vaccination defined as titer measured by Microneutralisation (MN) test ≥ 1:20

Further immunogenicity endpoints included the analysis of seroconversion, GM fold increase and GMT by MN assay and the evaluation by SRH assay.

Antibody response was analyzed for all subjects vaccinated with data available after the first and second vaccinations (ITT dataset). MN and SRH analyses were performed on 107 subjects for the first vaccination (55 vaccinated with the 3.75 µg dose, 52 vaccinated with the 7.5 µg dose), and 104 subjects after the second vaccination (52 vaccinated with the 3.75 µg dose, 52 vaccinated with the 7.5 µg dose).

Antibody response against the homologous strain A/Indonesia:
The neutralising antibody responses following the 2 doses against the homologous strain A/Indonesia are summarised in Table 23.

A neutralising antibody response defined as percentage with MN titres >= 1:20 21 days after the second vaccination for the vaccine strain, was found in 82.7% and 86.5% of subjects vaccinated with the 3.75µg or 7.5µg dose, respectively. Seroconversion defined as ≥4-fold increase in MN titer 21 days after vaccination as compared to baseline, was achieved after the first vaccination in 40.0% and 25.0% of subjects, and after the second vaccination in 82.7% and 86.5%, in the 3.75µg and 7.5µg dose groups, respectively. The GMT was 12.8 vs. 13.6 after the first and 34.5 vs. 36.0 after the second vaccination in the 3.75µg and 7.5µg dose groups, respectively. GM fold increase in MN titer was 3.0 vs. 3.1 after the first and 8.0 vs. 8.3 after the second vaccination in the 3.75µg dose group and in the 7.5µg dose group.

Table 23: Immunogenicity evaluation using the MN assay and wild type strain A/Indonesia (ITT dataset)

	Study groups					
	3.75µg non-adjuvanted			**7.5 µg non-adjuvanted**		
Seroneutralisation rates (MN titer >=1:20) 21 days after 1st/2nd vaccination						
Day	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**
0	0/55	0.0	0.0; 6.5	0/52	0.0	0.0; 6.8
21	20/55	36.4	23.8; 50.4	10/52	19.2	9.6; 32.5
42	43/52	82.7	69.7; 91.8	45/52	86.5	74.2; 94.4
Seroconversion rates 21 days after the 1st and 2nd vaccination as compared to baseline						
Day	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**
21	22/55	40.0	27.0; 54.1	13/52	25.0	14.0; 38.9
42	43/52	82.7	69.7; 91.8	45/52	86.5	74.2; 94.4
Geometric Mean fold Increase measured 21 days after 1st/2nd vaccination as compared to baseline						
Day	**N**	**GMI**	**95% CI**	**N**	**GMI**	**95% CI**
21	55	3.0	2.4 ; 3.7	52	3.1	2.6 ; 3.7
42	52	8.0	6.4 ; 10.1	52	8.3	6.8 ; 10.1

The antibody responses as measured by the SRH assay are given in Table 24. Antibody response associated with protection 21 days after the second vaccination for the vaccine strain, as defined by SRH area ≥25 mm^2 was determined in 71.2% and 69.2% of subjects vaccinated with the 3.75µg or 7.5µg dose, respectively. Seroconversion for the vaccine strain was shown in 38.2% vs. 38.5% after the first, and 71.2% vs. 67.3% of subjects after the second vaccination in the 3.75µg or 7.5µg dose groups, respectively. Antibody response determined by SRH assay, expressed as GM of haemolysis area (GMT) for the vaccine strain was also similar between the dose groups: 11.8 and 10.5 after the

first and 20.9 vs. 22.8 after the second vaccination in the 3.75µg and 7.5g dose groups, respectively. GM fold increase in antibody response measured by SRH in subjects in the 3.75µg and 7.5µg dose groups, respectively, with 2.8 vs. 2.5 after the first, and 5.0 vs. 5.4 after the second vaccination.

Table 24: Immunogenicity evaluation using the SRH assay and wild type strain A/Indonesia (ITT dataset)

	Study groups					
Seroprotection rates (SRH area >=25 mm^2) 21 days after 1st/2nd vaccination						
Day	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**
0	0/55	0.0	0.0; 6.5	1/52	1.9	0.0; 10.3
21	21/55	38.2	25.4; 52.3	21/52	40.4	27.0; 54.9
42	37/52	71.2	56.9; 82.9	36/52	69.2	54.9; 81.3
Seroconversion rates 21 days after the 1st and 2nd vaccination as compared to baseline						
Day	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**
21	21/55	38.2	25.4; 52.3	20/52	38.5	25.3; 53.0
42	37/52	71.2	56.9; 82.9	35/52	67.3	52.9; 79.7
Geometric Mean fold Increase measured 21 days after 1st/2nd vaccination as compared to baseline						
Day	**N**	**GM**	**95% CI**	**N**	**GM**	**95% CI**
21	55	2.8	2.1 ; 3.8	52	2.5	1.8 ; 3.4
42	52	5.0	3.8 ; 6.6	52	5.4	4.1 ; 7.1

In summary, the results of study 810701 indicate again that no true dose-response relation exists. The responsiveness of a lower dose of 3.75µg HA strain A/Indonesia is similar to a dose of 7.5µg HA strain A/Indonesia. Moreover the SPRs, SCRs and GMI determined by MN and SRH assay are consistent with the results of main study 810601. However, it should be noted that subjects enrolled in study 810701 had no baseline neutralising antibody titres and only 1 subject was positive as measured by SRH assay.

Cross-reactivity against A/Vietnam determined by MN

The rate of subjects with reciprocal MN titer ≥20 against a heterologous clade 1 strain (A/Vietnam/1203/2004) 21 days after the first and second vaccination is given in Table 25.

Table 25: Cross-Reactivity: Number of subjects with antibody titer ≥ 1:20, 21 days after the 1st/2nd vaccination measured by MN assay (ITT dataset)

Strain used for analysis	**Day**	**Study groups vaccinated with strain A/Indonesia**					
		3.75µg non-adjuvanted			**7.5 µg non-adjuvanted**		
		n/N	**%**	**95% CI**	**n/N**	**%**	**95% CI**
A/Vietnam	**0**	2/55	3.6	0.4; 12.5	1/52	1.9	0.0; 10.3
	21	11/55	20.0	10.4; 33.0	6/52	11.5	4.4; 23.4
	42	13/52	25.0	14.0; 38.9	11/52	21.2	11.1; 34.7

Clinical safety

- Patient exposure

Safety data are available from both clinical studies (810501 and 810601). In total 796 subjects were vaccinated with two doses of different vaccine formulations 21 days apart. 602 subjects received at least one dose of the vaccine formulation (7.5µg HA non-adjuvanted) intended for pandemic use.

- Adverse events

Special queried systemic and local adverse events were monitored by diary cards for 7 days after each vaccination. All adverse events were recorded for 21 days following each dose and for the time period 42 -180 days after first vaccination. For study 810601 all adverse events were reported for the time period 42 days after first vaccination for both age groups. Long-term 6-months follow-up data were provided during the procedure. Therefore the total number exposed is considered to be sufficient for a core dossier application as adverse reactions or events at a frequency of approximately 1% are detectable.

Study 810501

A total of 275 subjects received the first vaccination (on Day 0) and 257 subjects received the second vaccination (on Day 21) with the whole virion, Vero cell-derived influenza vaccine containing 3.75µg, 7.5µg, 15µg or 30µg H5N1 HA antigen/dose in an adjuvanted formulation with aluminium hydroxide, or 7.5µg or 15µg H5N1 HA antigen/dose in a non-adjuvanted formulation.

The occurrence of fever with onset within 7 days after the 1st and 2nd vaccination is provided in Table 26 and Table 27.

Table 26: Number of subjects with fever after 1st vaccination by severity grade (Study 810501)

			Severity of fever								
		NA	No reaction		Mild		Moderate		Severe		Total
Study group	N	%	N	%	N	%	N	%	N	%	N
3.75µg +Al	0	(0.0%)	44	(97.8%)	0	(0.0%)	1	(2.2%)	0	(0.0%)	45
7.5µg +Al	0	(0.0%)	43	(95.6%)	2	(4.4%)	0	(0.0%)	0	(0.0%)	45
15µg +Al	2	(4.3%)	42	(91.3%)	2	(4.3%)	0	(0.0%)	0	(0.0%)	46
30µg +Al	0	(0.0%)	48	(98.0%)	1	(2.0%)	0	(0.0%)	0	(0.0%)	49
7.5µg	0	(0.0%)	45	(100.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	45
15µg	1	(2.2%)	43	(95.6%)	1	(2.2%)	0	(0.0%)	0	(0.0%)	45
Total	3	(1.1%)	265	(96.4%)	6	(2.2%)	1	(0.4%)	0	(0.0%)	275

Table 27: Number of subjects with fever after 2nd vaccination by severity grade (St. 810501)

			Severity of fever								
		NA	No reaction		Mild		Moderate		Severe		Total
Study group	N	%	N	%	N	%	N	%	N	%	N
3.75µg +Al	0	(0.0%)	42	(100.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	42
7.5µg +Al	1	(2.4%)	40	(95.2%)	1	(2.4%)	0	(0.0%)	0	(0.0%)	42
15µg +Al	1	(2.3%)	42	(97.7%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	43
30µg +Al	0	(0.0%)	44	(97.8%)	1	(2.2%)	0	(0.0%)	0	(0.0%)	45
7.5µg	0	(0.0%)	40	(95.2%)	1	(2.4%)	1	(2.4%)	0	(0.0%)	42
15µg	2	(4.7%)	38	(88.4%)	3	(7.0%)	0	(0.0%)	0	(0.0%)	43
Total	4	(1.6%)	246	(95.7%)	6	(2.3%)	1	(0.4%)	0	(0.0%)	257

Specifically queried symptoms of local and systemic reactions that occurred within 7 days after the first and second immunisation are shown in Table 28 and Table 29.

Table 28: Specifically queried symptoms of local and systemic reactions (other than malaise and shivering) related to the 1st vaccination

Reported Term	Preferred Term	3.75µg +Al n (%) N=45	7.5µg +Al n (%) N=45	15µg +Al n (%) N=46	30µg +Al n (%) N=49	7.5µg n (%) N=45	15µg n (%) N=45
Swelling	Injection site swelling	0 (0.0%)	0 (0.0%)	1 (2.2%)	1 (2.0%)	0 (0.0%)	0 (0.0%)
Induration	Injection site induration	0 (0.0%)	1 (2.2%)	0 (0.0%)	1 (2.0%)	0 (0.0%)	2 (4.4%)
Redness	Injection site erythema	0 (0.0%)	1 (2.2%)	2 (4.3%)	0 (0.0%)	1 (2.2%)	0 (0.0%)
Injection Site Pain	Injection site pain	11 (24.4%)	8 (17.8%)	12 (26.1%)	11 (22.4%)	4 (8.9%)	8 (17.8%)
Ecchymosis	Injection site haemorrhage	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.0%)	0 (0.0%)	1 (2.2%)
Fatigue	Fatigue	5 (11.1%)	6 (13.3%)	7 (15.2%)	4 (8.2%)	3 (6.7%)	7 (15.6%)
Headache	Headache	11 (24.4%)	8 (17.8%)	5 (10.9%)	4 (8.2%)	5 (11.1%)	10 (22.2%)
Sweating	Hyperhidrosis	3 (6.7%)	3 (6.7%)	4 (8.7%)	2 (4.1%)	2 (4.4%)	2 (4.4%)
Muscle pain	Myalgia	4 (8.9%)	6 (13.3%)	4 (8.7%)	1 (2.0%)	2 (4.4%)	4 (8.9%)
Joint pain	Arthralgia	4 (8.9%)	4 (8.9%)	4 (8.7%)	2 (4.1%)	1 (2.2%)	3 (6.7%)
Fever with onset later than Day 7 after vacc.	Pyrexia	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 29: Specifically queried symptoms of local and systemic reactions (other than malaise and shivering) related to the 2nd vaccination

Reported Term	Preferred Term	3.75µg+Al n(%) N=42	7.5µg+Al n(%) N=42	15µg+Al n(%) N=43	30µg+Al n(%) N=45	7.5µg n(%) N=42	15µg n(%) N=43
Swelling	Injection site swelling	0 (0.0%)	1 (2.4%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Induration	Injection site induration	2 (4.8%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Redness	Injection site erythema	0 (0.0%)	1 (2.4%)	0 (0.0%)	0 (0.0%)	1 (2.4%)	0 (0.0%)
Injection Site Pain	Injection site pain	6 (14.3%)	4 (9.5%)	8 (18.6%)	5 (11.1%)	5 (11.9%)	7 (16.3%)
Ecchymosis	Injection site haemorrhage	0 (0.0%)	1 (2.4%)	0 (0.0%)	1 (2.2%)	0 (0.0%)	1 (2.3%)
Fatigue	Fatigue	3 (7.1%)	4 (9.5%)	5 (11.6%)	2 (4.4%)	2 (4.8%)	5 (11.6%)
Headache	Headache	7 (16.7%)	3 (7.1%)	4 (9.3%)	5 (11.1%)	1 (2.4%)	4 (9.3%)
Sweating	Hyperhidrosis	1 (2.4%)	2 (4.8%)	0 (0.0%)	1 (2.2%)	2 (4.8%)	2 (4.7%)
Muscle pain	Myalgia	5 (11.9%)	1 (2.4%)	1 (2.3%)	0 (0.0%)	1 (2.4%)	3 (7.0%)
Joint pain	Arthralgia	0 (0.0%)	2 (4.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Fever with onset later than Day 7 after vac.	Pyrexia	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

The analysis of the primary and secondary safety endpoints did not show any dose dependency or adjuvant effect, however, with respect to local reactions, there was a trend towards better tolerability in the absence of adjuvant. In the study group receiving 7.5 µg non-adjuvanted vaccine, the probability of occurrence of systemic reactions (including fever) was 24.4% and 14.3% after the first and second vaccinations, respectively. Fever was reported in this group in 0.0% of subjects after the first and in 4.8% after the second vaccination. No fever with onset later than Day 7 after vaccination was reported. Systemic reactions (excluding fever) were reported in 28.4% of subjects after the first and in 20.6% of subjects after the second vaccination. The severity of these reactions after the first and second vaccinations was mild in all but 4 (1.5%) and 1 (0.4%) subjects who reported moderate reactions after the first and second vaccinations, respectively. Malaise occurred in 9.5% of subjects after the first vaccination and in 6.6% of subjects after the second vaccination. The majority of cases were mild (8.4% and 6.2% after the first and second vaccination, respectively), with very few moderate cases reported. Shivering was reported less frequently: in 4.3% of subjects after the first and in 2.7% of subjects after the second vaccination. The most frequently reported queried symptoms of systemic reactions were headache, fatigue, and muscle pain.

All local reactions which occurred after the first and second vaccinations were mild in intensity and were reported in 22.5% and 15.2% of subjects, respectively. Injection site pain was the most frequently reported queried symptom of local reactions in all study groups. Among the other queried symptoms of local reactions (swelling, induration, erythema and ecchymosis) none occurred in more than a total of 4 subjects (0.0% to 4.4% of subjects per study group) after both the first and second vaccinations. As expected, between Day 42 and 180 (Part B of the study) there was a very low probability of occurrence of related AEs. Only one subject reported non-serious systemic symptoms

(diagnosed with upper respiratory tract infection 32 and 132 days after the second vaccination), which was judged as possibly related to study product.

Study 810601

A total of 561 subjects (281 adults and 280 elderly) received the first vaccination and 539 subjects (269 adults and 270 elderly) received the second vaccination 21 days later with the inactivated whole virion, Vero cell-derived vaccine containing 7.5µg H5N1 HA antigen, strain A/Vietnam/1203/2004.

The occurrence of fever with onset within 7 days after the 1st and 2nd vaccination is provided in Table 30 and Table 31.

Table 30: Number of subjects with fever with onset within 7 days after 1st vaccination by severity grade (full analysis dataset)

Age group		NAV	Severity of fever: No reaction		Mild		Moderate		Severe		Total
	N	%	N	%	N	%	N	%	N	%	N
18-59 yrs	5	(1.8%)	270	(96.1%)	4	(1.4%)	2	(0.7%)	0	(0.0%)	281
≥60 yrs	5	(1.8%)	272	(97.1%)	3	(1.1%)	0	(0.0%)	0	(0.0%)	280
Total	10	(1.8%)	542	(96.6%)	7	(1.2%)	2	(0.4%)	0	(0.0%)	561

Table 31: Number of subjects with fever with onset within 7 days after 2nd vaccination by severity grade (full analysis dataset)

Age group		NAV	Severity of fever: No reaction		Mild		Moderate		Severe		Total
	N	%	N	%	N	%	N	%	N	%	N
18-59 yrs	4	(1.5%)	264	(98.1%)	1	(0.4%)	0	(0.0%)	0	(0.0%)	269
≥60 yrs	2	(0.7%)	266	(98.5%)	1	(0.4%)	1	(0.4%)	0	(0.0%)	270
Total	6	(1.1%)	530	(98.3%)	2	(0.4%)	1	(0.2%)	0	(0.0%)	539

Specifically queried symptoms of local and systemic reactions that occurred within 7 days after the first and second immunisation are shown in Table 31 and Table 32.

Table 32: Specifically queried symptoms of local and systemic reactions (other than malaise and shivering) related to the 1st vaccination (full analysis dataset)

Reported Term	Preferred Term	Age group	
		18-59 yrs	≥60 yrs
		n/N (%) 95% C.I.	n/N (%) 95% C.I.
Swelling	Injection site swelling	2/281 (0.7%) (0.1% ; 2.5%)	4/280 (1.4%) (0.4% ; 3.6%)
Induration	Injection site induration	6/281 (2.1%) (0.8% ; 4.6%)	5/280 (1.8%) (0.6% ; 4.1%)
Redness	Injection site erythema	1/281 (0.4%) (0.0% ; 2.0%)	2/280 (0.7%) (0.1% ; 2.6%)
Injection Site Pain	Injection site pain	44/281 (15.7%) (11.6% ; 20.4%)	16/280 (5.7%) (3.3% ; 9.1%)
Ecchymosis	Injection site haemorrhage	4/281 (1.4%) (0.4% ; 3.6%)	0/280 (0.0%) (0.0% ; 1.3%)
Fatigue	Fatigue	23/281 (8.2%) (5.3% ; 12.0%)	21/280 (7.5%) (4.7% ; 11.2%)
Headache	Headache	27/281 (9.6%) (6.4% ; 13.7%)	27/280 (9.6%) (6.5% ; 13.7%)
Sweating	Hyperhidrosis	12/281 (4.3%) (2.2% ; 7.3%)	14/280 (5.0%) (2.8% ; 8.2%)
Muscle pain	Myalgia	11/281 (3.9%) (2.0% ; 6.9%)	9/280 (3.2%) (1.5% ; 6.0%)
Joint pain	Arthralgia	4/281 (1.4%) (0.4% ; 3.6%)	14/280 (5.0%) (2.8% ; 8.2%)
Fever with onset later than Day 7 after vaccination	Pyrexia	0/281 (0.0%) (0.0% ; 1.3%)	0/280 (0.0%) (0.0% ; 1.3%)

Table 33: Specifically queried symptoms of local and systemic reactions (other than malaise and shivering) related to the 2nd vaccination (full analysis dataset)

Reported Term	Preferred Term	Age group	
		18-59 yrs	≥60 yrs
		n/N (%) 95% C.I.	n/N (%) 95% C.I.
Swelling	Injection site swelling	1/269 (0.4%) (0.0% ; 2.1%)	4/270 (1.5%) (0.4% ; 3.7%)
Induration	Injection site induration	2/269 (0.7%) (0.1% ; 2.7%)	4/270 (1.5%) (0.4% ; 3.7%)
Redness	Injection site erythema	0/269 (0.0%) (0.0% ; 1.4%)	5/270 (1.9%) (0.6% ; 4.3%)
Injection Site Pain	Injection site pain	37/269 (13.8%) (9.9% ; 18.5%)	8/270 (3.0%) (1.3% ; 5.8%)
Ecchymosis	Injection site haemorrhage	1/269 (0.4%) (0.0% ; 2.1%)	1/270 (0.4%) (0.0% ; 2.0%)
Fatigue	Fatigue	18/269 (6.7%) (4.0% ; 10.4%)	12/270 (4.4%) (2.3% ; 7.6%)
Headache	Headache	14/269 (5.2%) (2.9% ; 8.6%)	17/270 (6.3%) (3.7% ; 9.9%)
Sweating	Hyperhidrosis	7/269 (2.6%) (1.1% ; 5.3%)	9/270 (3.3%) (1.5% ; 6.2%)
Muscle pain	Myalgia	6/269 (2.2%) (0.8% ; 4.8%)	9/270 (3.3%) (1.5% ; 6.2%)
Joint pain	Arthralgia	6/269 (2.2%) (0.8% ; 4.8%)	12/270 (4.4%) (2.3% ; 7.6%)
Fever with onset later than Day 7 after vaccination	Pyrexia	0/269 (0.0%) (0.0% ; 1.4%)	0/270 (0.0%) (0.0% ; 1.4%)

The probability of occurrence of systemic reactions (including fever) within 21 days after the first vaccination was 27.8% in adults and 23.3% in elderly subjects. The majority of subjects reported no fever within 7 days after the first and second vaccinations in both age strata. After the first vaccination, the occurrence of fever was 2.2% in the group of adults, and 1.1% in the elderly. After the second vaccination, the occurrence of fever within 7 days after vaccination was 0.4% and 0.7% in adults and elderly. No fever case lasted more than 2 days. Of the few fever cases reported, most were mild. There was no severe fever in either age stratum after either vaccination.

The probability of occurrence of malaise after the first vaccination was 6.4% in both age strata; after the second vaccination**,** 3.7% in adults, and 4.1% in elderly subjects. Malaise after the first vaccination in adults was reported mostly as mild (5.3%), 2 were moderate (0.7%), and 1 (0.4%) severe. The rates of malaise by severity were generally similar in elderly subjects (5.7% mild and 0.7% moderate), and none severe. After the second vaccination, mild or moderate malaise was reported in 6 (2.2%), and 4 adult subjects (1.5%), respectively, and 10 (3.7%) and 1 elderly subject (0.4%), respectively. The probability of occurrence of shivering after the first vaccination was 3.6% in adults and 4.6% in elderly; the rates were lower after the second vaccination: 1.1% and 1.9%, adult and elderly subjects, respectively. Reports of shivering were predominantly mild, with a few moderate cases reported, none were severe.

Local reactions after the first vaccination occurred at a rate of 17.1% in adults aged 18-59 years, and 8.6% in subjects 60 years and older, and in 14.5% and 6.3% of subjects after the second vaccination, respectively. Most of the local reactions were mild after each vaccination (15.7% and 8.2% after the first, and 13.8% and 5.9% after the second vaccination, respectively).

The follow-up data to 6 months after the first vaccination for all subjects were available during the procedure. None of the 503 subjects experienced systemic reactions and new adverse reaction in the period between day 42 and day 180. All systemic symptoms or diagnosis of AEs reported between Day 42 and 180 were considered unrelated to vaccination.

Systemic reactions within 21 days after the 6-months booster dose were mostly mild. One subject experienced moderate reactions (chills, nasopharyngitis, arthralgia and headache) in the group of adults. There were no severe systemic reactions. Safety data following the 12 to 15 months booster vaccinations (Part C of study 810601) did not raise any additional concerns.

- Serious adverse event/deaths/other significant events

Study 810501
During the 42 day and 180 day follow-up of the study, no SAEs related to the vaccination, deaths or other significant AEs were reported.

Study 810601
A total of 9 SAEs were reported during the 42 day follow-up of the study. Eight SAEs were considered unrelated to vaccination. One SAE (malaria tertiana reactivation) was judged related to vaccination by the investigator. The subject has a history of malaria tertiana since August 2006 and experienced an episode of reactivation of malaria tertiana previously in November 2006.

Within 21 days after the 6-month booster dose three subjects reported severe AEs (2 adults and 1 elderly subject), who suffered from nasopharyngitis, uveitis and spinal stenosis.

In addition, no deaths, no serious adverse events (SAEs) associated with the vaccines, and no unexpected AEs were noted following the 12 to 15 months booster vaccinations (Part C of the study).

- Laboratory findings

Alanine aminotransferase (ALT) values were tested in a subpopulation (N=51) in study 810601.
There were no clinically significant increases in ALT. Slightly elevated ALT values were detected in 3 subjects. All elevated ALT values were assessed as not related to vaccination by an independent DMC and the responsible investigators.

- Safety in special populations

A comparison of injection site reactions between the two age strata in Study 810601 showed that injection site pain was reported more often by the younger population than by the elderly. Joint pain and sweating was reported less often by the younger population than by the elderly.

- Safety related to drug-drug interactions and other interactions

Not applicable

- Discontinuation due to adverse events

810501: Two subjects stated adverse events experienced after the first vaccination as the reason for withdrawing their informed consent. These AEs were non-serious and were of mild or moderate severity, however, they were considered by the investigator to be related to the vaccination and included arthralgia, chills, eye discharge, fatigue, headache, hyperhidrosis, hypoesthesia, injection site

pain, malaise, myalgia, generalized pruritus and insomnia for one subject and arthralgia, myalgia, papular rash for another.

810601: One subject reported an AE as the reason for withdrawal. This subject experienced severe malaise and mild fatigue 3 days after the first vaccination which were considered to be probably related to vaccination and which lasted 7 days.

- Post marketing experience

There is no post-marketing experience at present.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The Pharmacovigilance system as described by the MAH fulfils the requirements and provides adequate evidence that the MAH has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

Risk Management Plan

An updated risk management plan for the A(H1N1)v vaccine was submitted before approval of the strain change variation. This was drafted in accordance with the CHMP core RMP for vaccines intended for use in a declared pandemic situation.

The CHMP, having considered the data submitted in the application of the variation to include the pandemic A(H1N1)v strain was of the opinion that the following activities are appropriate and necessary for the safe and effective use of the medicinal product:

- The MAH will conduct a prospective cohort safety study in at least 9,000 patients, in different age groups, including immunocompromised subjects, in accordance with the protocol submitted with the Risk Management Plan. Observed-to-Expected analyses will be performed. Interim and final results will be submitted in accordance with the protocol.

- The MAH commits to provide the details of the design and to provide the results of a study in a pregnancy registry. Details are to be submitted within one month of Commission Decision granting the Variation. Results are to be provided in the simplified PSUR.

- The MAH commits to establish mechanisms to promptly investigate issues affecting the benefit-risk balance of the vaccine. The design of additional studies for emerging benefit-risk evaluation is to be agreed with EMEA within 1 month of the Commission Decision granting the Variation.

- The MAH commits to submit the protocol and provide the results of the clinical effectiveness studies carried out in accordance with the study protocols published by ECDC.

- The MAH commits to provide an update of the RMP within one month of Commission Decision granting the Variation.

The details of the Risk Management plan are in Module 1.8.2. The MAH has committed to update it in line with Annex II.B of the opinion.

Summary of the risk management plan

A summary of safety concerns, Pharmacovigilance activities and Risk minimisation activities is presented below.

Identified/Potential safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
Important identified risk		
None	N/A	• N/A
Important potential risk		
Anaphylaxis	• Enhanced pharmacovigilance • Monitoring from observational study and on-going clinical studies. Incidence followed for 6 months after the 2nd vaccination..	• Contraindication for history of anaphylactic reaction to any constituent of the vaccine in the proposed labelling • Precaution in the proposed labelling regarding use in persons with known hypersensitivity, other than anaphylaxis, to vaccine components SPC, section 4.8, Post-marketing surveillance: *For cell-based influenza vaccines, post-marketing surveillance data are not yet available. From post-marketing surveillance with egg-derived interpandemic trivalent vaccines, the following serious adverse reactions have been reported:* *<u>Uncommon:</u>* *Generalised skin reactions including pruritus, urticaria, and non-specific rash.* *<u>Rare</u>:* *Neuralgia, paraesthesia, convulsions, transient thrombocytopenia.* *Allergic reactions, in rare cases leading to shock, have been reported.* *<u>Very rare:</u>* *Vasculitis with transient renal involvement.* *Neurological disorders, such as encephalomyelitis, neuritis and Guillain Barré syndrome.*
Bell's palsy	• Enhanced pharmacovigilance • Monitoring from observational study and on-going clinical studies. Incidence followed for 6 months after the 2nd vaccination..	
Convulsion	• Enhanced pharmacovigilance • Monitoring from observational study and on-going clinical studies. Incidence followed for 6 months after the 2nd vaccination..	
Demyelinating disorders	• Enhanced pharmacovigilance • Monitoring from observational study and on-going clinical studies. Incidence followed for 6 months after the 2nd vaccination..	
Encephalitis	• Enhanced pharmacovigilance • Monitoring from observational study and on-going clinical studies. Incidence followed for 6 months after the 2nd vaccination..	
Guillain-Barré syndrome	• Enhanced pharmacovigilance • Monitoring from observational study and on-going clinical studies. Incidence followed for 6 months after the 2nd vaccination..	
Neuritis	• Enhanced pharmacovigilance • Monitoring from observational study and on-going clinical studies. Incidence followed for 6 months after the 2nd vaccination..	
Vasculitis	• Enhanced pharmacovigilance • Monitoring from observational study and on-going clinical studies. Incidence followed for 6 months after the 2nd vaccination..	
Vaccination failure	• Enhanced pharmacovigilance • Monitoring from observational study and on-going clinical studies. Incidence followed for 6 months after the 2nd vaccination..	N/A
Effects of vaccine on liver function	• Investigation of ALT levels, as a marker of altered liver function, will be included in subgroups of Cohort 2 (immunocompromised patients) and Cohort 3 (chronically ill patients) of Study 810705 using mock-up H5N1 vaccine Further, in order to assess the risk of a potential negative effect of vaccination on liver functions in children, ALT investigation will also be included in a subset of the planned mock-up H5N1 vaccine paediatric Study 810706. • Observational cohort study (820901) will initially include patients at risk of influenza complication. Patients with a history of liver disease are included in the priority groups for vaccination and will be followed for safety after vaccination. Any safety signals regarding influence on liver functions will be monitored and communicated. • Monitoring of adverse event reports for abnormalities in liver function	SPC, section 5.3: *Non-Clinical data obtained with the pandemic vaccine using an H5N1 vaccine strain demonstrated alterations in liver enzymes and calcium levels in repeat dose toxicity studies in rats. Such alterations in liver function have not been seen to date in human clinical studies.*
Effects of vaccine on serum calcium levels	• Serum calcium levels will be examined in subgroups of subjects from cohort 1 (healthy subjects aged >18 years), cohort 2 (immunocompromised patients) and cohort 3 (chronically ill patients) of study mock-up H5N1 vaccine 810705. • Monitoring of adverse event reports for abnormalities in serum calcium levels	SPC, section 5.3 *Alterations in calcium metabolism have not been examined in human clinical studies.*
Important missing information		
Vaccine effectiveness	Baxter is in discussion with ECDC on participation in common	SPC; section 4.2:

	effectiveness study protocols.	*There is currently no clinical experience with Celvapan (H1N1) in adults, elderly, children or adolescents.*
Data in pregnant women	• Observational Study 820901will includes individuals in a variety of risk groups. This study will also include pregnant women in the second or third trimester • Spontaneous reports regarding AEs in pregnant women will be considered as medically significant, and will be followed up. • Baxter will collaborate with MHRA (post-marketing data) as well as pregnancy registries in the UK	SPC, section 4.6: *There are currently no data available on the use of Celvapan in pregnancy. Data from pregnant women vaccinated with different inactivated non-adjuvanted seasonal vaccines do not suggest malformations or foetal or neonatal toxicity.* *Animal studies with Celvapan do not indicate reproductive toxicity .* *The use of Celvapan may be considered during pregnancy if this is thought to be necessary, taking into account official recommendations. Celvapan may be used in lactating women.*
Data in children	• Paediatric study 820903 • Pandemic Observational Study 820901	SPC, section 4.2: *There is currently no clinical experience with Celvapan (H1N1) in adults, elderly, children or adolescents.*
No data in subjects with severe underlying medical conditions and immunocompromised	• Pre-pandemic Phase III study of H5N1 vaccine in adult and elderly population and specified risk groups • Observational Study 820901will include individuals in a variety of risk groups.	SPC, section 4.4.: *Antibody response in patients with endogenous or iatrogenic immunosuppression may be insufficient.*
Medication errors/misidentification of vaccine	• Monitoring of adverse event reports from both postmarketing and observational studies that may represent poor vaccine efficacy including vaccination failure.	1. Information provided to Health Care Professionals

2.6 Product Information

Further to the assessment and the scientific discussions held at the CHMP, changes to the SPC/Annex II/labelling/PL were implemented and details of the changes can be found in the final approved product information attached to this report.

2.7 Overall conclusions, risk/benefit assessment and recommendation

Clinical context

In April 2009, a new strain of human influenza A(H1N1) was identified and characterised.
On 11 June 2009 the WHO declared Phase 6 of the influenza pandemic.

Current estimates for the attack rate associated with the influenza A(H1N1)v virus over the first wave of infection vary from approximately 10-30 % in different geographical areas. There are no established criteria for classifying pandemics in terms of severity. The perceived severity can vary with geographical area, with sequential pandemic waves and in accordance with several other factors. Descriptions of severity based on factors such as rates of hospitalisation may be misleading due to different thresholds for this between countries and age groups.

The development of Celvapan was based on the guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application (CPMP/VEG/4717/03) and the guideline on submission of marketing authorisation applications for pandemic influenza vaccines through the centralised procedure (CPMP/VEG/4986/03). The core dossier procedure allows the insertion of the pandemic strain A(H1N1)v into the authorised mock-up vaccine as a strain change variation procedure.

This principle is based on the extrapolation of clinical safety and immunogenicity data obtained with the mock-up vaccine (containing H5N1 strains) to the same vaccine construct using the current influenza A(H1N1)v pandemic strain. It is expected that the insertion of the influenza A(H1N1)v

strain into the mock-up vaccine construct would result in a vaccine similarly or even more immunogenic than the H5N1 mock-up version and with a similar safety profile when used in a comparable population.

The specific commitments that accompany the strain change include collection of data from ongoing and planned clinical studies, which will provide safety and immunogenicity data.. These data will be submitted and reviewed on a rolling basis and updates to the Clinical Particulars in the SPC will be made as necessary.

Quality

The manufacture of the A(H1N1)v antigen and the A(H1N1)v formulated vials is well defined, controlled and is sufficiently validated. Adequate in-process controls, release and shelf life specifications have been set in line with relevant requirements (e.g. Ph. Eur.). The same manufacturing strategy was employed for the A(H1N1) vaccine as previously established and approved for the Mock-up A(H5N1) vaccine. All manufacturing sites are in compliance with current GMP requirements. Issues pertaining to the inactivation process, bacterial contamination, and the determination of the antigen content by SRD assay, which were initially raised as major concerns, have been addressed by the MAH. The MAH has committed to further address some minor outstanding issues as follow-up measure.

Non-clinical pharmacology and toxicology

At time of the strain variation most non-clinical data with Celvapan was generated with vaccine constructs that included an influenza A (H5N1) strain. Two studies (challenge and immunogenicity) in mice with A(H1N1) vaccine have been assessed.

Consistent pharmacology data generated with (H5N1) strains has been generated to support the potency of the vaccine, independent of the manufacturing scales and animal species tested, although a large body of data are from mice. The pharmacological program was in line with the Guideline on "core dossier approach to registration of pandemic influenza vaccines" (CPMP/VEG/4717/03).

The non-clinical toxicological testing program comprised a literature-based risk assessment of Tween 80 (Polysorbate 80), a non-GLP rabbit pyrogenicity study, a GLP single-dose toxicity study and a GLP pivotal repeat-dose toxicity study in which local tolerance assessment was included. This program is considered to sufficiently meet the requirements of Regulatory Guideline on "core dossier approach to registration of pandemic influenza vaccines" (CPMP/VEG/4717/03).
Non-clinical safety data reveal no special hazard for humans based on conventional studies of safety pharmacology, acute and repeated dose toxicity, local tolerance, embryo-foetal and postnatal toxicity (up to the end of the lactation period).

The initial immunogenicity dose-finding study in mice with the A(H1N1) strain demonstrated that the vaccine adjuvanted or not with 0.3% aluminum hydroxide, was immunogenic in CD1 mice. A clear dose-dependent antibody response was demonstrated. These initial data presented are regarded as preliminary.

A review of reproduction toxicity carried out with H5N1 antigen, based on the results of the studies with A/Indonesia/05/2005 and the A/Vietnam/1203/2004 candidate vaccines, it can be concluded that treatment of female CD rats with these Influenza vaccines on Days -42 and -14 before pairing and on Day 7 of gestation did not affect mating performance or fertility, embryo-fetal survival or growth or, pre- and post-natal survival and growth of the offspring or, adversely affect the pre- and post-natal development of the offspring up to 7 weeks of age.

The serological responses to the vaccine and exposure of fetuses to specific antibodies were demonstrated. According to the data presented, no vaccine-related harmful effects were seen on mating performance or female fertility, embryo-foetal survival and pre- and post-natal development.

Clinical

Most of the clinical data at time of the strain variation were generated with vaccine constructs that included an influenza A(H5N1) strain.

It is expected that the insertion of the influenza A(H1N1)v strain into the mock-up vaccine construct does not have a substantial effect on immunogenicity and safety compared to the corresponding mock-up vaccine when used in a comparable population.
Considerations for extrapolation of the clinical data include that the immunogenicity data available for the approved mock-up vaccines were generated using a strain to which the majority of subjects were immunologically naïve based on pre-vaccination testing for neutralising antibody and for antibody that inhibits haemagglutination.

It is assumed that the safety of the H5N1 mock-up vaccine is predictive for the influenza A(H1N1)v strain in the population it has been tested. Preliminary limited safety data from a clinical study investigating different H1N1 pandemic vaccine formulations in 387 healthy adult and elderly subjects were found to be acceptable. The safety profile is in general comparable with that observed for the H5N1 mock-up vaccine. However rare adverse reactions that might be specific to the influenza (H1N1)v strain can only be evaluated during very widespread usage.

Currently there is no data with Celvapan in children, pregnant women, immunocompromised patients and other risk groups or specific populations available.

Therefore in populations other than those in which it has been tested, data obtained with a mock-up vaccine would have to be extrapolated from the safety and immunogenicity of a corresponding construct.

Numerous safety, efficacy and effectiveness studies have been conducted with inactivated influenza vaccines since the 1960-ies in various age and risk categories, including children, the elderly, pregnant women and persons with underlying acute and chronic disease (Plotkin, Vaccines, Fourth Edition; 351 – 364). In particular, early studies with influenza vaccines were performed using whole virion preparations. Although clinical endpoints and outcomes were different in all these studies, immunogenicity was generally adequate and no specific safety signals were reported indicating that inactivated influenza vaccines based on whole virions do cause unacceptable adverse effects.
In conclusion, decades of experience with various preparations of inactivated influenza virus antigens do not provide evidence that those vaccines are harmful for individual age and risk categories. These evidences can be extrapolated to Celvapan, in particular, since the antigen amount used (7.5 µg) in Celvapan is significantly lower compared to the antigen amount used in the clinical trials mentioned above (usually 45 µg). Direct and indirect evidence available for the time being suggest that Celvapan is safe and efficacious also in groups not having been clinically investigated.

Data from ongoing and planned clinical trials as specified in the agreed pharmacovigilance/risk management plan using the Celvapan vaccine construct with the current pandemic influenza A(H1N1)v strain are reviewed on an ongoing basis. These studies will allow to obtain safety, immunogenicity and efficacy data for the influenza A(H1N1)v vaccine. The SPC will be updated accordingly as the CHMP considers necessary.

Efficacy

Clinical trials on protective efficacy for the mock-up vaccine were not possible as the strain causing the current pandemic as well as the subjects with a corresponding infection were not present at that time. Therefore a detailed characterisation of the immunological response has been performed.

Overall approximately 850 subjects (adults and elderly) were exposed to (H5N1) strains in clinical studies.
In the dose-response study 810501, four vaccine formulations adjuvanted with alum (3.5µg, 7.5µg, 15µg and 30µg) and 2 non-adjuvanted vaccine formulations (7.5, and 15µg) were evaluated in healthy

adults of 18-45 years of age. Based on the MN and SRH assay using the homologous vaccine strain (A/Vietnam) the highest immune responses were achieved and all CHMP requirements were fulfilled following the first and second immunisation with the non-adjuvanted 7.5µg vaccine formulation. Moreover cross-neutralisation experiments indicate a high responsiveness for the original prototype A/HongKong strain and a moderate cross-neutralising response for the further evolved strain A/Indonesia. The neutralising antibody responses against all three virus strains persist over 6 months with low to moderate decline rates.

In the pivotal trial 810601 the immunogenicity of the 7.5µg vaccine was investigated in healthy adults of 18-59 years of age and elderly 60 years of age and older. Following two vaccinations and based on the MN assay all three requirements were fulfilled in the age group of adults and 2 out of 3 requirements were met in the elderly. With regards to the group of adults a seroneutralisation rate of 72.5%, a seroconversion rate of 60.8% and a 4.7 fold GM increase was achieved. In the elderly a seroneutralisation rate of 74.1%, a seroconversion rate of 26.7% and a 2.8 fold increase was obtained. The results of the MN assay were generally confirmed by the SRH assay. Following two vaccinations 2 out of 3 three CHMP requirements were fulfilled in adults and all three 3 requirements were met in the elderly. In the group of the adults a seroprotection rate of 63.3%, a seroconversion rate of 60.2% and a 4.6 fold GM increase was achieved. In the elderly a seroprotection rate of 67.7%, a seroconversion rate of 62.4% and a 4.6 fold increase was obtained. Data on 6 months persistence of antibodies indicate a moderate decline in antibody responses.

Similar results were obtained in study 810701, where adults between 21 and 45 years of age received 2 doses of 3.75µg HA or 7.5µg HA of strain A/Indonesia/05/2005. With regard to the MN assay all three requirements were met regardless which antigen dose were administered. Based on the SRH assay nearly all CHMP criteria were fulfilled. While in the 3.75µg group a seroprotection rate of 71.2% was reached, it was slightly below the CHMP criterion for SPR in the 7.5µg group (69.2%).

Based on the MN and SRH assay the immunogenicity results obtained with the non-adjuvanted 7.5µg vaccine formulation are consistent throughout the three clinical studies suggesting that the Vero cell derived, inactivated whole virion H5N1 vaccine is suitable immunogenic.

Safety

The safety data provided with the mock up vaccine containing the (H5N1) strain does not raise any safety concerns as regards to frequency and nature of adverse events. The most commonly observed adverse reactions after administration of Celvapan were injection site pain, which was reported post dose 1 and 2. More rarely, local reactions such as injection site erythema and induration, as well as systemic reactions such as headache, fatigue, malaise, myalgia, chills, pharyngolaryngeal pain, pyrexia and arthralgia were reported after the first and second vaccination with the Vero cell-derived whole virion H5N1 pandemic vaccine. Symptoms normally abated without treatment after a few days. In general less systemic and local reactions were reported after the second vaccination compared to the first vaccination. The profile of adverse events after administration is not unusual and comparable to other licensed influenza vaccines. Adverse reactions that might be specific to the influenza A(H1N1)v strain can only be evaluated during post-approval usage. Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities are adequate and appropriate.

Preliminary analysis of safety data observed 21 days following the first vaccination of 387 adult and elderly subjects enrolled in a clinical trial investigating different H1N1 pandemic vaccine formulations was acceptable and the safety profile was found to be comparable with the safety profile reported for the H5N1 vaccine formulations. No safety issue was raised.

From the safety database all the adverse reactions reported in clinical trials have been included in the SPC.

In view of Risk Management, the MAH will submit on a monthly basis a simplified PSUR on all adverse reactions notified by patients and health care professionals. The Risk Management Plan

includes additional Pharmacovigilance activities to address important potential risks and important missing information. This includes the conduct of a study with at least 9,000 patients across different age groups, recruited at the start of the vaccination campaign, a specific monitoring of special populations such as pregnant women (through pregnancy registry in several EU countries), children and immunocompromised subjects, and the monitoring of adverse events of special interest. Effectiveness studies developed and conducted in accordance with the standard protocols published by the ECDC will be performed.

- User consultation

  The MAH performed readability testing ("user consultation") and a satisfactory report has been provided with the initial MAA.

Risk-benefit assessment

Benefits

The real benefits of Celvapan can only be assessed by its use during a pandemic. At present the benefit can only be evaluated based on detailed characterisation of immunological responses to vaccination with Celvapan containing the (H5N1) strains.

Based on the MN and SRH assays the immunogenicity results obtained with the non-adjuvanted 7.5µg vaccine formulation containing a H5N1 strain are consistent throughout the three clinical studies suggesting that the vaccine is suitably immunogenic.

Extrapolation of data collected with (H5N1) strains to the influenza A(H1N1)v strain is considered adequate.

Therefore the expected benefit of Celvapan is to provide protection against clinically-apparent infection and/or possibly against development of severe disease in case of an influenza A(H1N1)v 2009 infection.

Risks

Celvapan containing a H5N1 strain is commonly or very commonly associated with a range of local and systemic adverse reactions but these are not often of severe intensity and the safety profile would not preclude the use of the vaccine as described in the SPC.

The current safety database is considered to be sufficient to describe adverse reactions that occur commonly in the population in the clinical trials. However, there are some adverse reactions known to be uncommonly and rarely associated with influenza vaccines and it is currently not possible to predict if higher rates might be observed with Celvapan (H1N1)v compared with, for example, seasonal influenza vaccines.

The specific commitments that accompany the strain change variations, including collection of data from the ongoing and planned clinical studies and as specified in the Risk Management Plans, will provide safety and immunogenicity data on a rolling basis

Balance

Based on all the data that supported approval of the corresponding mock-up vaccine together with quality data specific to the pandemic influenza A(H1N1)v strain it is considered that in the current pandemic situation the benefits outweigh the risks that may be associated with the use of the vaccine in accordance with the SPC.

Recommendation

On the basis of the available data for Celvapan A(H1N1)v which is limited primarily to quality data and the data of the initially authorised medicinal product Celvapan H5N1, the CHMP considered by consensus that the risk-benefit balance of Celvapan for the prophylaxis of influenza in an officially declared pandemic situation, in accordance with official guidance, was favourable. Therefore CHMP recommended the variation to the marketing authorisation under exceptional circumstances in accordance with Article 8 of Commission Regulation (EC) No 1085/2003 to the terms of the Marketing Authorisation until specific conditions as defined in Annex II.C (points 1 and 2) are fulfilled.