22 February 2024
EMA/108199/2024
Committee for Medicinal Products for Human Use (CHMP)

CHMP assessment report

Incellipan

Pandemic influenza vaccine (H5N1) (surface antigen, inactivated, adjuvanted, prepared in cell cultures)

Procedure No. EMEA/H/C/006051/0000

Note

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

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List of abbreviations

AE	Adverse event
aH5N1c	Cell culture-derived MF59-adjuvanted H5N1 vaccine
AESI	Adverse event of special interest
ARDS	Acute respiratory distress syndrome
BARDA	Biomedical Advanced Research and Development Authority
BMI	Body mass index
BSE	Bovine spongiform encephalopathy
CBER	Center for Biologics Evaluation and Research
CPP	Critical process parameters
CQA	Clinical quality assurance
CFR	Case fatality rate
CI	Confidence interval
CMI	Cell-mediated immunity
CSR	Clinical study report
DHHS	Department of Health and Human Services
EMA	European Medicines Agency
ELISA	Enzyme-linked immunosorbent assay
ELLA	Enzyme-linked lectin assay
EU	European Union
FCC	Flu cell culture
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good clinical practice
GMP	Good manufacturing practice
GMR	Geometric mean ratio
GMT	Geometric mean titre
HA	haemagglutinin
HI	haemagglutination inhibition
HPAI	highly pathogenic avian influenza
ICH	International Conference on Harmonisation
IM	intramuscular
IPCs	In-process controls
IRB	Institutional Review Board
ISS	Integrated summary of safety
LL	Lower limit
MAA	Marketing authorisation application
MCB	Master cell bank
MDCK	Madin Darby Canine Kidney
MedDRA	Medical Dictionary for Regulatory Activities
MF59	MF59C.1 adjuvant
MN	microneutralisation
MPH	Monovalent pooled harvest
MPPS	Modified per protocol set
MS	Master seed
NA	Neuraminidase
NH	Northern hemisphere
NVD	Novartis Vaccines and Diagnostics

NOCD	New onset of chronic disease
PACMP	Post-approval change management protocol
PBS	Phosphate-buffered saline
PEP	Primary endpoint
Ph. Eur.	European Pharmacopoeia
PPQ	Process performance qualification
PPS	Per protocol set
PT	Preferred term
QA	Quality attributes
RH	Relative humidity
SAE	Serious adverse event
SD	Standard deviation
SEP	Secondary endpoint
SmPC	Summary of product characteristics
SRID	Single radial immunodiffusion
SRD	Single radial diffusion
SRH	Single radial haemolysis
TSE	Transmissible spongiform encephalopathies
UL	Upper limit
WCB	Working cell bank
WFI	Water for injection
WHO	World Health Organization
WS	Working seed

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Seqirus Netherlands B.V. submitted on 24 November 2022 an application for marketing authorisation to the European Medicines Agency (EMA) for Incellipan, through the centralised procedure falling within the Article 3(1) of Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication: Prophylaxis of influenza in an officially declared pandemic situation in persons 6 months and above. Incellipan should be used in accordance with Official Guidance.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

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

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

1.3. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0144/2022 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0144/2022 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's request(s) for consideration

1.5.1. Conditional marketing authorisation

The applicant requested consideration of its application for a Conditional marketing authorisation in accordance with Article 14-a of the above-mentioned Regulation.

1.6. Scientific advice

The applicant did not seek scientific advice from the CHMP.

1.7. Steps taken for the assessment of the product

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

Rapporteur: Daniela Philadelphy Co-Rapporteur: Ingrid Wang

The application was received by the EMA on	24 November 2022
The procedure started on	28 December 2022
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	20 March 2023
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	1 April 2023
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	3 April 2023
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	26 April 2023
The applicant submitted the responses to the CHMP consolidated List of Questions on	08 September 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	16 October 2023
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	26 October 2023
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	9 November 2023
The applicant submitted the responses to the CHMP List of Outstanding Issues on	23 January 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	07 February 2024
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 to Incellipan on	22 February 2024

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Influenza A is a member of the family of Orthomyxoviridae and is a natural pathogen of birds, although subtypes can infect humans and other mammals. Influenza A viruses are categorised into subtypes based on two surface antigens haemagglutinin (HA) and neuraminidase (NA). There are 18 HA types and 11 NA types (CDC 2018). Novel influenza viruses may emerge following the reassortment of two cocirculating viral strains (Belshe 2005) or from a series of genetic mutations in one strain (Taubenberger 2005; Tumpey 2005).

Pandemic influenza outbreaks occur when a new highly infectious virus strain enters a population with low immunity from previous exposure. A pandemic outbreak is expected to spread quickly and cause substantial global morbidity and mortality (Tumpey 2005; Johnson and Jürgen 2002). Infection with influenza virus usually occurs by droplet spread from infected people to uninfected people through inhalation. The virus can also be spread by hands contaminated with influenza viruses.

Preliminary findings have identified the H2, H5, H6, H7 and H9 subtypes of influenza A as those most likely to be transmitted to humans and therefore present a potential pandemic threat. Widespread circulation, pathogenicity and direct transmission of avian viruses to humans suggest that H5N1 has important pandemic potentialities, increased by the high case fatality rate. Although human transmission of H5N1 is rare, the mortality rate from H5N1 infection is high.

2.1.2. Epidemiology

According to the WHO, 863 human cases of H5N1 infection have been identified from 2003 through 31 March 2022, resulting in 455 deaths, and representing a case fatality rate (CFR) of 53% (WHO 2022). Most reported A/H5N1 influenza virus cases have been in children and adults younger than 40 years of age with the majority of cases in younger age groups:

- Approximately half of human A/H5N1 influenza cases have been in people younger than 20 years old.
- The age group of young adults aged 20 through 29 years comprises 22.4% of all A/H5N1 influenza cases reported since 2003 (Dudley and Mackay 2013).

Morbidity and mortality from A/H5N1 influenza virus infections are also age-specific, with the highest mortality rates reported for young people between 10 and 40 years of age. In a global patient registry of 193 children across 13 countries, the CFR associated with H5N1 infections declined with decreasing age among children who were younger than 5 years of age (Oner et al. 2012). The slightly improved CFR in the youngest affected may be related to earlier identification, and related treatment.

Outbreaks in both birds and mammals with influenza H5N1 clade 2.3.4.4b have been ongoing since 2020 in Africa, Asia and Europe and moved to North and South America in 2021. Additionally, there has been an increased spill over to non-avian species during 2022, including wild terrestrial and marine mammals and, more recently, the detection of an outbreak in a mink farm in Spain which included mink-to-mink transmission. According to a risk assessment published by the WHO (h5-risk-assessment-dec-2022.pdf (who.int)) From 2020 to December 2022, six human cases of influenza A(H5N1) belonging to the 2.3.4.4b clade were reported to WHO. There is still limited evidence for

mutations associated with adaptation to mammals and humans even when transmission in mammals has been reported.

2.1.3. Clinical presentation, diagnosis

Clinical signs and symptoms of A/H5N1 pandemic influenza may be characterised by the abrupt onset of fever (>38ºC), headache, myalgia, severe malaise, prostration, non-productive cough, sore throat and rhinitis. More severe illness can result when influenza virus moves from the tracheal epithelium to invade the lungs (primary viral pneumonia), or when secondary bacterial pneumonia occurs.

In some reports of infection with an A/H5N1 virus, diarrhoea and shortness of breath were prominent features. In severe cases the clinical picture can present with dyspnoea leading to acute respiratory distress syndrome (ARDS) which may lead to respiratory failure and death. Typical findings of chest radiographs in individuals infected with an H5N1 virus are interstitial infiltration, patchy lobar infiltrates in a variety of patterns (single lobe, multiple lobes, unilateral or bilateral distributions) progressing in some cases to a diffuse bilateral ground-glass appearance characteristic of ARDS. The median time from onset of fever to ARDS was reported as 6 days (range 4-13 days) (Chotpitayasunondh et al. 2005).

2.1.4. Management

Vaccination is the primary method for prevention of influenza and its severe complications. Immunisation with surface antigens, especially HA, reduces the likelihood of infection and severity of disease (Clements et al. 1986; Couch and Kasel 1983), and currently represents the most important measure for reducing the impact of influenza. Vaccination with seasonal influenza vaccine is associated with a reduction in influenza-related respiratory illness and physician visits at all ages, in hospitalisations and deaths among high-risk persons, otitis media among children, and work absenteeism among adults (Nichol et al. 1995; Campbell and Rumley 1997; Bridges et al. 2000; Patriarca 1986; Gross et al. 1995; Mullooly et al. 1994; Clements et al. 1995; Heikkinen et al. 1991; CDC 1999).

2.2. About the product

aH5N1c contains haemagglutinin (HA) and neuraminidase surface antigens derived from A/turkey/Turkey/1/2005 NIBRG-23, a reverse-genetic strain produced via a recombination of A/turkey/Turkey/1/2005 (H5N1) and influenza A PR8 strain. It is propagated in Madin Darby canine kidney (MDCK) cells, after which the antigens are purified and solubilised. The manufacturing process for monovalent bulk is derived from that developed for Flucelvax Tetra, an MDCK cell culture-derived quadrivalent, surface antigen, inactivated seasonal influenza vaccine also manufactured by the applicant.

MF59 is an oil-in-water emulsion composed of squalene, with surfactants polysorbate 80 and sorbitan trioleate, in citrate buffer. A "standard" dose of MF59 contains 9.75 mg squalene in 0.25 mL volume; smaller volumes contain proportionally less squalene.

The applicant aH5N1c vaccine is related to several other seasonal or pandemic influenza vaccines made by the applicant. Fluad (aTIV), which is an egg-derived, MF59-adjuvanted trivalent seasonal influenza vaccine, is currently licensed for use in the elderly in 12 countries, and in Canada also for use in children. The quadrivalent version of Fluad, Fluad Tetra (aQIV) is licensed for use in the elderly in the United States of America (USA), Australia, 27 European Union (EU) countries (EMEA/H/C/004993),

Iceland, Norway, Liechtenstein, Great Britain, and New Zealand. Flucelvax Tetra (QIVc), which is a quadrivalent seasonal influenza vaccine without adjuvant made in Madin Darby Canine Kidney (MDCK) cells, is licensed in the USA, Great Britain, Australia, New Zealand, Brazil, Argentina, Switzerland, Taiwan, and also in 27 EU countries (EMEA/H/C/004814), Iceland, Norway, and Liechtenstein. The trivalent version of Flucelvax (trade name Optaflu) was licensed in Europe (EMEA/H/C/000758), Switzerland, USA (trade name Flucelvax) and Australia. Celtura is a pandemic A/H1N1 cell culture-derived MF59-adjuvanted vaccine (aH1N1c), which was licensed in Germany and Switzerland in November 2009 and in Japan in January 2010.

MF59 was also the adjuvant present in the pandemic egg-based vaccine Focetria that was licensed in EU in 2007 as mock-up H5N1 vaccine (EMEA/H/C/000710) and underwent a pandemic strain change to H1N1 in 2009. Millions of doses of Focetria were distributed during the influenza pandemic in 2009 with approximately a quarter of those doses being used. During the pandemic in 2009 also the cell culture-derived vaccine Celtura was licensed in Germany, Switzerland and Japan; some millions of doses were distributed and approximately 10% of those doses were used. In addition, MF59 is the adjuvant present in the pandemic preparedness egg-based vaccine Foclivia (EMEA/H/C/001208) and in the zoonotic egg-based vaccine Aflunov (EMEA/H/C/2094), licensed in EU since 2009 and 2010, respectively.

The applicant is seeking licensure of aH5N1c based on immunogenicity and safety results from 5 clinical studies in subjects 6 months and older. In addition, safety data are presented from three dose-ranging studies with MF59 adjuvanted cell culture-derived influenza vaccines of other subtypes: key Study V110_04 (H1N1, paediatric subjects), supporting Study V129_01 (H3N2, paediatric subjects and adults/elderly), and supporting Study V131_01 (H7N9, adults).

2.3. Type of Application and aspects on development

Incellipan is a pandemic (pandemic preparedness) vaccine H5N1 strain A/turkey/Turkey/1/2005). Incellipan is submitted for a conditional marketing authorisation.

The applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14-a of the above-mentioned Regulation, based on the following criteria:

- The benefit-risk balance is positive.
- It is likely that the applicant will be able to provide comprehensive data. Soon after the declaration of the pandemic, the applicant will be initiating clinical trials with the strain matched vaccine in all age groups: adults, elderly and paediatrics (according to the approved PIP) which will be submitted to the EMA according to the pandemic strain change procedure laid down in Guideline on influenza vaccines – submission and procedural requirements EMA/56793/2014 Rev.1
- Unmet medical needs will be addressed. As per Article 21 of Regulation (EC) No 1234/2008, it may be exceptionally and temporarily acceptable that certain non-clinical or clinical data on the declared pandemic strain are missing however it is understood that the early availability of the pandemic influenza vaccine, even with limited data, will allow an early start of the vaccination campaign reducing the attack rate, hospitalization and disease-induced mortality. The availability in the EU of a pandemic preparedness vaccine manufactured in cell cultures is considered a critical aspect in the fight against an influenza pandemic, in fact some key challenges of influenza vaccine use, relate to production capacity and distribution. In addition, the method for influenza vaccine manufacture is constrained for most manufacturers by availability of influenza strains and also embryonated chicken eggs, which are required for viral production.

- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. All currently licensed pandemic and zoonotic vaccines are produced using egg-based manufacturing processes. A vaccine supply system solely dependent on an egg-based manufacturing process may be problematic as during a highly pathogenic avian influenza outbreak/pandemic both egg quantity and quality may be compromised. Furthermore, the cell-based vaccines will be a closer match to the circulating pandemic strain than the egg-based vaccines, as they do not undergo any process of egg adaptation and consequently can offer a higher protective effect.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as suspension for injection containing 7.5 micrograms per 0.5 ml dose of purified inactivated influenza virus surface antigens haemagglutinin (HA) and neuraminidase (NA) from a potential pandemic H5N1 virus strain candidate (A/turkey/Turkey/1/2005 (H5N1)-like strain (NIBRG-23) as active substance.

The adjuvant is MF59C.1, which is an oil-in-water emulsion containing squalene as the internal oil phase, sodium citrate – citric acid buffer as the external aqueous phase and polysorbate and sorbitan trioleate as emulsifiers. The MF59C.1 is the same adjuvant included in the seasonal, zoonotic or pandemic influenza vaccines Fluad Tetra, Aflunov and Foclivia.

Other ingredients are: sodium chloride, potassium chloride, magnesium chloride hexahydrate, disodium phosphate dihydrate, potassium dihydrogen phosphate and water for injections.

The product is available in pre-filled syringe (type I glass) with plunger-stopper (bromobutyl rubber) and packaged in sizes of 10 pre-filled syringes, without a needle.

Incellipan will undergo a pandemic strain change after declaration of a pandemic, resulting in a "final" vaccine with a different active substance.

2.4.2. Active Substance

2.4.2.1. General Information

The active substance of the applicant's adjuvanted cell culture-derived H5N1 subunit influenza virus vaccine aH5N1c is composed of a monovalent bulk containing inactivated influenza virus surface antigens haemagglutinin (HA) and neuraminidase (NA) of an A/H5N1-subtype virus. The representative strain for H5N1 is A/turkey/Turkey/1/2005 NIBRG-23, which is a strain that has been attenuated by reverse genetics.

Other viral proteins may be present in residual amounts. The monovalent bulk is prepared from influenza virus propagated in a suspension culture-adapted Madin Darby Canine Kidney (MDCK) cell line, MDCK 33016 PF.

Haemagglutinin is a transmembrane fusion glycoprotein with two major functions: 1) recognition of target cells by binding to sialic acid-containing receptors, and 2) fusion of the viral and the endosomal membranes following endocytosis. HA is the primary antigen of the active substance, for which vaccination elicits anti-HA antibodies and provide antigen memory. These antibodies will recognise this surface protein and neutralise the virus during natural infection.

The function of neuraminidase is to enzymatically remove sialic acid from glycoproteins thereby facilitating the release of progeny virus particles from the infected cell surfaces. This occurs during the budding processes to prevent self-aggregation and cell surface aggregation of virions by the removal of sialic acid from cell and viral proteins. It has also been suggested that NA helps the virus to penetrate the mucin layer in the respiratory tract to reach epithelial cells, which are the target cells for the virus.

2.4.2.2. Manufacture, process controls and characterisation

Manufacture

The monovalent bulk active substance is manufactured and release tested at the Seqirus, Inc. in Holly Springs (United States) in accordance with Good Manufacturing Practice (GMP).

The Seqirus facilities are also involved in active substance manufacture and testing as well as in manufacture and testing of the cell banks and the viral seeds Appropriate evidence of GMP compliance for all sites has been provided.

Description of manufacturing process and process controls

The proposed commercial active substance manufacturing process (Process 3.0) is derived from the process that was developed for Flucelvax Tetra (EMEA/H/C/004814), an MDCK cell culture-derived quadrivalent, surface antigen, inactivated seasonal influenza vaccine also manufactured by the applicant.

The active substance is prepared by propagation of influenza working seed virus in MDCK cell suspension culture. The active substance manufacturing process is divided into an upstream process and a downstream process. Cell expansion for the upstream non-infectious manufacturing process is conducted in cell culture medium, which is a chemically defined medium. Virus propagation for the upstream infectious manufacturing process is conducted in protein free medium. Following virus propagation, the virus/cell harvest is processed to remove cells and cell debris.

The downstream process consists of several steps designed to purify whole influenza virus from the clarified virus harvest, inactivate the virus, and then to purify and concentrate the viral surface proteins, haemagglutinin and neuraminidase.

In the downstream manufacturing process, clarified harvest is purified and buffer exchanged by chromatography and filtration and the virus concentrate is inactivated. Next, viral surface antigens are preferentially solubilised with the detergent followed by separation of the surface antigens from the viral core proteins. The process concludes with removal of detergent and buffer exchange of the surface antigens into the final formulation buffer yielding the influenza subunit HA and NA monovalent bulk.

The manufacture of active substance has been adequately described. The active substance manufacturing process is considered acceptable.

The applicant has performed a risk assessment to identify and evaluate the process parameters that may have an effect on the process performance (e.g., yield) or the critical quality attributes (CQAs) of the active substance.

Control of materials

Sufficient information on raw materials used in the active substance manufacturing process has been submitted. Compendial raw materials are tested in accordance with the corresponding monograph,

while specifications (including test methods) for non-compendial raw materials are presented. No human or animal-derived materials are used.

A brief description and qualitative composition of media is provided. A list of the chemicals, solvents, and solutions used to prepare in-house solutions prior to use during manufacturing and representative Certificates of Analysis are provided. Finally, the chromatography resin, filters and membranes used during upstream and downstream manufacturing are described.

Seed viruses

For influenza vaccines, the virus strains provided by the WHO collaborating centres are considered the candidate vaccine viruses (CVV); therefore, they are the reference seed from which the working seed lots are prepared. The applicant states that the CVVs are acquired from the WHO collaborating centres. The CVV can be isolated in eggs or directly in qualified cell lines, in line with the WHO document "Antigenic Characterization of Seasonal Influenza Viruses Isolated in Vaccine-qualified Cell Lines", published in 2015. This document describes the antigenic and genetic characterization of the cell culture candidate vaccine viruses (ccCVV) and makes reference to the adventitious agent safety evaluation of these ccCVV. Regarding the working seed virus, the applicant uses the same manufacturing process and control strategy for producing seed stock for both egg- and cell-derived CVVs. This is found acceptable, as long as the CVV obtained from the WHO collaborating centre has been suitably evaluated after receipt, to confirm it is antigenically identical to the CVV. Release criteria and in-process controls for testing of the working virus seeds have been provided. All test methods are considered validated and validation reports are provided.

Cell substrates

The cell substrate and the cell banks have been appropriately described. The monovalent bulk is manufactured using a continuous, mammalian cell line (Madin Darby Canine Kidney (MDCK) cells) to propagate the virus. This cell line was established from the kidney of a normal male dog at the University of California, Berkeley in 1958. The origin, source and early history of this cell line has been briefly presented. The adaption of this cell line has been summarised. A description of the cell bank system is included in the dossier. Of note during cell line development two Master Cell Banks (MCBs), and several Working Cell Banks (WCBs), and EoP cell banks have been established and characterised. The cell bank characterisation and testing has been summarised and is in line with the appropriate ICH guidance. Additional working cell banks will be routinely prepared from the MCB according to a specified protocol and agreed specifications. Acceptance criteria for future WCBs is included.

Control strategy

The active substance control strategy is based on a planned set of controls derived from current product and process understanding that ensures process performance and product quality. The controls can include parameters and attributes related to active substance and finished product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control. Critical process parameters (CPPs) (process input) as well as critical process controls (CPCs) (output variables) have been summarised. A well-established control system is in place which ensures that the active substance manufacturing process is stable and remains under control.

Control of critical steps and intermediates

A set of process parameters (input variables or conditions of the manufacturing process that can be directly controlled in the process) classified as critical, key and non-key process parameters, as well as of process controls (output variables or outcomes that are indicative that the process performed as

expected) is in place to ensure that the process is consistent and under control. The applicant has differentiated the critical and non-critical process parameters and process controls in the dossier.

In conclusion, the control system is considered adequate to monitor and control the active substance manufacturing process with regards to critical, non-critical operational parameters and in-process controls. Actions taken if limits are exceeded are specified. The methods used for the control of critical steps and intermediates have been described and validated. This approach is acceptable.

Process validation

All process validation activities have been successfully completed according to the active substance process validation master plan. It was demonstrated that the Flu cell culture (FCC) active substance manufacturing process meets established pre-determined criteria. To ensure that the process remains in a validated state during commercial manufacturing, a continuous process verification (CPV) scheme has been established. The validation of the virus seed stock production process was performed, operating parameters were within their normal operating ranges and performance parameters met all acceptance criteria. Based on this assessment, the data confirm that the virus seed production process was successfully validated and can reproducibly produce material that meets acceptance criteria. The process validation report for the virus seed production manufacturing process is provided. It is agreed that a repetition of virus strain or subtype independent validation is not required. Summaries with the essential details of the conducted validation work were provided.

Regarding the process performance qualification (PPQ), separate process validation reports have been provided for the individual "process parts" of the current, intended commercial processes. This approach is considered to be acceptable.

A brief summary of the conducted non-product solution hold time validation work was provided, the results reported and finally the validated maximum hold time for the individual solutions was provided.

Details regarding the clearance of process-related impurities were provided.

The inactivation of the vaccine virus is considered to be a critical process. In accordance with the Guideline on Influenza vaccines – Quality module, EMA/CHMP/BWP/310834/2012 Rev.1, verification of virus inactivation was performed for the PPQ batches tested under the inactivation verification protocol. Samples taken from the PPQ batches were tested for residual infectious influenza virus, and all passed with no viable virus detectable for three consecutive executions of the inactivation verification study. Consequently, inactivation of the vaccine virus has been sufficiently addressed.

Validation of the splitting step was performed and results of this validation showed that all acceptance criteria were met.

Lifetime validation of the resin and membranes is conducted according to lifetime protocols. The maximum number of reuse cycles are stated for the resin and the membranes.

Additional process validation activities such as aseptic process validation, mixing and filter validation, validation of the reprocessing (re-filtering), and a risk assessment to identify potential extractable and leachable have been performed. Finally, the applicant indicates that continued process verification will be established to ensure that the process remains in a validated state during commercial manufacturing.

Overall, appropriate process validation has been conducted which indicates that the active substance manufacturing process can perform effectively and reproducibly to produce active substance meeting its predetermined specifications and quality attributes.

Manufacturing process development

Development of this cell-based influenza vaccine was started by Behring Vaccine GmbH & Co (Marburg, Germany), later named Chiron Behring GmbH & Co KG (Chiron Vaccines) and then Novartis Vaccines and Diagnostics (NV&D/Novartis Vaccines). In 2015 Novartis Influenza Vaccines was acquired by CSL. Subsequently, the bioCSL arm of CSL group was merged with the influenza vaccines division of Novartis. The new corporate entity is referred to as Seqirus. Several documents included in the dossier still bear the names of Seqirus predecessor companies.

The active substance process development at Holly Springs started with Process 2.0, which was based on the Process 1.1 seasonal influenza vaccine manufactured in Marburg, Germany and transferred to Holly Springs. The pandemic active substance process was changed from Process 2.0 to Process 1.1 to align with the licensed seasonal influenza vaccine manufacturing process (Flucevax Tetra), and then Process 3.0, the currently licensed seasonal influenza vaccine process and the proposed commercial process for pandemic. The Phase 2 clinical trial material was manufactured using Process 2.0, while the Phase 3 clinical trial material was manufactured using Process 1.1.

The proposed and validated commercial process for active substance is Process 3.0, which is the currently licensed process for the seasonal influenza vaccine, Flucelvax Tetra.

A summary of the process versions is provided and the process changes introduced have been sufficiently described.

Comparability of Process 1.0 to Process 2.0

Process 1.0 was manufactured in Marburg, Germany and used for the Phase 1 clinical trial material. The Process 1.0 manufacturing process was a 2500 L production scale at the virus infection stage, while Process 2.0 was a 5000 L scale.

The downstream process for Process 1.0 utilized Sulfate Chromatography as the primary virus purification step, Process 2.0 utilized Ultracentrifugation. Additionally, for Process 1.0, the adsorption step was performed in batch mode. The method of buffer exchange into the final formulation buffer, phosphate buffered saline (PBS), was also modified slightly for Process 2.0. Initially the monovalent bulk was stored in Tris, followed by a buffer exchange into PBS immediately prior to blending. For Process 2.0, the buffer exchange immediately followed the chromatography step.

Comparability of Process 2.0 to Process 1.1

The process and analytical comparability of the H5N1 monovalent pandemic bulk material produced using Process 1.1 and Process 2.0 was demonstrated using quantitative and qualitative analyses of the data generated through routine manufacturing, in-process testing and release testing of monovalent bulks. To demonstrate comparability of Process 2.0 and Process 1.1, performance attributes (output parameters) as defined in the process comparability protocol met their respective acceptance criteria during representative cGMP lots produced in Holly Springs. Each parameter is compared between Process 2.0 and Process 1.1. To demonstrate comparability of Process 2.0 and Process 1.1, routine release testing, routine in-process control (IPC) testing, and supplemental characterization testing met their respective acceptance criteria. The comparability assessment data demonstrated that the monovalent bulk produced using Process 1.1 and Process 2.0 were equivalent with respect to safety, integrity, strength, purity, and quality, and demonstrated comparability.

Process 3.0

The fundamental principles of the cell-culture expansion process remain unchanged in the Upstream manufacturing process from Process 1.1 to Process 3.0with no changes to the MDCK cell-line used, the basic culture conditions (e.g. temperature, agitation, control of dissolved oxygen and carbon dioxide),

and the type of vessels used for the cell culture expansion process (e.g. shake flasks, Wave bioreactors, stainless-steel stirred-tank bioreactors). Moreover, the evaluation of process controls such as final cell viability at each cell-expansion step and final PDL limit at the terminal cell-expansion step was maintained between Process 1.1 and 3.0, to ensure comparability of the cell-substrate.

Comparability of Process 3.0 to Process 1.1

Comparability studies (including an evaluation of process comparability and analytical comparability) were performed between Process 1.1 and Process 3.0 to support the manufacture of Drug Substance using Process 3.0. Process 1.1 was the originally licensed Drug Substance process for Flucelvax, while Process 3.0 is the currently licensed Drug Substance process, and the proposed commercial process for pandemic. The results of the comparability studies confirmed that the manufacturing process changes did not impact product quality, and therefore it is concluded that drug substance manufactured using Process 3.0 is comparable to that manufactured by Process 1.1. Consequently, the process changes are not expected to have any adverse impact on the immunogenicity or reactogenicity of the product in patients.

Comparability of Process 2.0 to Process 3.0

Comparability studies (including an evaluation of process comparability and analytical comparability) were performed between Process 2.0 and Process 3.0 to support the manufacture changes between the two processes. Process 2.0 was the original pandemic process at the Holly Springs facility when the seasonal influenza process was transferred from Marburg, while Process 3.0 is the currently licensed seasonal influenza process, and the proposed commercial process for pandemic. Comparability between Process 2.0 and Process 1.1 was demonstrated, as well as between Process 1.1 and Process 3.0. Therefore, comparability between the original process at Holly Springs (Process 2.0) and the proposed commercial process (Process 3.0) was also demonstrated. As the process and equipment between Process 2.0 and Process 1.1 were the same, most of the comparability between Process 1.1 and Process 3.0 also applies to the comparability of Process 2.0 to Process 3.0. The results of the comparability studies confirmed that the manufacturing process changes did not impact product quality, and therefore it is concluded that drug substance manufactured using Process 3.0 is comparable to that manufactured by Process 2.0. Consequently, the process changes are not expected to have any adverse impact on the immunogenicity or reactogenicity of the product in patients.

Comparability of the individual process versions has been demonstrated. The reduced specific potency observed in batches from Process 1.1 has been sufficiently justified.

Characterisation

Elucidation of structure and other characteristics

Elucidation of structure and other characteristics was performed on antigens from influenza strains H1N1, H3N2 and the B-strain: monovalent MDCK cell culture derived lots A/New Caledonia (H1N1), A/New York (H3N2), and B/Jiangsu as well as on finished trivalent product comprised of antigens from A/New Caledonia (H1N1), A/Wyoming (H3N2), and B/Jiangsu. A special focus was put on the comparison of the MDCK cell culture derived antigen versus the egg derived antigen. In addition, the applicant has provided also data from other virus strains (H1N1, H3N2 and H7N9) as supportive evidence that the platform works with other subtypes. Upon request, the applicant provided characterisation data from the monovalent cell culture-derived H5N1 subunit influenza virus vaccine. The biological, immunological and physicochemical properties of the HA antigen was verified using a wide range of state-of-the-art analytical methods, which are considered adequate.

The presence of HA is confirmed in both the active substance and finished product via appropriate activity and quantity by Single radial immunodiffusion assay (SRD or SRID). The presence of NA is confirmed in the active substance by an identity test.

In summary, characterisation made use of a broad panel of standard and state-of-the art analytical methods. The relevant biological, immunological and physicochemical properties of MDCK cell culture derived influenza antigens have been addressed. The method principle for each method used for characterisation and a brief description of the methods and evidence that the methods are suitable for their intended use was provided. The methods are considered adequate.

Impurities

The potential impurities have been extensively discussed. Regarding the product-related impurities the dossier states that no new product-related impurities other than those previously described are contained within the FCC monovalent bulk that would have an impact on activity, efficacy, and safety. In summary, the product related impurities consisting of oligomerisation states of influenza HA, do not impact activity, efficacy, and safety.

Process-related impurities include residuals associated with the cell substrate as well as residuals associated with the manufacturing process. For the process-related impurities common between Process 1.1 and Process 3.0, acceptance criteria were based on data previously obtained from seasonal and pre-pandemic sub-type H5N1 lots used in preclinical and clinical studies and manufacturing consistency lots and remain unchanged. This strategy is acceptable.

For most of the listed process-related impurities, removal has been demonstrated through product development and process validation. Lots manufactured during seasonal and H5N1 process validation were analysed at various process steps to demonstrate the consistent removal of HCP. The Guideline on Influenza vaccines – Quality module, EMA/CHMP/BWP/310834/2012 Rev.1, states that 'where the production method for cell culture-derived vaccine is validated (using a wide range of influenza strains to demonstrate suitable reduction of residual DNA/ host-cell protein), routine testing for residual DNA and host-cell proteins may be omitted'. Therefore, the proposal to omit release testing for HCP is acceptable. The
enzyme-linked immunosorbent assay (ELISA) method and its validation has been submitted.
In summary, a sufficient and appropriate discussion on the impurities has been provided.

2.4.2.3. Specification and analytical methods

The active substance release specifications, as proposed by the applicant, are acceptable as they cover the main characteristics of the product: identity, purity and potency.

Analytical methods

The active substance release specification is provided for the H5N1 strain. The set of specifications cover identity, purity and potency. The proposed testing protocol for the monovalent bulk complies with the Ph.Eur. monograph. Potency of the active substance is determined by the quantification of the haemagglutinin antigen using the single radial diffusion (SRD) assay. The analytical procedures (principle, equipment, standards/solution, procedure, measurement/ evaluation) are concisely described and the validation reports provided. Additional information was also requested for some of the methods.

Taken together, proposed analytical methods are considered suitable to control critical quality attributes of this kind of product and were validated according to guideline ICH Q2(R1). Thus, the presented active substance release testing strategy is acceptable.

Justification of specifications

During the procedure, it was noted that the limit for HA content is lower than available batch data and the applicant was invited to further justify or tighten the release specification for HA content to ensure a batch released at the lower limit of the acceptance criteria will remain within stability specification throughout the proposed shelf life. The applicant further justified the acceptance criteria based on the requirements in the Ph. Eur. monograph for influenza vaccine that further tightening is not needed. In addition, an additional time point in the stability protocol to verify the stability was included.

The specifications for Identification of HA and residual infectious influenza virus are as indicated in Ph. Eur. monograph 2149, and for sterility as indicated in Ph. Eur. 2.6.1. The applicant acknowledged the limited data set and as part of the continuous process verification process, data will continue to be trended and re-assessed. Identity test for strain-specific neuraminidase was performed on the first three monovalent bulks and compared with the WHO reference standard. The demonstration of type-specific neuraminidase ensures that lots produced with new seeds will reliably result in detectable neuraminidase expression.

Batch analysis

Batch analysis data for three H5N1c active substance Process Performance Qualification (PPQ) batches manufactured in 2017 are provided. All acceptance criteria were met. In addition, the dossier has been updated to include batch data from development and clinical batches. With the continuous process verification process, data will continue to be trended and re-assessed by the applicant.

Reference standards or materials

Information about critical reagents and reference standards are included.

Concerning the SRD assay, the reference antigen and antiserum (sheep origin) are provided by Centre for Biologics Evaluation and Research (CBER), or the applicable WHO Collaborating Centre for Influenza or Essential Regulatory Laboratory (ERL). The reference antigen is cell-derived from whole virus preparation in MDCK cells but if these are unavailable, it can also be egg-based. These references are calibrated by the ERLs. The qualification of reagents includes optimisation of antiserum through screening, qualification of the reference antigen and antiserum, and compatibility confirmation of the reagents with the monovalent FCC production material. To qualify a lot of reference antigen from an existing, qualified strain, the use of the optimised antiserum concentration is used to test the new reference antigen lot in parallel with the current antigen lot for comparison of results. The assay acceptance criteria must be met and the percent difference between the mean potency values must be ≤ 15%. The qualification of a lot of reference antigen from an existing, qualified strain is acceptable.

Concerning the neuraminidase antigen assay, the applicant briefly described the qualification of the positive control. Neuraminidase type specific antiserum is provided by the applicable WHO Collaborating Centre for Influenza or ERL.

For purity testing the precision of the standard protein is stated and the acceptance criteria of the assay control (monovalent bulk material) is defined by a qualification procedure.

Overall, sufficient information about the reference materials used for the active substance assays are provided.

Container closure system

The monovalent bulk is stored in a 50-L polypropylene (PP) carboy with a stainless-steel dip-tube system integrated into a white polypropylene screwcap closure fitted with a silicone gasket.

2.4.2.4. Stability

The choice of the tested parameters has been accepted because they are stability-indicating parameters as verified in the forced degradation studies. The stability data is considered appropriate to support the proposed shelf life and the post-approval stability protocol and stability commitments are also acceptable

A shelf life of 18 months is proposed for the active substance when stored at 2-8°C.

This claim is based on the data obtained with 3 representative active substance batches with A/turkey/Turkey/1/2005 NIBRG-23 strain. The utilised batches are not components of the finished product batches used in finished product stability study. The stability studies were performed at the recommended storage condition (2-8°C).

The rationale for the choice of the tested parameters is acceptable, since it is in line with the forced degradation studies. This justification is acceptable.

The long-term stability study is finished. Overall, the active substance stability batches have met the acceptance criteria for all the parameters tested in long-term stability studies. In addition, the accelerated stability results for all batches evaluated have met all specifications through study completion.

The stability results indicate that the active substance is sufficiently stable and justify the proposed shelf life in the proposed container when stored at the recommended storage condition.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Description and composition of the product

Influenza A (H5N1) virus vaccine, cell-derived and adjuvanted with MF59C.1, also referenced as aH5N1c, is an inactivated influenza vaccine containing predominantly haemagglutinin and neuraminidase surface antigens from a MDCK cell-derived H5N1 virus subtype. The potency of the vaccine is expressed as the concentration of the HA protein. Phosphate buffered saline (PBS) is included in the formulation to stabilise the product and to adjust the osmolality.

The final product is adjuvanted with MF59C.1, an oil-in water suspension containing squalene oil as the dispersed phase and low ionic strength citrate buffer as the continuous phase. The suspension is stabilised by the inclusion of two non-ionic surfactants (polysorbate 80 and sorbitan trioleate).

Excipients, in addition to the adjuvant are sodium chloride (isotonicity agent), potassium chloride (isotonicity agent), magnesium chloride hexahydrate (stabiliser), disodium phosphate dihydrate (buffer), potassium dihydrogen phosphate (buffer) and water for injection (diluent). All components comply with the current edition of the USP and Ph. Eur. monographs, as applicable. There are no novel excipients used. The composition of the adjuvanted H5N1c Influenza vaccine is described in Table 1.

The vaccine is presented as a liquid for injection, in a Type I glass pre-filled syringe ready for use, containing a 0.5 mL dose. The vaccine is milky-white homogeneous fluid in appearance and is preservative-free. Each syringe is intended for a single use.

A sufficient overfill is included in the syringe to ensure the withdrawal of a nominal volume of 0.5 mL per human dose.

Table 1. Composition of Adjuvanted H5N1c Influenza Vaccine

Ingredients	Quantity per Adult Dose (0.5 mL/dose)	Quantity per mL	Function	Reference to Standards
Active Ingredient				
H5N1 monovalent bulk	7.5 µg[1]	15 µg	HA antigen (active ingredient)	NIBSC
Adjuvant				
Squalene	9.75 mg	19.50 mg	Oil phase	Ph. Eur.
Polysorbate 80	1.175 mg	2.350 mg	Surfactant	USP/Ph. Eur.
Sorbitan trioleate	1.175 mg	2.350 mg	Surfactant	Ph. Eur.
Sodium citrate	0.66 mg	1.32 mg	Buffer	USP/Ph. Eur.
Citric acid	0.04 mg	0.08 mg	Buffer	USP/Ph. Eur.
Excipients: Phosphate-Buffered Saline,				
Sodium chloride	mg	mg	Tonicity agent	USP/Ph. Eur.
Potassium chloride	mg	mg	Tonicity agent	USP/Ph. Eur.
Potassium dihydrogen phosphate	mg	mg	Buffer	USP/Ph. Eur.
Disodium phosphate dihydrate	mg	mg	Buffer	USP/Ph. Eur.
Magnesium chloride hexahydrate	mg	mg	Stabiliser	USP/Ph. Eur.
Water for injection	q.s. to 0.5 mL	q.s. to 1mL	Diluent	USP/Ph. Eur.

HA: Haemagglutinin Antigen; NIBSC: National Institute for Biological Standards and Control; Ph. Eur.: European Pharmacopoeia; q.s.: Quantity Sufficient; USP: United States Pharmacopeia

[1]Nominal concentration is 7.5 µg HA per 0.5 mL dose.

The information presented is considered acceptable.

Pharmaceutical development

The formulation of the finished product has been adapted to the aH5N1c format, starting from the licensed cell-culture based Flucelvax Tetra (EMEA/H/C/004814) and the adjuvanted Fluad Tetra (EMEA/H/C/004993) vaccines. The formulation of the final formulated bulk product was developed in accordance with the European Pharmacopoeia.

The concentration per dose is ≥ 7.5 µg haemagglutinin antigen (HA) for the aH5N1c strain contained in a phosphate buffered saline (PBS) buffer.

MF59C.1 adjuvant

The formulated adjuvant (with a squalene concentration of 36 to 42 mg/mL) is a white, homogeneous liquid, pH 6.0 to 7.0, which is free from visible foreign particulates.

The following solutions are prepared prior to the emulsification process: sodium citrate buffer, polysorbate 80 in water for injection and squalene in sorbitan trioleate. To prepare the emulsification, water for injection is added to the pre-mixing tank followed by the solutions. The material is passed through a mixer to form a crude premix. The crude premix is then passed through a microfluidiser to produce a fine emulsion. The microfluidised bulk is filtered and filled into a sterile Flexbag containers. The product contact layer of the flexible bags is compliant with Ph. Eur. requirements for containers and tubing for parenteral nutrition preparations and is certified transmissible spongiform encephalopathies (TSE)/ bovine spongiform encephalopathy (BSE) free. The filtered bulk is stored at 2-8ºC. The manufacturing process at both sites is sufficiently described.

A list of the materials used in the production of MF59C.1 bulk adjuvant is provided. With the exception of squalene (derived from shark liver oil), these materials do not contain any human or animal-derived components. All other materials are compendial.

The manufacturing process of MF59C.1 consists of a series of manufacturing steps including: raw materials dispensing and blending, premixing, emulsification, sizing filtration and filling into flex bags. Appropriate process controls (including critical process parameters and in-process controls) are defined for its manufacture.

Testing for leachables and extractables was performed. The actual risk of extractables/leachables arising from the product contact materials of construction used in the bulk MF59C.1 adjuvant process was considered to be low. Additionally, the results of the leachables and extractables assessment indicate that the MF59C.1 is chemically compatible with materials of construction used during processing.

The MF59C.1 bulk adjuvant process validation has been successfully completed with three consecutive batches at representative scale at Holly Springs. The process validation parameters were within the respective normal operating ranges and met all acceptance criteria specified.

At the Liverpool site the three validation runs had to be repeated because of the growth of negative bacteria. Further engineering runs indicated no contamination for an extended period of time, allowing for closure of the deviation report and performance of three repeat runs. The process validation parameters of the repeated runs were within the specified parameters confirming the process is reproducible and produced product meets the approved specifications.

A process characterisation of the microfluidisation and filtration steps was performed at Chiron's manufacturing site in St Louis, Missouri, USA. Since the same equipment was transferred from St Louis to Chiron's manufacturing site in Marburg, Germany, this study is considered applicable to the current manufacturing process. Three consistency lots were manufactured, and the adjuvant product was characterised for emulsion components, bioburden, endotoxin level, pH, and appearance. The consistency in the characteristic of the three manufactured lots demonstrated that the manufacturing process is reproducible.

Additionally, a transport validation procedure was executed, and it demonstrated the sterility of materials shipped in the Satorius Bio-Process Container. Sufficient information for the transport validation at 2-8ºC was submitted.

MF59C.1 Manufacturing Process Development

Holly Springs:

The MF59C.1 manufacturing process was transferred from Marburg site to Holly Springs where it has been validated. . Manufacturing process, facility, equipment and raw materials are considered comparable. The comparability between Marburg and Holy Spring sites was sufficiently demonstrated.

Liverpool:

From 2016 till 2019 the applicant conducted the technology transfer of bulk MF59C.1 manufacturing from the Marburg facility to the Liverpool site 4 facility. Historical batch analysis data from Marburg site were compared with release data of batches from Liverpool site. Based on the comparison of the input materials, equipment and process, alongside the information generated from the batches manufactured at Liverpool, it can be considered that the manufacturing processes between Liverpool and Marburg sites are comparable.

MF59C.1 Specification and Analytical Methods

MF59 Specification and analytical methods include chemical composition, physical characteristics (e.g., visual appearance, viscosity, pH, size and size distribution), biochemical characteristics, and purity (e.g., endotoxin content, bioburden, manufacturing residuals).

For Holly spring site assay validation data of quantification and identification of carbonyl compounds in MF59C.1 adjuvant and MF59C.1 formulated products have been submitted.

Additionally, method transfer report for the measurement of quantification of particles in MF59C.1 adjuvant formulations and transfer of quantitation of polysorbate 80 and sorbitan trioleate in MF59C.1 adjuvant from Marburg, Germany to Holly Springs has been submitted. The execution of the method transfer protocol demonstrated that the receiving laboratory can perform this assay with comparable results.

The method for the quantification and identification of polysorbate 80 and sorbitan trioleate in MF59C.1 bulk and polysorbate 80 solutions was validated.

The assay for squalene content was validated and demonstrated to be fit for purpose for the influenza platform.

With a statistical evaluation of the analytical methods for release and stability testing of MF59C.1 adjuvant between the Seqirus sites comparability of the methods has been demonstrated.

MF59C.1 Batch Analysis Data

Batch analyses are presented for batches) produced at the Seqirus sites. All batches complied with the specifications approved at the time of release. Manufacture has also been appropriately validated. Prior to use in finished product formulation, the MF59C.1 adjuvant is sterile filtered into flexible bags. Sterile MF59C.1 is stored at 2-8ºC.

The batch analysis data for three MF59C.1 bulk adjuvant lots produced at the Liverpool facility are provided. The information provided is considered sufficient.

MF59C.1 Reference Standard Materials

Reference standards for squalene, polysorbate 80, sorbitan trioleate, and MF59C.1 bulk adjuvant are presented in the dossier. Reference standards are drawn from production lots released in-house as per release specification. The reference lots are drawn from the same materials used for production of the MF59C.1 bulk adjuvant lot being assayed to prevent a lot-to-lot variability. This procedure is acceptable.

MF59C.1 Container Closure System

MF59C.1 bulk adjuvant is stored in flexible polymer bags (flex bags).

Leachables/extractables studies performed on bags which are in contact with MF59C.1 bulk adjuvant showed that the calculated amounts of marker compounds in a vaccine dose were all below the established safety thresholds after the full exposure time.

Long-term stability studies are currently on-going utilising process validation material. The long-term storage condition has previously been demonstrated.

A study on stability/compatibility of the plastic material of the flex bag with MF59C.1 adjuvant bulk has been performed in order to support the storage of the final bulk from formulation date, at the recommended storage condition of 2-8ºC, protected from light. Safety and purity data are demonstrated by extractables and leachables studies performed on the adjuvant as provided in the toxicological assessment.

Overall sufficient information about the container closure material used for both manufacturing sites was submitted.

MF59C.1 Stability

A shelf life is proposed for the MF59C.1 bulk adjuvant at 2-8°C, protected from light.

Long term stability data at 2ºC to 8ºC were provided. Results of these stability studies showed that tested parameters were within the defined acceptance criteria.

Stability studies have been initiated on batches manufactured as part of the PPQ performed as part of the equipment and manufacturing technical transfer of MF59C.1 bulk manufacture. All parameters tested remained within the specifications .

The provided data support the storage conditions of 2°C to 8°C) of MF59C.1 bulk adjuvant.

Updated long term stability data of MF59C.1 bulk adjuvant from the Liverpool site support a shelf life of MF59C.1 Bulk Adjuvant.

Photostability experiments were performed to determine the sensitivity of MF59C.1 bulk adjuvant to light. Preliminary results indicated that protection from light is necessary for the MF59C.1 bulk adjuvant.

Manufacturing process development

The applicant described the different process development steps from phase 1.0 to 3.0.

In phase I of the manufacturing process evolution the finished product was filled and packaged in Rosia, Italy. The FCC process and finished product manufacturing processes were subsequently transferred from the Novartis Vaccines and Diagnostics (NVD) Marburg and Rosia facilities to NVD Holly Springs, North Carolina (now Seqirus).

Process 1.0 of phase I at 2008 used H5N1 Strain A/Indonesia/5/2005 at the NVD Marburg and Rosia sites to determine the best dose of antigen and adjuvant MF59C.1. Under Process 2.0 in phase II the manufacturing process was transferred from Marburg site to Holy Springs site and the formulation and filling was performed at Rosia site. In 2016 the materials for the Phase III clinical trial were manufactured with monovalent bulk using the H5N1 Virus Strain A/H5N1/turkey/Turkey/1/2005 NIBRG-23, formulated and filled into syringes for the clinical study (Process 1.1).

With Process 3.0 the applicant upgraded the active substance manufacturing process at the Holly Springs manufacturing facility. Furthermore, the Phase III clinical trial material was also formulated and filled at the Holly Springs site by using a scaled-up formulation and filling process. The formulation and filling process of phase II from Rosia site was compared with phase III from Holy Spring site (using a scaled-up formulation and filling process). The differences in the formulation and filling processes from Phase II (Rosia) to Phase III (Holly Springs) are mostly justified by upscaling steps. Assessments were performed to evaluate facilities and equipment; process (including in-process

checks, in-process controls and hold times); analytical methods and specifications; stability; and validation (including filter validations, process qualification, extractables/leachables, and mixing). It could be demonstrated that equipment, operational parameters, in-process checks, controls and hold times are comparable at both sites (Rosia for Phase II and Holly Springs for Phase III).

Analytical methods were modified in Phase III for harmonization with other licensed influenza vaccines. Comparability studies demonstrated that the process design, process performance, and analytical attributes for Phase III manufactured at Holly Springs were comparable to the Phase II manufactured at Marburg/Rosia. Overall, the changes made from Phase III to commercial manufacturing for inspection and packaging are considered comparable.

As Process 1.1 and Process 3.0 active substance materials have been demonstrated to be equivalent, the applicant does not plan to perform any additional process validation studies for the pre-filled syringe formulation and filling processes. However, one pre-filled syringe finished product lot has been manufactured with Process 3.0 H5N1c active substance material and has been placed on stability (referred to as the stability lot). The applicant states that one pre-filled syringe lot (lot 251069) and three multi-dose vial lots were manufactured with active substance from process 3.0, for a total of four lots, and used for comparability to the finished product manufactured with active substance from process 1.1 (phase III clinical trial material). Data for the multidose vial lots is presented, and a comparability exercise of data from the commercial and phase III finished product lots are presented. The provided information is considered acceptable.

Container closure system

The primary packaging consists of a 1 mL syringe with a plastic rigid tip cap of rubber formulation FM30 (not made with natural rubber latex) that is closed with plunger stopper. The syringe and plastic rigid tip cap are manufactured as a luer lock syringe. No needle is present on the syringe. The tip cap is lodged in a rigid plastic shell and screwed into the luer lock adaptor; the plastic shell protects the tip cap from damage.

The nature of materials of each container closure component is described and comply with Ph. Eur. and EC requirements. Detailed drawings of the syringes without a needle is provided. A certificate of conformity is provided for each component.

A Notified Body opinion for the integral medical device (syringe) from the Notified Body BSI (Netherlands) has been submitted. The technical documentation was reviewed in accordance with Annex I of Regulation (EU) 2017/745. The Notified Body`s assessment has been performed for the purpose of an initial application and the objectives of the assessment were found to have been met. The technical documentation for the device is considered adequate to support compliance with the General Safety and Performance Requirements of the MDR. Consequently, the major objection raised during the procedure is solved.

The compatibility of the finished product with the container has been confirmed by the stability studies. The data submitted do not seem to be of concern regarding the safety of the container closure system.

Microbiological attributes

The finished product is a sterile solution. Both formulation and filling of the syringes are carried out in controlled conditions, and the filled final product is tested for sterility. No preservatives are used in the finished product.

2.4.3.2. Manufacture of the product and process controls

The finished product is manufactured, controlled and stored by Seqirus Inc (Holly Springs, USA) in accordance with cGMPs. Final release is performed by Seqirus Netherlands B.V. (Amsterdam, Netherlands). Further quality control sites are in Ireland and the Netherlands. Appropriate evidence of GMP compliance for all sites has been provided.

Batch formula

The batch formula for the finished product depends on the haemagglutinin concentration (potency) and density of the monovalent bulk. Based on the HA concentration of the monovalent bulk, the weight of HA antigen is calculated and the amount of buffer is adjusted according to a defined quantitative formulation.

Manufacturing process and process controls

Formulation of the monovalent aH5N1c final bulk with, water for injection, and sterile MF59C.1 adjuvant is carried out using a closed system in a controlled zone.

The various components are connected by pre-sterilised, disposable assemblies with connectors, or by steam-in-place piping. A flow chart completed with process parameters and in-process controls was submitted. All the process controls described by the applicant are agreed to be sufficient.

The adjuvant MF59C.1 is sterile filtered into bags prior to use in formulation. Sterile Filtration can either be carried out as an in-line step with the manufacture of bulk MF59C.1 or through the process of pooling. Sterile filtration and in-line sterile filtration of MF59C.1 is sufficiently described.

The packaging of the finished product consists of a 1 mL Luer-LokTM syringe and a plunger stopper and tip cap with plastic rigid tip cap overseal. The filling process has been validated. The inspection, labelling and packaging procedures are all found acceptable.

Controls of critical steps and intermediates

A quality risk analysis was performed to assess the critical quality attributes and critical process parameters for the formulation, filling and packaging manufacturing process. Critical process parameters for the intermediate (the sterile-filtered MF59C.1 into flex bags) and the formulation steps to formulate the H5N1c bulk are identified. Further critical- and in-process controls for the sterile filtered MF59C.1 and the formulation of the aH5N1c bulk are implemented. Release specifications at the end of formulation must be met for batch release. There are no intermediates during the filling and packaging process. Critical process parameters and in-process controls are clearly identified for the formulation and filling process. Release specifications for filled product must be met for batch release. Overall, consistent manufacture has been demonstrated.

Process validation and/or evaluation

Different process validation activities associated with the sterile and in-line sterile filtration of MF59C.1 were performed with multiple runs. The filter used for sterile filtration was validated with regards to chemical compatibility, integrity, bacterial retention capability, and extractables and leachables. Aseptic simulation of aseptic steps was performed and showed satisfactory results. Hold time limits for MF59C.1 have been demonstrated by chemical stability data and aseptic process simulations.

The results of the submitted validation studies for MF59C.1 support the conclusion that manufacturing process of MF59C.1 is sufficiently validated.

The formulation of aH5N1c bulk was validated using the seasonal active substance manufacturing Process 1.1 material for the PPQ Phase III clinical lots. One further finished product lot and three multi-

dose vial lots utilizing Process 3.0 H5N1c active substance material for formulation were also filled into pre-filled syringes and placed on stability. The applicant showed that the active substance Process 1.1 to Process 3.0 is comparable therefore the applicant does not plan to perform any additional process validation on the PFS formulation and filling processes with lots manufactured with 3.0 H5N1c active substance material. The approach is acceptable.

Monovalent bulk antigen, and water for injection were aseptically added to the formulation vessel via sterile filters. Sterile-Filtered MF59C.1 was aseptically added to the formulation vessel without passing through a filter. The output of the formulated process is a final finished product bulk solution to be transferred for filling into syringes.

Analytical results of the three PPQ Phase III clinical lots were all within the defined specifications. Operational parameters include key or critical process parameters met the acceptable normal operating range. Deviations in the validation process were identified and described. The impact of the deviations for the process were assessed.

Further validations and formulation process-associated studies were performed, including the following: filter validation, leachables and extractable on all direct and indirect product contact materials, mixing validation, aseptic process simulations, and hold time validation.

The PPQ for filling and inspection steps was performed on clinical (PPQ) lots. A minimum fill target) of the clinical Phase III PPQ batches was filled and inspected.. To support approval of the aH5N1c fill finish process various validations and associated studies for the filling and packaging processes were performed: syringe inspection system, an evaluation of PPQ lots formulated, labelling, packaging and shipping validation. All fill uniformity data met the acceptance criteria for the 3 Phase III clinical (PPQ) lots. The results presented are reassuring about the validation of syringe filling, labelling and packaging.

Shipping validation was performed with water, which aligns with the shipping validation master plan, and is considered sufficient.

Control of excipients

Phosphate buffered saline and a stabilizing solution containing magnesium chloride and calcium chloride are excipients used for the aH5N1c formulation. All components comply with the current edition of the USP and Ph. Eur. monographs, as applicable. Water for injections, used as a formulation vehicle, complies with the requirements of the current edition of the USP and Ph. Eur. monographs.

The compendial assays were verified and carried out in accordance with the current Ph. Eur. requirements. Since the methods are compendial, further validation was not required. However, for completeness, the corresponding USP / Ph. Eur. monograph numbers were added to each buffer component and to each compendial method. Squalene, derived from shark liver, is the only substance of animal origin used in the finished product. There are no novel excipients.

2.4.3.3. Product specification

The specifications for release of the final formulated bulk, and filled product are provided. Specifications include appropriate tests for physicochemical characteristics, potency, identity and purity, and are supplemented with tests to control the adjuvant characteristics, i.e. squalene identity and content, mean particle diameter and number of large particles with a diameter of > 1.2 µm.

Process related impurities of H5N1 monovalent bulk are listed and come from the active substance manufacturing process. Reference is made to active substance characterisation of impurities. There are no product-related impurities derived from the H5N1 monovalent bulk.

The adjuvant MF59C.1 has no process-related impurities since all raw materials added during the manufacturing process are components of the formulated adjuvant. Product-related impurities are inherent in squalene (not introduced during the purification process of squalene or during the MF59C.1 manufacturing process).

Justification of specification is based on Ph. Eur. monograph recommendations or WHO requirements for influenza vaccine prepared in cell cultures. Overall justification of specifications are sufficiently given.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed (as requested) considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided [in Nitrosamine Risk Assessment Report for Audenz (US registered cell culture, adjuvanted monovalent, influenza pandemic vaccine (aH5N1c))] it is accepted that no risk was identified on the possible presence of nitrosamine impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

The applicant submitted in the dossier a risk assessment report for elemental impurities. In this report the conclusion was that the risk of elemental impurities is inherently low and poses no safety concerns or risk to the patient. The risk assessment was performed for the US registered cell culture, adjuvanted monovalent, influenza pandemic vaccine (aH5N1c), Audenz. This study is acceptable because the vaccine manufacturing process is comparable with Incellipan.

Analytical methods

Validation summaries for the non-compendial methods and verifications of compendial methods are provided. The appearance test is compendial according of Ph. Eur. 2.9.20 Particulate contamination: visible particles. In line with Ph. Eur., a test for residual infectious virus and visible and sub-visible particle is included for finished product.

The applicant has provided an update on ongoing efforts to desist the use of for horseshoe crab derived material in endotoxin testing, which is appreciated.

The parameters evaluated for the non-compendial methods are in line with ICH Q2 guideline: linearity, specificity, intermediate precision and accuracy for the quantitative methods, specificity for the identity tests and limit of quantification for the impurities methods.

The results of the validated assays met all of the pre-determined acceptance criteria and method transfer reports have been provided for non-compendial methods.

Batch analysis

Batch analysis data for three Phase III clinical lots manufactured with active substance Process 1.1 were presented. All test results of the final bulks and final finished products for PPQ batches are compliant with the product specifications. Furthermore, batch analysis results of one batch manufactured with Process 3.0 are well within the product specifications.

Reference standards or materials

Reference standards have been assessed during the method validation and transfer process.

The reference antigen and antiserum reagents used to calibrate the SRD assay are obtained by WHO Collaborating Centres.

2.4.3.4. Stability of the product

A 12 month shelf life is proposed for the finished product when stored at 2-8°C.

Long term stability data at 2-8°C for 24 months from three clinical phase III batches (PPQ batches) manufactured with active substance process 1.1 are provided. All results were consistently within the acceptance criteria. The MAH could sufficiently justify the out-of-specification result.

Accelerated stability data for almost all tested finished product batches were within the defined specifications with one exception. Out-of-specification results were observed for all PPQ lots for HA by SRD. The out-of-specification result has no influence for the request of shelf life of 12 months at 2-8°C.

Regarding the post-approval stability protocol and stability commitment, a minimum of three production batches will be placed on stability for a minimum of 18 months at the recommended storage condition of 2-8°C and the same analytical parameters will be assessed as for the above-mentioned lots.

The stability studies and the data from their corresponding results are considered acceptable.

A 12 month shelf life for the finished product at 2-8°C, protected from light, as stated in the SmPC is supported by the provided data and is therefore considered acceptable.

2.4.3.5. Adventitious agents

The adventitious agent management programme for the FCC vaccine reflects the ICH recommended complementary approach to controlling potential viral contamination of biotechnology products. The MDCK cell line and other raw materials, including media components, have been selected and tested for the absence of undesirable viruses which may be infectious or pathogenic to humans.

Regarding MCB and cells used for vaccine production, no animal-derived product is used. The harvested virus material is inactivated and undergoes detergent treatment and purification to yield a surface antigen subunit vaccine. Regarding MDCK cells and TSE, the applicant states that EMA indicated that there is no TSE-related concern over the acceptability of the MDCK master cell bank. In addition, the MDCK cells were not in contact with animal-derived material since 1996. Several tests (normal canine prion) and risk assessment were carried out relating to TSE risk for these cells, which show that it is extremely low.

The manufacturing process has been assessed for its capacity to clear infectious viruses (influenza, herpes simplex virus 2, murine leukaemia virus, reovirus type 3, SV-40 and porcine parvovirus). Viral and Non-Viral Safety Data for the cell substrate was assessed by literature and experimental.

Product intermediates and the final container are tested at appropriate intervals to assure the absence of contaminating infectious viruses.

Due to the changes from process 1.1 to process 3.0 a comprehensive adventitious agent safety assessment was performed to identify potential gaps and drive appropriate resolution. The overall microbial control strategy in the FCC 3.0 manufacturing process has not changed from the FCC 1.1 process. The ability to effective and substantially eliminate more virus than is estimated to be present in a single-dose- equivalent of unprocessed bulk (ICH guideline Q5A) with Process 1.1 is also valid for Process 3.0.

The chromatography resin, mode of operation, and cleaning were assessed to ensure no virus carryover between chromatography runs. All acceptance criteria for the studies were met, demonstrating absence of virus carryover between chromatography cycles.

It is concluded that the changes made to the FCC manufacturing process from process 1.1 to process 3.0 do not increase the risk to viral safety, but rather improve the management of adventitious agents in the manufacturing process. Overall, the viral safety package is comprehensive and there are no concerns.

2.4.4. Discussion and conclusions on chemical, pharmaceutical and biological aspects

Overall, the Quality Module 3 is of acceptable quality and a number of deficiencies raised were satisfactorily addressed.

The active substance and finished product manufacturing processes, its validation and control are sufficiently described. Characterisation made use of a broad panel of standard and state-of-the art analytical methods. The relevant biological, immunological and physicochemical properties of MDCK cell culture derived influenza antigens have been addressed. The control strategy, including in-process controls and release testing specifications of active substance and finished product, are acceptable. The proposed shelf life for active substance and final product are supported by data and is considered acceptable.

The adventitious agent management programme for the FCC vaccine reflects the ICH recommended complementary approach to controlling potential viral contamination of biotechnology products. The MDCK cell line and other raw materials, including media components, have been selected and tested for the absence of undesirable viruses which may be infectious or pathogenic to humans. Overall, the viral safety package is comprehensive and there are no concerns.

The manufacturing process of the adjuvant MF59C.1 is well documented. The general characteristics and structure of the adjuvant MF59C.1, whose main component is squalene are described by the applicant. Concerning the process-related impurities, those arising from the manufacturing process of the MF59C.1 adjuvant are controlled in the adjuvant bulk, as they are not expected to increase in the formulated product. The provided stability data support the proposed expiry and storage conditions (2°C to 8°C, up to 60 months) of MF59C.1 bulk adjuvant manufactured at both sites Holy Springs and Liverpool.

During the evaluation procedure, a major objection was raised on the lack of a Notified Body opinion for the pre-filled syringe integral medical device confirming compliance with the relevant General Safety and Performance Requirements in Annex I of Regulation (EU) 2017/745. The applicant provided the Notified body opinion addressing the major objection.

Questions were raised on the characterisation data with the H5N1 antigen, specification (particularly the SRID potency assay), validation reports and finished product stability that were appropriately solved.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.4.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends some points for investigation.

2.5. Non-clinical aspects

2.5.1. Introduction

The Influenza A (H5N1) Monovalent Vaccine, Adjuvanted (called aH5N1c in this submission) is intended for active immunisation for the prevention of disease caused by the influenza A virus H5N1 subtype contained in the vaccine.

aH5N1c is a surface antigen, inactivated, influenza vaccine adjuvanted with MF59C.1 (MF59). The vaccine contains purified haemagglutinin (HA) and neuraminidase (NA) antigens from the NIBRG-23 virus, a reverse genetics-derived reference strain supplied by NIBSC UK. NIBRG-23 contains HA and NA from the H5N1 strain A/turkey/Turkey/1/2005. The virus is grown in Madin Darby Canine Kidney (MDCK) cells and inactivated before purification of the surface antigens and final formulation.

The manufacturing process for the drug substance is based on the Flucelvax Tetra (QIVc) process. Flucelvax Tetra is an MDCK cell culture-derived quadrivalent surface antigen, inactivated seasonal influenza vaccine manufactured by the applicant. This quadrivalent product was licensed in Europe in December 2018 (EMEA/H/C/004814), the trivalent version was licensed in Europe in June 2007 under the trade name Optaflu (EMEA/H/C/000758).

The drug substance is formulated with MF59 (MF59C.1 proprietary adjuvant), to produce drug product. MF59 is an oil-in-water emulsion, composed mainly of squalene, stabilised with the surfactants polysorbate 80 and sorbitan trioleate, in citrate buffer. MF59 is the adjuvant present in the seasonal trivalent influenza vaccine Fluad which has been licensed in several European countries via Mutual Recognition Procedure since May 1997 (IT/H/104/001), and in the seasonal quadrivalent influenza vaccine Fluad Tetra licensed in Europe in May 2020 (EMEA/H/C/004993).

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Mice data provided from literature confirmed the positive effect of MF59 by showing a comparison of the immune response elicited by seasonal influenza antigens with and without the adjuvant. In the study data provided, cell culture-derived trivalent influence vaccine (TIVc) was combined with various adjuvants, including MF59. Immunogenic response after intramuscular administration was evaluated

by hemagglutination inhibition (HI) assays, IgG titres, virus neutralisation and T-cell response. For all of these end points, addition of MF59 enhanced the immune response.

In the GLP-compliant repeat-dose and developmental toxicity studies in rabbit conducted with aH5N1c using the intended clinical administration route, antibody response was assessed either via hemagglutination inhibition assay or by ELISA.

In the repeat-dose toxicity study, rabbits received three intramuscular doses of either saline, MF59, or aH5N1c (15 µg antigen; 0.25 mL of MF59; total volume of 0.5 mL per dose) on days 1, 15 and 29. The vaccine strain used was A/Indonesia/05/2005 (H5N1)/PR-8-IBCDC-RG02. Immunogenicity was assessed using a heterologous (Vietnam; Clade 1) virus strain because of restrictions in place at the time of analysis affecting the availability of the homologous strain (Indonesia; Clade 2). Whereas no antibody response was detectable in control and MF59-alone treated group, all rabbits receiving two doses of aH5N1c showed titres ≥160 in the HI assay to the heterologous strain from day 29 onwards.

In the GLP reproductive and developmental toxicity study, female rabbits received the clinical dose of vaccine (7.5 µg HA + 0.25 ml MF59) twice prior to mating, and twice during gestation. Immunogenic response was investigated by ELISA in female rabbits, fetuses and offspring. Data confirmed aH5N1c to be immunogenic in female rabbits, and antibodies in vaccine-treated does were shown to be transferred to fetuses and measurable in offspring through the lactation period.

Additional data supporting the nonclinical immunogenicity of aH5N1c were provided with a similar formulation using another similar cell-culture derived influenza strain (H1N1) with or without MF59 in a ferret challenge study. This formulation, aH1N1c, was shown to be immunogenic in ferrets and protective against intratracheal challenge with 106TCID50 of A/H1N1 virus (A/Netherlands/602/2009).

2.5.2.2. Secondary pharmacodynamic studies

In accordance with guidelines on the nonclinical development of vaccines, no secondary pharmacodynamics studies were performed.

2.5.2.3. Safety pharmacology programme

Dedicated safety pharmacology studies have not been performed with aH5N1c. Data from nonclinical and clinical studies, and post-marketing experience do not indicate that MF59-adjuvanted vaccines affect central nervous system, respiratory, cardiovascular and renal functions. Inactivated purified influenza antigens do not fall into categories 'of concern' for possessing unexpected innate pharmacological activities. Therefore, the programme of studies performed to support the registration of aH5N1c did not include standard safety pharmacology studies.

2.5.2.4. Pharmacodynamic drug interactions

In accordance with guidelines on the nonclinical development of vaccines, no pharmacodynamic drug interaction studies were performed.

2.5.3. Pharmacokinetics

According to the EMA Guideline on Influenza Vaccines - Non-clinical and Clinical Module (EMA/CHMP/VWP/457259/2014) the determination of serum concentrations of antigens is not needed. The applicant's approach to not conduct pharmacokinetics studies is therefore accepted.

The haemagglutination inhibition assay used in rabbit studies was qualified with respect to its repeatability, intermediate precision, dilutional linearity, limit of detection and qualification and specificity. All parameters tested were within the acceptable limits and the assay provided consistent results throughout the qualification. The LoD/LoQ were established at 2 HAI and 4 HAI, respectively. Haemolytic or lipemic samples did not impact the HAI titres. The haemagglutination assay was thus considered qualified for detection of antibody titres in rabbit sera.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

The applicant did not conduct single-dose toxicity studies but evaluated acute effects after the first dose in the repeat-dose toxicity study. This approach is in line with the EMA Guideline on Influenza Vaccines - Non-clinical and Clinical Module (EMA/CHMP/VWP/457259/2014).

2.5.4.2. Repeat dose toxicity

A GLP-compliant 6-week combined pharmacology and repeat-dose toxicity study was conducted in New Zealand White Rabbits (Study No. 466122). Eight animals (4 main and 4 recovery animals) of each sex were allocated to control (PBS + Thiomersal), adjuvant control (MF59 + PBS + Thiomersal) and vaccine groups (15 µg H5N1 FCC + 0.25 mL MF59 + 50 µg Thiomersal) and i.m. administered 0.5 mL of the respective test substance on study days 1, 15 and 29. The applicant stated that a contamination with HA from the A/turkey/Turkey strain was detected in addition to the intended A/Indonesia strain in this lot. Main animals were terminated 2 days after the last dose, recovery animals 14 days after the last dose. Post-dose examination included clinical signs, skin reactions at the injection site, body weight, food consumption, ophthalmoscopy (comparatively pre- and posttest), heart and respiratory rate, body temperature, haematology, clinical chemistry as well as macroscopy, organ weights and histopathology after termination. No adverse findings were made regarding any of the investigated parameters. The only observations were increased fibrinogen levels and decreased prothrombin times in animals treated with the vaccine or adjuvant control on days 17 and 31. Both values returned to normal during the recovery period with the exception of prothrombin time in female animals treated with adjuvant control which remained slightly below baseline. The applicant argues that elevated fibrinogen levels resulted from an inflammatory response to adjuvant and vaccine which was also reflected by slightly increased body temperatures in male animals after having received the third vaccine dose. In addition, total protein levels were increased which was attributed to elevated globulin levels as a consequence of the therapeutic effect.

2.5.4.3. Genotoxicity

No studies on the genotoxicity of aH5N1c were performed which is in line with the EMA *Guideline on Influenza Vaccines - Non-clinical and Clinical Module* (EMA/CHMP/VWP/457259/2014).

2.5.4.4. Carcinogenicity

No studies on the carcinogenicity of aH5N1c were performed which is in line with the EMA *Guideline on Influenza Vaccines - Non-clinical and Clinical Module* (EMA/CHMP/VWP/457259/2014).

2.5.4.5. Reproductive and developmental toxicity

A GLP-compliant developmental toxicity study was conducted in New Zealand White rabbits (Study No. AB20852). Female animals were treated with four human doses of a Phase 3 clinical batch 21 and 7 days before mating as well as on days 7 and 20 of gestation. Litters were investigated either at day 29 of gestation after caesarean section or after delivery at the end of the gestation period. Maternal toxicity including reproductive parameters and behaviour in the case of natural delivery as well as embryo-fetal survival, weights and sex were examined. In addition, external, visceral and skeletal fetal abnormalities as well as behaviour for the naturally delivered pups were evaluated. Observations included slightly lower ovarian weights and slightly higher fetal weights – presumably due to the slightly lower litter size - in the treatment group subject to caesarean section as compared to the vehicle control group. However, all observed findings were within the historical control range. No observations were made in the group allowed for natural delivery. Common to both groups was the slightly higher proportion of male offspring in the vaccinated group. The presence of strain-specific antibodies was confirmed in both mothers and offspring of the vaccinated group.

2.5.4.6. Local tolerance

Evaluation of the local tolerance of aH5N1c was evaluated in the scope of both toxicity studies. Observations included on the histopathological level minimal to mild panniculus muscle fiber degeneration, occasional haemorrhage, leucocyte infiltration and inflammation in some animals of the repeat-dose toxicity study. Females subject to the reproductive and developmental toxicity study showed occasional slight oedema, haematoma and erythema.

2.5.4.7. Other toxicity studies

The applicant discussed the toxicological risk of potential presence of trace amounts of dioxins and/or polychlorinated biphenyls (PCBs) originating from the fish-derived squalene in the MF59 formulation. Assuming a Tolerable Daily Intake (TDI) of 1-4 pg Toxic Equivalents (TEQ) of dioxins/dioxin-like PCBs and a specification limit of ≤ 661 pg/g for dioxin and PCBs in squalene, the worst-case exposure for a 60 kg adult would be 0.107 pg TEQ/kg.

With regard to the safety of the MF59-adjuvant the applicant provided a literature-based discussion which included non-clinical and clinical safety observations. No findings beyond reversible local inflammatory reactions that were not exacerbated by the combination of MF59 with antigen were reported.

For the safety of the MDCK cell platform the applicant referred to the MAA procedure of Optaflu (EMEA/H/C/000758).

No new excipients are used in the formulation of aH5N1c. Regarding polysorbate 80 and cetyltrimethylammonium bromide (CTAB) the applicant refers to licensed Flucelvax Tetra seasonal influenza vaccine that contains similar or higher amounts of these excipients, respectively.

2.5.5. Ecotoxicity/environmental risk assessment

No ERA studies were conducted for aH5N1c which is acceptable as according to the "Guideline on the environmental risk assessment of medicinal products for human use" - EMEA/CHMP/SWP/4447/00 corr 2 and the "Guideline on Influenza Vaccines - Non-clinical and Clinical Module" (EMA/CHMP/VWP/457259/2014) inactivated vaccines are exempted from the submission of specific ERA studies.

2.5.6. Discussion on non-clinical aspects

To confirm pharmacological effect / immunogenicity the applicant submitted data from mice, rabbit and ferret, which are generally considered adequate animal models to study influenza vaccine immunogenicity and viral infection. The extent of the pharmacological investigations is limited but considered sufficient to perform a non-clinical pharmacological characterisation. While immunogenicity in rabbits was investigated using the respective formulation, aH5N1c (including MF59), intended for clinical use as part of the conducted repeat-dose and developmental toxicity studies, data in mice and ferrets were provided from literature and previously conducted nonclinical studies using similar formulations (including also MF59 as adjuvant), but differing with respect to the included cell-culture derived influenza strains. These latter data can thus formally only be considered supportive of the nonclinical immunogenicity of aH5N1c. However, these data are generally still regarded representative and of value to confirm the immunogenic potential of cell culture-derived influenza antigens adjuvanted with MF59. The studies performed included analyses of the humoral (using HI and ELISA) and cellular immune response as well as proof of concept (challenge with pathogenic virus) studies.

The use of MF59 as an adjuvant for influenza and other vaccines is well established and MF59 is generally regarded safe and well tolerated in humans. MF59 was shown to be a very potent single adjuvant and to significantly enhance HI and neutralising titres (humoral response), as well as T-cell response (cytokine positive CD4+ cells).

Due to the broad experience of the applicant with similar vaccine formulations (influenza antigens with and without MF59), the immunogenic response after intramuscular administration of aH5N1c to rabbits was only investigated on the humoral level (i.e. presence of antibodies pre- and post-dose) in both studies conducted. Dedicated data on cellular immune response was not provided for aH5N1c, however data from previous nonclinical programs and literature submitted clearly support the assumption that cell-culture derived influenza antigens adjuvanted with MF59 elicit not only humoral but also cellular immune response.

Although the strain and dosing regimen in the non-GLP ferret challenge study differed slightly from the intended clinical dosing with aH5N1c (a single dose of 15 µg aH1N1c was administered compared to two times 7.5 µg HA as planned for aH5N1c and some animals were primed with a dose of seasonal vaccine), these data can be regarded supportive for the actual MAA as it provides further confirmation of the immunogenic potential of cell-culture derived influenza antigens, their protective effect against virus challenge (shown by reduction of lung titres and viral shedding), and that the addition of MF59 enhances the immunogenic response when combined with.

Despite the use of a heterologous strain in the HI assay for the conducted repeat-dose toxicity study, no further non-clinical data or discussion on heterologous immunogenicity was provided. The current Guideline on Influenza Vaccines (EMA/CHMP/VWP/457259/2014) states that 'Data on cross-neutralizing antibodies and cross-reactivity should be obtained from serological studies using heterologous viruses for pandemic, zoonotic or adjuvanted seasonal vaccines.' With regard to the lack of nonclinical data on heterologous immunogenicity, please refer to the clinical discussion on heterologous immune response to aH5N1c.

Altogether, nonclinical immunogenicity data from mice, rabbits, and ferrets provided in this dossier from dedicated studies using aH5N1c or from literature and previously conducted nonclinical studies using similar formulations/virus strains with and without MF59, demonstrated immunogenic response to cell-culture derived influenza antigens adjuvanted with MF59 (proof-of-concept) and specifically immunogenicity of aH5N1c. These data support the use of aH5N1c in the targeted clinical indication.

The non-clinical toxicology programme with aH5N1c consists of a repeat-dose toxicity study and a reproductive and developmental toxicity study. Both pivotal studies were conducted in New Zealand

White rabbits and in compliance with GLP. The extent of the non-clinical toxicology data package is in accordance with the relevant guidelines and thus supported.

The repeat-dose toxicity study was conducted with a non-clinical batch, whereas the reproductive and developmental toxicity study used the Phase 3 clinical batch. The clinical test material is preservative-free, whereas the preservative thiomersal was included in the preclinical batch. Nevertheless, since toxicity related to immunogenicity often is species specific and the applicant has obtained clinical safety data from Phase III studies, it is not considered appropriate to perform additional repeat-dose toxicity studies with the applied clinical batch of aH5N1c. Moreover, the reproductive and developmental toxicity study used the Phase 3 clinical batch formulation with multiple administrations of a clinically relevant vaccine dose and included several of the endpoints applied in repeat-dose toxicity study.

In a GLP-compliant 6-week combined pharmacology and repeat-dose toxicity study New Zealand White (NZW) Rabbits received three clinical doses of the aH5N1c vaccine, adjuvant control or vehicle control using the i.m. clinical route of administration. Rabbit was chosen as the animal model as it is an accepted species for non-clinical toxicity testing and has shown to develop immune responses to influenza antigens. The contamination with HA from the A/turkey/Turkey strain in addition to the A/Indonesia strain in the lot used for this study is not regarded critical and the applicant's argumentation that these strains are highly related and no impact on the toxicity is exerted by this contamination is fully supported. No toxicities were recorded throughout the observation period. Transient elevated fibrinogen levels and reduced prothrombin times were observed in animals that received the vaccine or vehicle control. It is agreed with the applicant that such findings are attributable to the inflammatory response secondary to the vaccination and, thus, actually related to the pharmacodynamic effect. Similarly, an increase in total protein levels due to an elevated content of globulin is a conceivable effect of the immunisation. Overall, three doses corresponding to 40x and 20x the clinical amount of antigen and adjuvant per dose, respectively, administered to rabbits were well tolerated and did not result in any unexpected findings.

In order to evaluate adverse effects on pregnancy and embryo-foetal development, female NZW rabbits were vaccinated with a total of four human doses of a late clinical batch of aH5N1c. Two doses were administered before mating and two during pregnancy. The study design with regards to species selection, dosing levels, duration and investigated endpoints is supported. The formation of strain-specific antibody in response to the vaccinations was confirmed in the sera of mothers and their offspring. Maternal animals were either subjected to caesarean section or allowed to deliver naturally. Minor observations regarding lower ovarian weights and higher foetal weights were made in the vaccinated caesarean group as compared to the control group. However, it was argued that the deviations were within the historical control range. Overall, no effects on foetal growth, survival or morphological development were observed.

In line with the EMA *Guideline on Influenza Vaccines - Non-clinical and Clinical Module* (EMA/CHMP/VWP/457259/2014) evaluation of local tolerance was included in the repeat-dose toxicity study as well as in the developmental toxicity study. Overall, none of the findings deviates from expectations after i.m. administration of an influenza vaccine or even vehicle and therefore, local tolerance for aH5N1c is considered unremarkable.

A thorough discussion on the safety of impurities and the adjuvant was provided. Reference to other procedures without the provision of study reports – as done for the safety of the MDCK platform - is generally not acceptable. However, the cell platform has been sufficiently characterised in Module 3 of the current procedure so that no safety concerns exist. From the data presented it can be concluded that no risk is exerted by the MF59 adjuvant, the platform cell line or excipients.

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, aH5N1c is not expected to pose a risk to the environment.

2.5.7. Conclusion on the non-clinical aspects

The provided nonclinical data supports use of aH5N1c in the intended clinical indication. Incellipan is considered approvable from a non-clinical point of view.

2.6. Clinical aspects

2.6.1. Introduction

The purpose of this Marketing Authorization Application (MAA) is to seek approval of a Pandemic Influenza Vaccine for the active immunisation against influenza in an officially declared pandemic.

This vaccine represents a pandemic preparedness vaccine, i.e. it is authorised in advance of a pandemic based on a core dossier that includes a minimum set of data to define the benefit risk balance, with appropriate specific obligations that the applicant is requested to fulfil at the time of the next pandemic. Thus the vaccine can only be administered after a pandemic is duly recognised. When a pandemic is recognised by WHO or the EU, a variation application will be submitted to include the declared pandemic strain in the vaccine. This strategy ensures a faster availability of pandemic vaccines during a pandemic.

GCP aspects

Clinical trials were mainly carried out outside the European Union. Of the five main clinical studies (V89_P1, V89_04, V89_11, V89_13, V89_18) only V89_P1 included sites in Europe (Germany).

According to the applicant, all trials were carried out in accordance with Good Clinical Practice (GCP) according to the International Conference on Harmonization (ICH) guidelines as well as national regulatory requirements, which cover ethical requirements of Directive 2001/20/EC which was in place at the time the trials were conducted.

GCP inspections were carried out by the FDA for studies V89_11 (2 sites in Thailand in 2019) and V89_18 (4 sites in USA, one in 2017 and the remaining 3 sites in 2019).

No GCP inspections were carried out or planned for studies V89_P1 or V89_13 or V89_04

Regarding supportive studies, no GCP inspections were done or planned for study V110_04 (H1N1).

No information is provided for the remaining supportive studies V129_01 ; V131_01.

- **Tabular overview of clinical studies**

Seqirus is seeking licensure of aH5N1c based on immunogenicity and safety results from 5 clinical studies in subjects 6 months and older (see Table 2 for a summary of the clinical development programme). In addition, data are presented from three dose-ranging studies with MF59 adjuvanted cell culture-derived influenza vaccines of other subtypes: key Study V110_04 (H1N1, paediatric subjects), supporting Study V129_01 (H3N2, paediatric subjects and adults/elderly), and supporting Study V131_01 (H7N9, adults).

Table 2. Summary of aH5N1c Clinical Development Programme

Study	Study Description	Phase	Age Categories of Subjects
V89_18	Lot-to-lot consistency, placebo-controlled (7.5 µg HA dose)	3	Adult and elderly (≥18 years of age)
V89_04	7.5 µg vs. 3.75 µg HA dose comparison	2	Adults (18 to <65 years of age)
V89_13	7.5 µg vs. 3.75 µg HA dose comparison	2	Elderly (≥65 years of age)
V89P1	Dose ranging study	1/2	Adults (18 to ≤40 years of age)
V89_11	7.5 µg vs. 3.75 µg HA dose comparison	2	Paediatrics (6 months to ≤17 years of age)

- Source: ISS SAP v5.0
- Abbreviations: HA = haemagglutinin; ISS = integrated summary of safety; SAP = statistical analysis plan.

Ongoing Study V89_18E1: to investigate whether one or two heterologous booster vaccinations with an MF59-adjuvanted H5N6 cell culture derived vaccine (aH5N6c) administered 3 weeks apart elicit immune responses in subjects primed by previous vaccination with aH5N1c (more than 5 years ago) to the antigens used for priming (H5N1) and boosting (H5N6). Study V89_18E1 is being conducted in the US in approximately 260 adult subjects.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

As is routine in vaccine applications, studies on bioavailability, relative bioavailability and bioequivalence are not pertinent to formulation development; therefore, these types of study were not performed. Since kinetic properties of injectable vaccines do not provide information useful for establishing adequate dosing recommendations, pharmacokinetic studies are generally not required.

2.6.2.2. Pharmacodynamics

The pharmacodynamic profile of vaccines is defined by their immunogenicity, as detailed in the CHMP guideline "Guideline on Clinical Evaluation of Vaccines" (EMEA/CHMP/VWP/164653/05 Rev. 1).

Mechanism of action

aH5N1c is a monovalent, cell culture-derived, inactivated influenza vaccine that comprises surface antigens from a potential pandemic H5N1 virus strain candidate (A/turkey/Turkey/1/2005 [H5N1] NIBRG-23 strain) and the adjuvant MF59 (MF59C.1 proprietary adjuvant). The prefix "a" indicates that this is an adjuvanted vaccine, using MF59 oil-in-water emulsion adjuvant to augment the immune

response to viral antigen. The suffix "c" indicates that this is a cell culture-derived vaccine which does not require eggs for production.

The efficacy of aH5N1c was assessed using the immunogenicity criteria as outlined in the Guidance for Industry "Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines" (CBER 2007) and the European Medicines Agency's (EMA) Committee for Medicinal Products for Human Use (CHMP) former criteria (EMA 1997). The former CHMP criteria were in place at the time the trials were conducted.

Prophylaxis against a pandemic strain of influenza cannot be evaluated prospectively. HA antibody response as a measure of immunogenicity is recognised widely as a predictive marker of influenza vaccine efficacy. The section on clinical efficacy assesses HA antibody response data as a surrogate for evaluation of aH5N1c efficacy. In the event of a flu pandemic, it is anticipated that the vaccine strain of the pandemic preparedness vaccine will be matched to the circulating pandemic influenza strain via a strain change supplement.

Table 3. Comparison of (former) CHMP and CBER Criteria for Evaluation of Influenza Vaccines

CHMP Criteria	**Adults 18 to <60 Years**	**Adults ≥60 Years**
Percentage of subjects with HI antibody titer ≥1:40	>70%	>60%
Geometric Mean Ratio	>2.5	>2.0
Percentage of subjects with seroconversion[a]	>40%	>30%
CBER Criteria	**Adults 18 to <65 Years**	**Adults ≥65 Years**
Lower-limit of the 95% CI for percentage of subjects with HI antibody titer ≥1:40	≥70%	≥60%
Lower-limit of the 2-sided 95% CI for percentage of subjects with seroconversion[a]	≥40%	≥30%

Abbreviations: CBER= Center for Biologics Evaluation and Research; CHMP=Committee for human medicinal products; CI=confidence interval; HI= hemagglutination inhibition.
[a] Seroconversion=either a prevaccination (baseline) HI titer <1:10 and post vaccination HI titer ≥1:40, or a prevaccination HI titer ≥1:10 and a ≥4-fold increase in post vaccination HI antibody titer.

Primary and secondary pharmacology

Haemagglutinin inhibition assay

The applicant has implemented a modified hemagglutination inhibition (HI) assay according to a protocol of World Health Organization. In brief, the modified HI is run using horse erythrocytes that solely express α 2,3-Gal-linked N-acetylneuraminic acid on their surface, in contrast to traditionally used chicken erythrocytes that express a mixture of (α 2,3-Gal) linkages and (α 2,6-Gal) linkages, and that are insensitive for the detection of antibody responses against avian Influenza antigens. Serum samples are pre-treated with sialidase and serially diluted and incubated with a defined amount of virus. Subsequently horse red blood cells are added. If the serum dilution contains enough antibodies to neutralise the challenge virus, the red blood cells will not agglutinate but form a red, dot-shaped sediment. However, in the presence of excess virus, the Influenza virus is able to agglutinate the red blood cells which stay in solution. The HI titre is reported as the last dilution step, in which agglutination is still completely inhibited. For the evaluation of immunogenicity of pandemic Influenza vaccines the amount of haemagglutination inhibiting influenza antibodies in serum of subjects needs to be quantified prior to and after vaccination.

The assay was implemented and validated in 2007 at Novartis Vaccines and Diagnostics using virus material of H5N1 strain A/Vietnam/1203/2004VNH5N1PR8CDC-RG, and improved due to a lack of robustness. It was transferred and re-validated at Viroclinical Biosciences BV on April 2008. When the SW-H1N1 outbreak occurred in April 2009 the assay was adapted for the use of SW H1N1 strains and re-validated for the use against egg-derived A/Vietnam vaccination strain and in 2012 upon request from the EMA for the use against further egg derived A/turkey, A/Indonesia and A/Anhui H5N1 influenza strains. To fulfil expectations of the guidance for industry on Bioanalytical Method Validation issued in September, 2013, additional validation experiments were successfully performed. The assay was re-validated in 2019 with the aim to characterise and quantify the specificity of HAI titres for two H1N1 human antiserum pools against A/California/07/2009 virus and A/turkey/Turkey/1/05 H5N1-like (NIBRG-23) virus to check for absence of cross reactivity and for sera raised against B/Brisbane/60/2008, A/Texas/50/2012 (H3N2) and B/Massachusetts/02/2012.

Micro-neutralisation assay

The applicant has established and validated a micro neutralisation assay to quantify functional antibodies against avian influenza A type H5N1 in human sera. In brief, heat-inactivated serum samples are serially diluted and incubated with a defined amount of virus (100 TCID50). Subsequently MDCK cell are added and the mixture is incubated over night. After fixation of cells, the presence of Influenza A virus nucleoprotein is detected in infected cells using an ELISA. When sufficient amount of antibodies are present in the serum to neutralise the challenge virus, the MDCK cells remain intact and no virus nucleoprotein can be detected. In case there is an excess of virus, the virus is able to infect the MDCK cells. The neutralizing titre is defined as the reciprocal value of the factor 2 serum dilution that is able to elicit an at least 50% neutralisation of 100 TCID50 of the challenge virus. The assay is considered highly representative for the vaccines mechanism of action.

The MN was successfully set up and validated in 2007 at Novartis Behring for the H5N1 A/Vietnam H5N1 clade1 strain and re-validated in 2011 upon EMA request for additional circulating H5N1 phylogenetically distinct H5N1 strains A/turkey (clade 2.2), A/Indonesia clade (2.1.3) and A/Anhui (2.3.4). The validation confirmed specificity of the MN for influenza antibodies of the A/H5N1 subtype also shows a good linearity for all assessed strains. Repeatability and intermediate precision results demonstrated that titre results can be well retrieved when tested repeatedly. Assay variability was found to be smaller than one titre step at all conditions. The assay was established and re-validated at Viroclinics Biosciences BV in 2016, and a range extension validation was successfully concluded in 2022. Inter-laboratory consistency was confirmed for clinical serology, Novartis Behring and in the labs of Health Protection Agency, Colindale, UK.

2.6.3. Discussion on clinical pharmacology

Human pharmacokinetic (PK) studies are considered not informative for the evaluation of vaccines. Therefore, it is acceptable that no PK data were collected. Furthermore, the MF59 adjuvant has been used in a number of seasonal influenza vaccines (such as Fluad Tetra (aQIV)), zoonotic vaccines (Aflunov) and pre-pandemic vaccines (Foclivia) authorised in the EU. As well as Celtura (A/H1N1) cell based (aH1N1c) licensed in Germany and Switzerland.

The pharmacodynamics of vaccines are investigated by immunogenicity studies, which characterise the immune response to the vaccine. To determine the immunogenicity of aH5N1c, a modified haemagglutination inhibition (HI) assay and a Microneutralisation (MN) assay were utilised. Of note, a Single Radial Haemolysis (SRH) assay was part of the primary objective of the Phase 1 study, V89P1

which used a different vaccine strain (A/Indonesia/5/2005/PR8-IBCDC-RG2). No reports regarding the SRH-assay have been provided. Therefore, the SRH data are only considered exploratory.

The use of the haemagglutination inhibition (HI) assay as the primary assay to assess vaccine immunogenicity in clinical trials is in line with the recommendations of the Guideline on Influenza Vaccines (EMA/CHMP/VWP/457259/2014). While HI titres are not a true surrogate marker, it has been widely shown that higher HI titres tend to correlate with better protection. The choice of H5N1 to investigate the immunogenicity of the vaccine construct for this pandemic preparedness vaccine is also agreed, since this is a subtype which is considered poorly immunogenic and to which the vast majority of humans are naïve.

Comparing HI titres in terms of GMTs/GMRs is considered adequate. Similarly, the definition of seroconversion rate defined as, the percentage of subjects achieving either: 1) a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40 after vaccination; or 2) a prevaccination (baseline) HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI titre) is also considered appropriate.

The former criteria recommended by CHMP to assess immunogenicity of influenza vaccines and the current criteria recommended by CBER (2007) are deemed informative, but not critical for the immunogenicity assessment of the vaccine. The totality of all provided immunogenicity data should be sufficiently convincing to ensure CHMP that the candidate vaccine is likely to be efficacious.

No data on cell-mediated immunity and anti-neuraminidase (NA) antibodies have been provided.

For inactivated influenza vaccines containing viral HA, an HI titre of 1:40 was previously suggested to represent a reasonable statistical correlate for an efficacy of 50-70% against clinical symptoms of influenza based on challenge studies in healthy adults. However, since then, evidence has emerged to indicate there remains a need to better define correlates of protection against influenza, which potentially may vary according to individual characteristics, populations, specific age groups (e.g. paediatric population) and vaccine types (EMEA/CHMP/VWP/457259/2014). Applicants should therefore make every effort to obtain data that can support identification and validation of correlates of protection against clinically manifest influenza. Analyses should be conducted to explore the correlation between immune response parameters and protection against disease. While it is appreciated that obtaining efficacy data against H5N1 that does not normally infect humans is problematic, some non-clinical data could have been considered in later phases of clinical development, for example, challenge studies in a suitable animal model such as ferrets. Alternatively, comparing data, for example, using H1N1 as the vaccine strain to an approved H1N1 vaccine where efficacy has been demonstrated in similar target populations. The V110_04 study in the paediatric population was carried out between 2009 and 2011 and used H1N1 as the vaccine strain. The primary endpoint was safety and tolerability with immunogenicity as a secondary endpoint. No endpoints appear to have been considered relating to symptomatic influenza, even though the study was carried out when H1N1 was circulating.

The primary analyses in the clinical studies were based on the A/turkey/Turkey/1/2005 (H5N1) NIBRG-23 strain, except for Study V89P1, where the primary analysis was based on the A/Indonesia/5/2005 strain. In studies V89_04, V89_11 and V89_13 heterologous testing using HI and MN assays was performed against 5 additional H5N1 strains: A/Anhui/1/2005, A/Egypt/N03072/2010, A/Hubei/1/2010, A/Indonesia/5/2005 and A/Vietnam/1203/2004. These strains were chosen to cover various genetic clades of H5N1 that differed from the vaccine strain, to assess whether the aH5N1c vaccine may provide some level of protection against influenza caused by drifted strains. The strain choices were agreed between the applicant and the Biomedical Advanced Research and Development Authority (BARDA) of the US Government. No scientific advice was sought from EMA.

The HI assay presented was considered suitable to assess the success of vaccination and seems to provide valid data within its working range. Method validation was performed according to ICH guideline Q2 (R1). Validation plans were provided, and acceptance criteria were met. Deviations were explained and discussed in enough detail. The range and limit of quantitation of the assay were adapted based on the performance of the assay. Assay matrix, positive and negative controls were representatives, and reproducibility of the HI assay across different laboratories throughout the clinical development programme was confirmed.

The submitted dossier includes microneutralisation (MN) data from all studies, but MN assays have only been performed in relatively small subsets of participants. Of note, for the phase 3 study, microneutralisation was determined post hoc.

Validation protocols, validation reports, SOPs and raw data were available or well summarised. Assay matrix, positive and negative controls were carefully selected, and are considered representatives. Deviations from the protocol were summarised and sufficiently discussed. Critical materials were listed and well described. The validation activities were carried out according to ICH guidelines Q2 (R1) and upon request of health agencies. Thus, the assay is considered validated for its precision, dilutional linearity, robustness and specificity the seasonal H1N1, H3N2 and B strains as well as pandemic H5N1 strains. An interlaboratory comparison show concordance between MN titres found between two laboratory sites. Different passage numbers of the MDCK-SIAT1 target cell line showed comparable results and confirmed cell line stability over the clinical development programme.

The primary analyses in the clinical studies were based on the A/turkey/Turkey/1/2005 (H5N1) NIBRG-23 strain, except for Study V89P1, where the primary analysis was based on the A/Indonesia/5/2005 strain. In studies V89_04, V89_11 and V89_13 heterologous testing using HI and MN assays was performed against 5 additional H5N1 strains: A/Anhui/1/2005, A/Egypt/N03072/2010, A/Hubei/1/2010, A/Indonesia/5/2005 and A/Vietnam/1203/2004. It should be noted that not all strains tested in the clinical trials were represented in the validation reports such as A/Egypt/N03072/2010, A/Hubei/1/2010. The five strains were chosen to cover various genetic clades of H5N1 that differed from the vaccine strain, to assess whether the aH5N1c vaccine may provide some level of protection against influenza caused by drifted strains.

2.6.4. Conclusions on clinical pharmacology

The CHMP considers that all aspects dealing with clinical pharmacology have been well addressed by the applicant.

2.6.5. Clinical efficacy

2.6.5.1. Dose response studies

Study V89P1

Study Design: Study V89P1 was a Phase 1/2, randomised, observer-blind, dose-ranging, factorial design, multicentre study conducted between 2008 and 2010 to evaluate the immunogenicity and safety of 2 IM injections of aH5N1c (A/Indonesia/5/2005 strain) administered 3 weeks apart in healthy subjects aged 18 to ≤40 years.

This study was performed over a period of approximately 2 years at 6 investigational sites, in the United States and in Germany. Approximately 720 healthy subjects were planned to be enrolled in the study, 60 in each of 12 vaccination groups (as summarised in the Table below).

Table 4. Vaccination groups

H5N1 Antigen	MF59 dose level 0%	25%	50%	100%
3.75 µg	Group A0	Group A1	Group A2	Group A3
7.5 µg	Group B0	Group B1	Group B2	Group B3
15 µg	Group C0	Group C1	Group C2	Group C3

Blood was taken on Days 1, 22, 43 and 366 for the evaluation of immunogenicity. Immunogenicity was evaluated in serum by haemagglutination inhibition (HI), microneutralization (MN), and single radial haemolysis (SRH).

Study Participants: In total, 753 subjects were randomised into the study in the USA (5 study centres) and Germany (1 study centre), 752 subjects received at least one vaccination (range 61-65 per vaccination group) and 705 subjects received the second vaccination on Day 22 (however, 10 subjects received a different vaccine than the first vaccination). Overall, 530 subjects completed the study up to Day 546, and 223 subjects withdrew prematurely (range for the different treatment groups: 17% to 41% for the randomised set, with the highest drop-out rate in the 15 µg 0% MF59 [41%] and 15 µg 100% MF59 [40%]). The main reason for premature withdrawal was 'lost to follow-up' (10% to 23% among treatment groups), followed by 'withdrawal of consent' (2% to 14%). Three subjects died during the study (1 subject each in the 3.75 µg, 7.5 µg and 15 µg 0% MF59 groups) and 3 subjects withdrew due to AEs (1 subject each in the 3.75 µg 50% MF59 group and 7.5 µg 0% and 100% MF59 groups).

The study subjects were randomised equally to 1 of 12 treatment groups. Between 61 and 64 subjects were enrolled in each group (Table 5) and between 47 and 59 subjects were included in the MPPS (modified per protocol set) for each group.

Table 5. Study V89P1 – Overview of Subject Populations

	3.75 µg HA	3.75 µg HA + 0.063 mL MF59	3.75 µg HA + 0.125 mL MF59[a]	3.75 µg HA + 0.25 mL MF59	7.5 µg HA	7.5 µg HA + 0.063 mL MF59	7.5 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59[b]	15 µg HA	15 µg HA + 0.063 mL MF59	15 µg HA + 0.125 mL MF59	15 µg HA + 0.25 mL MF59
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
All enrolled	62 (100)	63 (100)	63 (100)	62 (100)	64 (100)	62 (100)	62 (100)	64 (100)	64 (100)	61 (100)	63 (100)	63 (100)
Enrolled as treated	63 (100)	64 (100)	62 (100)	62 (100)	63 (100)	61 (100)	61 (100)	64 (100	65 (100)	63 (100)	63 (100)	62 (100)
FAS Day 1	58 (94)	60 (95)	62 (98)	58 (94)	61 (95)	57 (92)	56 (90)	59 (92)	60 (94)	57 (93)	61 (97)	56 (89)
FAS Day 22	57 (92)	60 (95)	61 (97)	58 (94)	60 (94)	57 (92)	56 (90)	58 (91)	60 (94)	57 (93)	61 (97)	55 (87)
FAS Day 43	56 (90)	58 (92)	61 (97)	56 (90)	57 (89)	56 (90)	55 (89)	59 (92)	59 (92)	54 (89)	60 (95)	51 (81)
PPS Day 43	41 (66)	47 (75)	44 (70)	43 (69)	43 (67)	40 (65)	45 (73)	44 (69)	53 (83)	45 (74)	52 (83)	42 (67)
MPPS Day 43	55 (87)	55 (86)	53 (85)	51 (82)	51 (81)	51 (84)	53 (87)	50 (78)	58 (89)	50 (79)	59 (94)	47 (76)

Source: CSR V89P1, Table 14.1.1.1.1.1, Table 14.1.1.1.1.2, Table 14.1.1.1.1.3, Table 14.1.1.1.2.1, Table 14.1.1.1.2.2, Table 14.1.1.1.2.3.
Abbreviations: FAS = full analysis set; HA = hemagglutinin; PPS = per protocol set; MPPS = modified per protocol set; n = number of subjects in category.
[a] Corresponds to the half dose group in Section 2.7.2.3.
[b] Corresponds to the full dose group in Section 2.7.2.3.

Overall mean age was 28.0 years, ranging from 26.5 to 29.8 years within the 12 vaccine groups. The majority of subjects were Black (41%) or Caucasian (38%). Mean height, weight and BMI were similar for all groups. Nearly all subjects (overall 99%, 98% to 100% for the different vaccine groups) met the inclusion criteria. Overall 51% of the subjects were male and 49% were female (within the different vaccine groups the percentages ranged from 41% to 61% for males and from 39% to 58% for females).

Primary Objectives:

- To assess the immunogenicity of a Cell Culture-Derived H5N1 Subunit Influenza Virus Vaccine containing different amounts of antigen and adjuvant.
- To identify the optimal adjuvant-antigen dose combination considering antibody titres against the H5N1 strain observed three weeks after two intramuscular (IM) doses in healthy adults.

All statistical analyses for HI, SRH and MN were performed on the logarithmically (base 10) transformed values. Individual HI and MN titres below detection limit were set to half that limit. The lower detection limit of the SRH test is at an area of 4 mm2. All areas below the lower limit of detection were set to 4 mm2 for the immunogenicity analysis.

Prior to unblinding it was decided that the primary analysis of the immunogenicity results was to be based on the MPPS and not as initially planned on the FAS. This was documented in an amendment to the analysis plan. All immunogenicity analysis tables were done also for the PPS and the FAS, if the respective sets differed by more than 10% in the number of subjects included.

HI assay

After 2 vaccinations, at Day 43, the seroconversion criterion for CBER was met for all adjuvanted groups except for the 3.75 µg group adjuvanted with 0.063 mL MF59, while none of the study groups reached the CBER criterion for HI titre ≥1:40.

The adjuvanted formulations tested in trial V89P1 at Day 43 either met all 3 or at least 2 of the CHMP criteria set for HI. None of the nonadjuvanted formulations met any of the CHMP criteria at either Day 22 or Day 43. The lowest dose formulation which met all CHMP licensing criteria for HI was 3.75 µg + 0.125 mL MF59 (half dose).

While GMTs did not rise markedly 3 weeks after first vaccination (Day 22) in any group, a noticeable increase in GMTs was observed at Day 43 in the adjuvanted groups only, as shown by geometric mean ratios (GMRs) (Day 43/Day 1) ranging from 5.52 to 30, whereas GMRs ranged from 1.45 to 1.48 in the nonadjuvanted groups. At Day 43, the CHMP criterion for GMR was met by all adjuvanted groups (Table 6).

Table 6.

Study V89P1 - Percentages of Subjects with Seroconversion, with HI Titre ≥1:40 and GMT and GMR per Vaccine Group at Days 1, 22 and 43 - Age 18 to ≤40 Years - MPPS

HA µg	3.75				7.5				15			
MF59 mL	0	0.063	0.125	0.25	0	0.063	0.125	0.25	0	0.063	0.125	0.25
N	55	55	53	51	51	51	53	50	58	50	59	47
Seroconversion (95% CI)												
Day 22	4 (0; 13)	5 (1; 15)	13 (6; 26)	12 (4; 24)	2 (0.05; 11)	12 (4; 24)	11 (4; 23)	18 (9; 32)	2 (0.04; 9)	6 (1; 17)	8 (3; 19)	17 (8; 31)
Day 43	9 (3; 20)	***51*** **(37; 65)**	***77*** **(64; 88)**	***65*** **(50; 78)**	10 (3; 21)	***55*** **(40; 69)**	***70*** **(56; 82)**	***78*** **(64; 88)**	12 (5; 23)	***56*** **(41; 70)**	***64*** **(51; 76)**	***83*** **(69; 92)**
HI Titre ≥1:40 (95% CI)												
Day 1	0 (0; 6)	0 (0; 6)	0 (0; 7)	0 (0; 7)	0 (0; 7)	0 (0; 7)	2 (0.048; 10)	0 (0; 7)	0 (0; 6)	2 (0.051; 11)	0 (0; 6)	0 (0; 8)
Day 22	4 (0; 13)	5 (1; 15)	13 (6; 26)	12 (4; 24)	2 (0.05; 11)	12 (4; 24)	11 (4; 23)	18 (9; 32)	2 (0.04; 9)	8 (2; 19)	8 (3; 19)	17 (8; 31)
Day 43	9 (3; 20)	51 (37; 65)	***77*** (64; 88)	67 (52; 79)	10 (3; 21)	55 (40; 69)	70 (56; 82)	***78*** (64; 88)	12 (5; 23)	58 (43; 72)	64 (51; 76)	***83*** (69; 92)
GMT and GMR												
Day 1	5	5	5	5.17	5	5	5.41	5	5	5.25	5	5
Day 22	6.1	6.15	9.11	7.57	5.51	7.62	7.9	8.93	5.31	6.37	6.95	8.38
GMR	1.22	1.23	1.82	1.46	1.1	1.52	1.46	1.79	1.06	1.21	1.39	1.68
Day 43	7.3	28	103	68	7.27	41	79	131	7.42	37	58	151
GMR	1.46	***5.52***	***21***	***13***	1.45	***8.22***	***15***	***26***	1.48	***7.06***	***12***	***30***

Source: CSR V89P1: Table 14.2.1.2.3.1, 14.2.1.2.3.2, 14.2.1.2.3.3, Table 14.2.1.1.3.1, Table 14.2.1.1.3.2, Table 14.2.1.1.3.3

Bold: meeting CBER criterion (lower bound of CI); ***Bold italic***: meeting CHMP criterion (see Table 3).

Abbreviations: CI = confidence interval; GMR = geometric mean ratio; GMT = geometric mean titre; HA = haemagglutinin; HI = haemagglutination inhibition; MPPS = modified per protocol set; N = total number of subjects.

MN Assay

At baseline, the GMTs were similar in all vaccine groups (range 10 - 11). On Day 22, the adjuvanted vaccine groups showed a higher increase in GMTs (GMR above baseline of 2.04 to 7.24) when compared to the non-adjuvanted vaccine groups (GMR range 1.16 to 1.28). On Day 43, GMR in the non-adjuvanted groups increased to 3.57 to 5.67 and remarkably higher in the adjuvanted groups (GMR range between 20 and 64). For all pairwise comparisons at the same antigen level, formulations with adjuvant showed higher GMTs on Day 43 than those without adjuvant ($P < 0.001$, thus the added value of the adjuvant was demonstrated.

On Day 366 GMTs returned to baseline values in the non-adjuvanted vaccine groups (GMRs of 0.55 to 0.75). In the adjuvanted groups GMR ranged between 1.36 and 3.16, with the lowest GMR observed in the 3.75 µg 25% MF59 group and the highest GMR in the 15 µg 100% MF59 group.

Based on the results of Study V89P1, the "half dose" (3.75 µg HA antigen + 0.125 mL MF59) and "full dose" (7.5 µg HA antigen + 0.25 mL MF59) were selected for further investigation.

Study V89_04

Study design: Study V89_04 was a Phase 2, randomised, controlled, observer-blind, multicentre study conducted to evaluate the safety, tolerability and immunogenicity of aH5N1c in nonelderly adults 18 to <65 years of age. Subjects were randomly assigned (1:1) to receive a series of 2 IM injections administered 3 weeks apart, of aH5N1c containing either 3.75 µg of the H5N1 (A/turkey/Turkey/1/2005 NIBRG-23 strain) influenza antigen with 0.125 mL MF59 (half dose) or 7.5 µg of the H5N1 influenza antigen with 0.25 mL MF59 (full dose). A total of 975 healthy nonelderly adults were vaccinated, of whom 490 received the half dose (3.75 µg HA) and 485 received the full dose (7.5 µg HA).

Primary Immunogenicity Objective: To select the vaccine (low dose or high dose aH5N1c) to be tested in phase 3 based on achievement of Center for Biologics Evaluation and Research (CBER) criteria 3

weeks after the second vaccine administration as measured by strain-specific hemagglutination inhibition (HI) assays.

Secondary Immunogenicity Objectives:

- For each aH5N1c vaccine (low dose or high dose), to evaluate achievement of all CHMP criteria 3 weeks after the second vaccine administration as measured by strain-specific HI assay.
- For each aH5N1c vaccine (low dose or high dose), to evaluate achievement of CBER and CHMP criteria 3 weeks after the first vaccine administration as measured by strain-specific HI assay.
- To evaluate the immunogenicity of each aH5N1c vaccine (low dose or high dose) 12 months after the primary 2-dose course with respect to CBER and CHMP criteria, as measured by strain-specific HI assays.

Criteria for evaluation: The measures of immunogenicity included seroconversion rate, the percentage of subjects achieving HI titres ≥1:40 and GMTs. In addition, antibody responses against heterologous and homologous influenza strain(s) were evaluated using the MN assay. Results were analysed according to CBER and CHMP criteria.

Number of Subjects (planned and analysed): Approximately 972 subjects were planned to be randomised at a 1:1 ratio, stratified by site, to receive either low dose or high dose aH5N1c vaccine. All subjects were to receive 2 doses of study vaccine 3 weeks apart. In total 979 subjects were enrolled into the study and out of which 975 subjects were exposed to at least one dose of the study vaccine. The full analysis set (FAS), the primary population for immunogenicity in this study, consisted of 891 subjects at day 43. At the completion of the study at day 387, the FAS for immunogenicity was 806 subjects (82% of enrolled).

Demography and baseline characteristics of the 979 enrolled subjects were balanced between the half and full dose groups.

Results

Seroconversion: After 3 weeks of first vaccination (day 22), a higher percentage of subjects in the high dose group (48% of subjects) showed seroconversion against Influenza A H5N1 Turkey/2005 CC Ab strain than in the low dose group (27% of subjects) and similar trends were observed 3 weeks after the second vaccination (day 43; Table 7).

Table 7.

Number (%) of Subjects (97.5% CI) with Seroconversion[a] Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) Strain – HI Assay – FAS

Vaccine Group	Number (%) of Subjects	
	Low Dose N = 461	High Dose N = 464
Day 22	124 (27%) (22%-32%)	225 (48%) (43%-54%)
Day 43	269 (61%) (56%-66%) N = 440	373 (83%) (78%-87%) N = 451
Day 387	35 (9%) (6%-13%) N = 395	90 (22%) (17%-27%) N = 411

Source: Table 14.2.1.2.
Abbreviations: CI, confidence interval; HI, hemagglutination inhibition; FAS, full analysis set; N, number of subjects.
[a]Seroconversion is defined as either a prevaccination HI < 1:10 and a postvaccination HI ≥ 1:40 or a prevaccination HI ≥ 1:10 and a minimum 4-fold rise in postvaccination HI antibody titer.

Percentage of Subjects with HI ≥ 1:40: Three weeks following the first vaccination (day 22), there was an increase in the percentages of subjects achieving HI ≥ 1:40 over baseline in low dose and high dose groups, including a majority of subjects in the high dose group (52% of subjects). Similar trends were observed 3 weeks after second vaccination (day 43;Table 8).

Table 8.

Number (%) (97.5% CI) of Subjects with HI ≥ 1:40 Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) – HI Assay – FAS

Vaccine Group	Number (%) of Subjects (97.5% CI)	
	Low Dose N = 483	High Dose N = 478
Day 1	18 (4%) (2%-6%)	20 (4%) (2%-7%)
Day 22	140 (30%) (26%-35%) N = 461	239 (52%) (46%-57%) N = 464
Day 43	278 (63%) (58%-68%) N = 440	382 (85%) (81%-88%) N = 451
Day 387	45 (11%) (8%-15%) N = 395	109 (27%) (22%-32%) N = 411

Source: Table 14.2.1.1.
Abbreviations: N, number of subjects; CI, confidence interval; HI, hemagglutination inhibition; FAS, full analysis set.

GMTs and GMRs: Baseline titres against Influenza A H5N1 Turkey/2005 CC Ab among the vaccine groups bordered the limit of detection for the HI serology assay (titres below the limit of detection of the HI assay (< 1:10) were imputed as 5. Three weeks following the first vaccination (day 22), titres (GMRs) were increased over baseline in both low dose (2.43-fold) and high dose (5.37-fold) groups.

Three weeks after the second vaccination (day 43), the magnitude of increase from day 1 was higher in high dose group (41-fold) than low dose group (11-fold; Table 9).

Table 9.

Geometric Mean Titers and Geometric Mean Ratios (97.5% CI) on Day 43 Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) – HI Assay – FAS

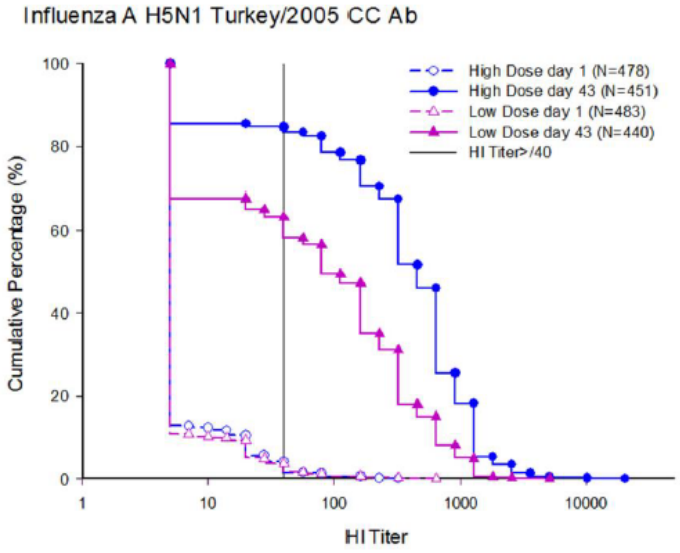
Vaccine Group	Low Dose	High Dose
	N = 483	N = 478
GMT Day 1	5.95 (5.63-6.29)	6.11 (5.78-6.46)
GMT Day 22	15 (13-17) N = 461	33 (28-39) N = 464
GMR Day 22/Day 1	2.43 (2.07-2.84) N = 461	5.37 (4.6-6.27) N = 464
GMT Day 43	64 (53-77) N = 440	250 (208-302) N = 451
GMR Day 43/Day 1	11 (8.68-13) N = 440	41 (34-49) N = 451
GMT Day 387	7.63 (6.79-8.57 N = 395	12 (11-14) N = 411
GMR Day 387/Day 1	1.24 (1.1-1.4) N = 395	1.95 (1.73-2.19) N = 411

Source: Table 14.2.1.3.
Abbreviations: CI, confidence interval; HI, hemagglutination inhibition, FAS, full analysis set; GMT, geometric mean titer; GMR, geometric mean ratio; N, number of subjects.

Reverse cumulative distribution frequency curve (RCDF) of HI titres for low dose and high dose against the Influenza A H5N1 Turkey/2005 CC Ab strain at baseline (day 1) and at 3 weeks following the second vaccination (day 43) is provided in Figure 1.

Figure 1.

Reverse Cumulative Distribution Frequency of HI Titers Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) at Day 1 and Day 43 – HI Assay – FAS



Source: Figure 14.2.3.3.7.

Of note, antibody responses against heterologous H5N1 strains as measured by HI and against heterologous H5N1 strains and homologous strain as measured by MN assay (in a subset of participants) are presented in section 3.3.4.6 (analysis performed across trials). These data were only collected for the high dose group after initial results were available, because the low dose vaccine did not achieve all CBER criteria. In short, the immunogenicity profile obtained from the HI assay was confirmed by MN results against the homologous strain in the full dose group. Cross-reactive antibody responses against heterologous strains were less robust than for the homologous strain as measured by HI assay, and generally higher heterologous responses were measured using the MN assay (see section 3.3.4.6 for detailed results).

The dose-response was also investigated in two additional dedicated phase 2 studies for the elderly (V89_13) and paediatric (V89_11) populations for the same two dose levels ("full dose" and "half dose"). In elderly, the higher dose led to a higher GMT/GMR and a significantly higher proportion of participants who met the definition for seroconversion at Day 43, which further supports the chosen dose. In the paediatric phase 2 trial, both dose levels achieved relatively high (vs. adults) seroconversion rates and GMTs/GMRs and not surprisingly, the higher dose did again perform better. These results are presented in more detail in section 3.3.4.4 (Clinical studies in special populations).

2.6.5.2. Main study

Study V89_18 (adult participants, including elderly)

Methods

Study V89_18 was a Phase 3, stratified, randomised, observer-blind, multi-centre, placebo-controlled study to evaluate safety, immunogenicity and lot-to-lot consistency of aH5N1c in healthy adult subjects ≥18 years of age. Subjects were randomly assigned (1:1:1:1) to receive a series of 2 IM

injections administered 3 weeks apart, of 1 of 3 lots aH5N1c containing 7.5 µg of the H5N1 (A/turkey/Turkey/1/2005 NIBRG-23 strain) influenza antigen with 0.25 mL MF59 (full dose) or placebo. The randomisation was stratified by age (18 to <65 years of age and ≥65 years of age) with a goal to enrol 50% into each age cohort.

Table below outlines the study design as well as the number of planned and enrolled subjects.

Table 10. Study Design

Treatment Arm	Schedule of Vaccine Administration	Planned/Actual Number of Subjects	Enrolled/Exposed by Age Group		Subjects Included in Per Protocol Set
			Age 18 to <65 years	Age ≥65 years	
Group A: aH5N1c Lot #1	Day 1, Day 22	798/804	403/403	401/402	761
Group B: aH5N1c Lot #2	Day 1, Day 22	798/799	399/398	400/399	747
Group C: aH5N1c Lot #3	Day 1, Day 22	798/795	397/397	398/396	741
Group D: Placebo	Day 1, Day 22	798/798	398/398	400/398	N/A
Total		3192/3196	1597/1596	1599/1595	

After the second vaccine administration, subjects were to be monitored for approximately 6 months for antibody persistence, and approximately 12 months for safety, for a total study duration of approximately 13 months per subject.

- **Study Participants**

This study was conducted at 26 centres in the USA.

Inclusion Criteria

In order to participate in this study, all individuals were required to meet ALL of the inclusion criteria described.

1. Males and females 18 years of age or greater.

2. Individuals who had given written consent after the nature of the study had been explained according to local regulatory requirements.

3. Individuals in good health as determined by the outcome of medical history, physical examination and clinical judgment of the Investigator.

Key exclusion criteria

1. Individuals with known or suspected impairment of the immune system.

2. Individuals who were pregnant or breastfeeding. Female subjects of childbearing potential must have had a negative pregnancy test prior to study vaccines being administered.

3. Females of childbearing potential who refused to use an acceptable method of birth control from Day 1 (first vaccination) to 3 weeks after the second study vaccination, and, if sexually active, who were not using a reliable birth control method for at least 2 months prior to study entry.

4. Individuals who received any type of influenza vaccine (e.g., "seasonal") within 7 days prior to enrolment in this study or who were planning to receive any type of influenza vaccine within 7 days (before or after) from the study vaccines.

5. Individuals who received any other licensed vaccine within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to enrolment in this study or who were planning to receive any (noninfluenza) vaccine within 28 days (before or after) from the study vaccines.

6. Individuals who had a previous confirmed or suspected illness from avian flu caused by an H5N1 virus.

7. Individuals who received any prior H5N1c vaccine.

8. Individuals with any progressive or severe neurologic disorder, seizure disorder, or history of Guillain-Barré syndrome.

9. Individuals who had a malignancy (excluding nonmelanotic skin cancer) or lymphoproliferative disorder within the past 5 years from Day 1.

10. Individuals with a body mass index (BMI) >35 kg/m2.

11. Individuals with a history of illness or with an ongoing illness that, in the opinion of the Investigator, might pose additional risk to the subject if he/she participated in the study.

- **Treatments**

The aH5N1c vaccine used for this study was an MF59-adjuvanted cell culture-derived H5N1 subunit influenza virus (A/turkey/Turkey/1/2005 NIBRG-23 strain). Each 0.5 mL dose contained 7.5 µg H5 haemagglutinin + 0.25 mL MF59. The aH5N1c vaccine was manufactured by Seqirus, Inc (formerly Novartis Vaccines and Diagnostics Inc). Placebo control consisted of 0.5 mL sterile saline (0.9% NaCl).

The study vaccine and placebo control were administered intramuscularly in the deltoid muscle. Vaccine was administered in an observer-blind manner.

Blood was drawn from each subject for immunogenicity assessments before each vaccination (two vaccinations, 3 weeks apart, Day 1 and Day 22) and also at clinic visits on Days 43 and 183.

- **Objectives**

Co-Primary Immunogenicity Objectives:

- To demonstrate lot-to-lot consistency across 3 consecutively produced lots of aH5N1c vaccine, as assessed by the ratio of geometric mean titres (GMTs) of hemagglutination inhibition (HI) antibody responses to the H5N1 vaccine strain 3 weeks after the second vaccine administration (Day 43) in healthy adult subjects ≥18 years of age.
- After lot-to-lot consistency was demonstrated, the populations of all H5N1c vaccine recipients were pooled in order to evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by Center for Biologics Evaluation and Research (CBER) guidance 3 weeks after the second vaccine administration (Day 43) as measured by age cohort and by strain-specific HI assay.

Criteria for lot-to-lot consistency:

Lot-to-lot consistency would be demonstrated if the limits of the 2-sided 95% confidence intervals (CI) for the GMT ratio were within the predefined equivalence range of 0.67 to 1.5.

Secondary Immunogenicity Objectives:

After lot-to-lot consistency was demonstrated, the populations of vaccine recipients administered aH5N1c lots were pooled, and the following objectives assessed:

- To evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CHMP recommendations 3 weeks after the second vaccine administration (Day 43) in healthy adult subjects ≥18 years of age by age cohort, as measured by strain-specific HI assay.
- To evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CBER and CHMP recommendations 3 weeks after the first vaccine administration (Day 22) in healthy adult subjects ≥18 years of age by age cohort, as measured by strain-specific HI assay.
- To evaluate immune responses to aH5N1c vaccine 6 months after the first vaccine administration (Day 183) in healthy adult subjects ≥18 years of age, by age cohort, as measured by strain-specific HI assay.

Both CBER and CHMP criteria were evaluated separately for each age cohort (18 to <65 years and ≥ 65 years, and 18 to <60 years and ≥ 60 years respectively).

Additional (***post hoc***) objective (based on the requirements described in the Guideline on Influenza Vaccines [EMA/CHMP/VWP/457259/2014], requesting investigation of neutralizing antibody titres at least in a representative subset of the study population in all studies):

To evaluate MN antibody responses elicited with the applicant's MF59 adjuvanted, cell culture derived H5N1 vaccine (aH5N1c) against the H5N1 virus contained within the vaccine (A/turkey/Turkey/1/2005), 3 weeks after the second vaccine administration (Day 43) in healthy adult subject 18 to <65 years of age.

- **Outcomes/endpoints**

Primary Immunogenicity Endpoints

The primary immunogenicity endpoints were based on HI antibody responses to the H5N1 vaccine strain for subjects ≥18 years of age, in the aH5N1c vaccine groups only.

- GMTs at Day 43 by lot.
- Percentage of subjects with HI titre ≥1:40 on Day 43 by age cohort (18 to <65 years of age and ≥ 65 years of age) in all lots, pooled.

Secondary Immunogenicity Endpoints

The secondary measures of immunogenicity, as determined by the HI assay, against the H5N1 homologous strain included the following:

- GMT at Day 1, Day 22, Day 43 and Day 183 by treatment group (aH5N1c or placebo) and by age cohort (18 to <65 years of age and ≥65 years of age).
- Percentage of subjects with HI titre ≥1:40 at Day 1, Day 22 and Day 183 by treatment group (aH5N1c or placebo) and by age cohort (18 to <65 years of age and ≥65 years of age).
- Percentage of subjects achieving seroconversion (defined as: HI titre ≥1:40 for subjects negative at baseline [HI titre <1:10]; or a minimum 4-fold increase in HI titre for subjects positive at baseline [HI titre ≥1:10]) on Day 22 and Day 43, respectively by treatment group (aH5N1c or placebo) and by age cohort (18 to <65 years of age and ≥65 years of age).
- GMR of HI titre: Day 22/Day 1, Day 43/Day 1 by treatment group (aH5N1c or placebo) and by age cohort (18 to <60 years of age and ≥60 years of age).

Additional immunogenicity endpoints (post hoc analysis):

The measures of immunogenicity (endpoints), as determined by the MN assay, against the H5N1 homologous strain by treatment group (aH5N1c or placebo) included the following:

- MN geometric mean titres (GMTs) at Day 1 (prevaccination) and Day 43 (3 weeks after the second vaccination)
- Percentage of subjects achieving seroconversion
- Geometric mean ratio (GMR), defined as the within subject fold increase in MN GMT postvaccination (Day 43) compared with prevaccination (Day 1)
- Percentage of subjects with MN titres ≥ 1:40, ≥ 1:80, and ≥ 1:160 at Day 1 and Day 43

- **Sample size**

Data observed in previous studies in similar populations (V89_04 in subjects 18-64 years of age; V89_13 in subjects >65 years of age) showed a variability of the postvaccination titres ranging from 0.75 to 0.88 (in the log10 scale). Assuming a SD of 0.85 for the log10 antibody titres (for each vaccine lot), approximate pairwise equivalence of factor 1 and independency (2 group t-test with equivalence limit 0.176 and expected difference 0), a single equivalence test based on 718 subjects per lot group has a power of 95% with alpha of .025. The resulting overall power is approximately 86% (0.95^3), because the total number of comparisons is 3. To account for dropouts (approximately 10%), a total recruitment of n=798 per lot was planned.

Data observed in previous studies in similar populations (V89_04 in subjects 18-<65 years of age; V89_13 in subjects > 65 years of age) showed a proportion of 86% of subjects aged 18 to <65 years and a proportion of 81% of subjects aged 65 years and older achieving an HI antibody titre of ≥1:40. With a sample size of 1077 evaluable subjects from the pooled lots there is an overall power of approximately 98% (.99*.99) to achieve the CBER criteria (HI antibody titre ≥1:40 for at least 70% of the subjects aged 18 to <65 years, and for at least 60% of the subjects aged 65 years and older). To account for dropouts (approximately 10%), a minimum sample size of n=1197 for all lots combined was needed. The combined overall power was 84%.

- **Randomisation and blinding (masking)**

After giving informed consent, and if an individual was determined to be eligible for study participation, the subject was enrolled in an Electronic Data Capture (EDC) system using the screening number assigned by the Investigator and then randomised. At randomisation, the subject was automatically assigned a unique subject identification (ID). Enrolled subjects were randomly assigned to the study groups, either one of 3 lots of aH5N1c or placebo, in a ratio of 1:1:1:1. Randomisation was stratified by centre and by age cohort (18 to <65 years and ≥65 years).

Randomisation and unblinding was to be managed by the PAREXEL Informatics group through Clinphone Interactive Response Technology (IRT).

The study was an observer-blind study. During the Treatment Administration Days (Day 1 and Day 22), designated unblinded nurse(s), physician(s), or other qualified personnel were responsible for administering the study vaccines to the subjects. They were instructed not to reveal the identity of the study vaccines either to the subject or the investigative site personnel involved in the subject assessments, except in an emergency. The designated unblinded nurse(s) or physician(s) did not take part in evaluating the subject(s) for safety or collect study data after the study vaccine administration. Except in the case of medical necessity, a subject's treatment should not have been unblinded without the approval of the Sponsor.

For this study using IRT, vaccine was to be packed using the 'scrambled pack numbering' methodology. Specifically, a kit list was generated and uploaded into the Interactive Response Technology (IRT). This list assigned a scrambled pack identification number to each treatment type. The system was programmed to assign the appropriate kit, based on the randomisation information entered for a subject. The outer carton of each kit was blinded and each pack was to be given a unique pack number. Once the unblinded site staff performing the vaccination has opened the outer pack, this person was unblinded for that subject.

A formal analysis of immunogenicity was planned to be carried out by an independent statistician and programmer (to preserve the blind) after Day 43 immunogenicity data were available for all subjects. Investigators, other site, CRO and Sponsor personnel, and the serology laboratory should remain blinded.

- **Statistical methods**

Analysis sets

All Enrolled Set: All screened subjects who provided informed consent and provided demographic and/or other baseline screening assessments, regardless of the subject's randomisation and treatment status in the study, and received a subject identification.

Exposed Set: All subjects in the All Enrolled Set who received a study vaccination.

The Full Analysis Set (FAS) included all subjects in the All Enrolled Set who:

- Actually received at least one dose of study vaccination, and
- Provided at least one evaluable serum sample at relevant timepoints.

FAS was defined by timepoint and objective:

- FAS-1 included all subjects who received at least one dose of study vaccine and provided at least one immunogenicity result at Day 1 and Day 43.
- FAS-2 included all subjects who received at least one dose of study vaccine and provided at least one immunogenicity result at Day 1 and Day 22.
- FAS-3 included all subjects who received at least one dose of study vaccine and provided at least one immunogenicity result at Day 1 and Day 183.

The Per Protocol Set (PPS) included all subjects in the FAS Immunogenicity who:

- Correctly received the vaccine ie, received the vaccine to which the subject was randomised and at the scheduled time points.
- Had no major protocol deviation leading to exclusion as defined prior to unblinding/analysis.
- Were not excluded due to other reasons (eg, subjects who withdrew informed consent) defined prior to unblinding or analysis.

Similarly to FAS, PPS was also defined per objective and time point.

Exclusions needed to be considered by objective/time point, ie, sometimes not all data of a subject but only part of the subject's data were excluded from the PPS analysis.

Analysis of Demographic and Baseline Characteristics

Age, height, weight, and BMI were summarised by reporting the mean, standard deviation (SD), median and range, and were calculated by vaccine lot/group and overall. The frequencies and percentages of subjects by sex, ethnic origin, race, and previous influenza vaccination (in the past 12

months) were presented by vaccine lot/group and overall. Demographic data were tabulated by vaccine lot/group and overall for the All Enrolled, PPS, and Overall Safety Set. The frequencies and percentages of subjects with medical history were tabulated for the Enrolled Set, PPS and Overall Safety Set. If the percentage of subjects excluded from the PPS had been greater than 5%, the demographic data and medical history data would have also been tabulated for the FAS.

Analysis of Co-primary Endpoints

The primary immunogenicity endpoints were based on HI antibody responses to the H5N1 vaccine strain for subjects ≥18 years of age, in the aH5N1c vaccine groups only.

For lot-to-lot consistency at Day 43, the following equivalence hypotheses were tested simultaneously:

H0: $(\mu_{lot\ A} - \mu_{lot\ B}) \leq -0.176$ or $(\mu_{lot\ A} - \mu_{lot\ B}) \geq 0.176$ or

$(\mu_{lot\ A} - \mu_{lot\ C}) \leq -0.176$ or $(\mu_{lot\ A} - \mu_{lot\ C}) \geq 0.176$ or

$(\mu_{lot\ B} - \mu_{lot\ C}) \leq -0.176$ or $(\mu_{lot\ B} - \mu_{lot\ C}) \geq 0.176$

vs.

H1: $(\mu_{lot\ A} - \mu_{lot\ B}) > -0.176$ and $(\mu_{lot\ A} - \mu_{lot\ B}) < 0.176$ and

$(\mu_{lot\ A} - \mu_{lot\ C}) > -0.176$ and $(\mu_{lot\ A} - \mu_{lot\ C}) < 0.176$ and

$(\mu_{lot\ B} - \mu_{lot\ C}) > -0.176$ and $(\mu_{lot\ B} - \mu_{lot\ C}) < 0.176$.

Here, H1 referred to the alternative hypothesis of pairwise equivalence (consistency) transformed to the log10 scale. Accordingly, $\mu_{lot\ A}$, $\mu_{lot\ B}$, and $\mu_{lot\ C}$ denoted the means of log10-transformed Day 43 titres of the corresponding lot groups. The lot-to-lot consistency of the immune response across lots would be claimed if the 2-sided 95% confidence intervals (CIs) of all the 3 pairwise comparisons of the GMT ratios were within the equivalence ranges of 0.667 and 1.5. Significance level to all these tests was $\alpha = 5\%$, which needed no adjustment for multiplicity as all hypotheses had to be rejected (union-intersection principle).

Adjusted estimates of ratio of GMTs, and their associated 95% CIs at Day 43 were computed for each pair among the 3 lots using analysis of covariance (ANCOVA) with factors for lot, age groups (18 to <65 years, ≥65 years), centre and a covariate for the effect defined by the log-transformed prevaccination antibody titre. Influenza-antibody GMTs (Day 43), associated 2-sided 95% CIs and median, minimal, and maximal area/titre values were determined and presented together with N (number of subjects), by lot (for the first primary objective). This model included only immunogenicity data from the 3 lots of aH5N1.

For evaluation of immune response to aH5N1c vaccine (percentage of subjects with HI titre ≥1:40) on Day 43 by age cohort (18 to <65 years of age and ≥65 years of age) according to CBER criteria:

The lower bound of the adjusted 2-sided 95% CI for the percentage of subjects achieving an HI antibody titre ≥1:40 should meet or exceed 70% and 60% respectively for the 2 different age cohorts (18 to <65 years of age and ≥65 years of age), the following hypothesis will be: H0: $(\pi_i - \pi_0) \leq 0$ vs. H1: $(\pi_i - \pi_0) > 0$ where H1 referred to the alternative hypothesis. π0 denoted the threshold for proportion of subjects with a HI titre ≥1:40 ($\pi_0 = 0.7$ for subjects 18 to <65 years of age and $\pi_0 = 0.6$ for subjects ≥65 years of age). Significance levels to these tests was $\alpha = 5\%$ which needed no adjustment for multiplicity as all hypothesis had to be rejected.

Binary data (proportion of subjects with HI titre ≥ 1:40) was to be analysed using separate log-linear models for age groups (8-64, ≥ 65 years) with a factor for dose group adjusted for centre, at Day 43.

Proportions and two sided 95% CIs were calculated based on these models. Models might have been simplified in case of convergence problems.

The primary analysis population for testing the null hypotheses was the PPS. If the percentage of subjects excluded from the PPS for analysis of immunogenicity was greater than 5% of subjects in the FAS, a supporting analysis based on the FAS was to be performed to complement the analysis based on the PPS.

The study was considered a success if both lot-to-lot consistency for subjects aged ≥ 18 years, and CBER criteria (HI antibody titre ≥ 1:40) for both age cohorts (18 to <65 years and ≥ 65 years) were met.

All statistical analyses for HI were performed on the logarithmically (base 10) transformed values. Individual HI titres below the detection limit were set to half that limit.

Analysis of Secondary Endpoints

Adjusted estimates of geometric mean titres (GMTs), and their associated 95% CIs at Days 1, 22, 43 and 183 were determined using analysis of covariance (ANCOVA) with factors for vaccine dose group and centre, by age cohort. GMTs at Days 22, 43 and 183 were adjusted by the pre-vaccination antibody titre. Influenza-antibody GMTs, associated two-sided 95% CIs and median, minimal, and maximal area/titre values will be determined and presented together with N (number of subjects), by vaccine group in both age cohorts (for CBER and CHMP objectives). Statistical analyses were performed on the logarithmically (base 10) transformed titre values, while median, minimum, and maximum values will be obtained on the actual titre values.

Binary data (proportion of subjects with HI titre ≥ 1:40 and proportions of subjects achieving seroconversion) were analysed using separate log-linear models for age groups (8-64, ≥ 65 years) with a factor for treatment group adjusted for centre, at Days 1, 22, 43 and 183. Proportions and two-sided 95% CIs were calculated based on these models. Models might have been simplified in case of convergence problems. The same statistical model as the one used for the primary objective was applied.

To assess CBER criteria, lower limit of the 95% CI of the two endpoints (proportion of Subjects with HI titre ≥ 1:40 and proportions of subjects achieving seroconversion) was compared with the pre-specified thresholds, in the two age cohorts (18-64, ≥ 65 years). Similarly, CHMP criteria were to be assessed.

In addition, results collected at Day 22 were evaluated against the CBER criteria for both age cohorts. Results collected at Day 22 were also evaluated against the CHMP criteria.

The analysis was based on the PPS. If the percentage of subjects excluded from the PPS for analysis of immunogenicity was greater than 5%, a supporting analysis based on the FAS was to be performed to complement the analysis based on the PPS.

Missing Data

Missing immunogenicity values were considered 'missing completely at random' (MCAR) and where therefore not supposed to contain information that impacted the result of the analysis. Imputation methods were therefore not used.

Interim Analysis

The interim analysis was to be performed on serology data, i.e., serum HI titre results for all subjects through the Day 43 visit (excluding those lost to follow-up or withdrawn for other reasons), by independent individuals who were not involved in clinical study conduct or any day-to-day study

activities. This analysis would minimally include demographic, baseline characteristics, medical history, vaccination administration, and immunogenicity (HI titre). The unblinded results were not to be depicted as individual subject results; results were to be presented by treatment arm only.

The interim analysis was to be performed after all necessary Day 43 data are available, i.e., approximately 11 months prior to reporting the complete study. The interim analysis of immunogenicity was to be performed on cleaned serology data, however the snapshot of other clinical data may not be cleaned at the time of interim analysis, and represented the final results through day 43. The analysis performed at interim analysis was to be refreshed at the final analysis on the fully cleaned and locked database.

Results

- **Participant flow**

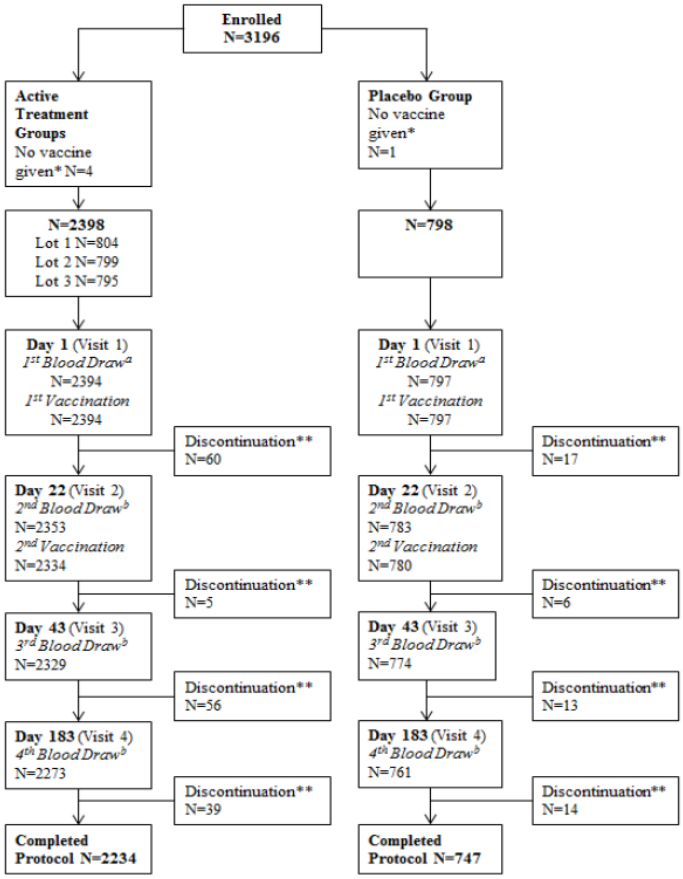
Disposition of Subjects: In total, 3196 subjects were enrolled into the study and 2981 (93%) completed the study (Table 11). The proportions of subjects who were exposed to any of the 3 lots of aH5N1c or placebo were balanced. The most commonly reported primary reasons for discontinuation from the study was lost to follow-up (3.2%) and withdrawal by subjects (2.2%). Study disposition flowchart is provided in Figure 2.

Table 11. Summary of Study Terminations throughout the Study – As Randomised – All Enrolled Set

Treatment Group	aH5N1c Lot #1 (N=804)	aH5N1c Lot #2 (N=799)	aH5N1c Lot #3 (N=795)	Active Treatment Groups (N=2398)	Placebo (N=798)	Total (N=3196)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Total Number of Subjects Enrolled	804 (100.0)	799 (100.0)	795 (100.0)	2398 (100.0)	798 (100.0)	3196 (100.0)
Total Number of Subjects Exposed	804 (100.0)	797 (99.7)	793 (99.7)	2394 (99.8)	797 (99.9)	3191 (99.8)
Completed Protocol	746 (92.8)	741 (92.7)	747 (94.0)	2234 (93.2)	747 (93.6)	2981 (93.3)
Primary Reason for Discontinuation from Study						
Adverse Event	0	1 (0.1)	2 (0.3)	3 (0.1)	2 (0.3)	5 (0.2)
Death	4 (0.5)	6 (0.8)	1 (0.1)	11 (0.5)	1 (0.1)	12 (0.4)
Withdrawal by Subject	27 (3.4)	19 (2.4)	11 (1.4)	57 (2.4)	13 (1.6)	70 (2.2)
Lost to Follow-up	22 (2.7)	27 (3.4)	29 (3.6)	78 (3.3)	25 (3.1)	103 (3.2)
Administrative Reason	0	0	0	0	1 (0.1)	1 (0.0)
Protocol Deviation/Violation	1 (0.1)	2 (0.3)	2 (0.3)	5 (0.2)	3 (0.4)	8 (0.3)
Other	4 (0.5)	3 (0.4)	3 (0.4)	10 (0.4)	6 (0.8)	16 (0.5)

Source: Table 14.1.1.2
Abbreviations: FAS=Full Analysis Set; ID=identification; n=number of subjects with values in category; N=total number of subjects; PPS=Per Protocol Set.
A total of 5 subjects were enrolled and randomized in error by sites (subject numbers 1120099, 1130027, 1200001, 1230012, and 1260028). These subjects were deemed screen or randomization failures due to violation of the inclusion and/or exclusion criteria. Study vaccine was not administered to the 5 subjects. These 5 subjects were therefore excluded from the FAS, PPS, and Safety populations due to a protocol deviation in the category Investigational Product Administration/Study Treatment: "Study Vaccine Was Not Administered at All".
Enrolled subjects are all screened subjects who provided informed consent and provided demographic and/or other baseline screening measurements, regardless of the subject's randomization and vaccination status in the study and received a subject ID.
Exposed subjects are all enrolled subjects who received a study vaccination.
As randomized: according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received.
Percentages are based on the number of subjects in each treatment group.

Figure 2. Study Disposition Flowchart



Source: Table 14.1.1.1, Table 14.1.1.2, Table 14.1.1.5 and Table 14.1.1.6
Abbreviation: N=number of subjects.
[a] Includes subjects for whom blood was drawn but not on Day 1.
[b] Includes subjects for whom blood was drawn out of the time window specified in the protocol, version 3.0, dated 15 March 2016.
* Details provided in Table 7 footnote.
** Reasons for discontinuation from the study are summarized in Table 7.

- **Recruitment**

Date of first enrolment: 11 July 2016

Date of last completed: 04 October 2017

Date of CSR: 20 September 2018

The dossier includes a CSR Addendum dated with 10 October 2022 describing post hoc microneutralisation (MN) data from 76 subjects who received aH5N1c and 24 subjects who received placebo. According to the CSR, the assay was performed in the same lab as the HI assay. Of note, immunogenicity assays from earlier trials were performed in a different laboratory.

- **Conduct of the study**

Amendments: There were 2 amendments to the original protocol (Protocol Version 1.0 dated 09 June 2014). No subject was treated under the original protocol and Protocol Amendment 1 (Protocol Version 2.0 dated 11 January 2016). At the time of first subject first visit, on 11 July 2016, Protocol Amendment 2 (Protocol Version 3.0 dated 15 March 2016) was in place.

Key change in Protocol Amendment 1 (dated 11 January 2016; Protocol Version 2.0) was the introduction of an interim analysis of immunogenicity results using data obtained 3 weeks after the second vaccination for H5N1 influenza (see description in the methods section above).

Minor changes were made in Protocol Amendment 2 (dated 15 March 2016; Protocol Version 3.0)

Protocol deviations: Overall, 384 (12.0%) subjects reported one or more major protocol deviations. The proportion of subjects with protocol deviations was similar across the 4 treatment groups. The most commonly reported protocol deviation was in the category Procedure/Tests, 'did not comply with blood draw schedule' (8.1%). This protocol deviation was equally distributed across the 18 to <65 years and ≥65 years of age groups and also across the treatment groups.

Table 12. Protocol Deviations – As Randomised - All Enrolled Set

Treatment Group	aH5N1c Lot #1 (N=804)	aH5N1c Lot #2 (N=799)	aH5N1c Lot #3 (N=795)	Placebo (N=798)	Total (N=3196)
	n (%)	n (%)	n (%)	n (%)	n (%)
Any Protocol Deviation	88 (10.9)	100 (12.5)	83 (10.4)	113 (14.2)	384 (12.0)
Inclusion/Exclusion Criteria	9 (1.1)	11 (1.4)	5 (0.6)	9 (1.1)	34 (1.1)
Subject Did not Meet Inclusion Criteria	1 (0.1)	0	0	0	1 (0.0)
Subject Met Exclusion Criteria	8 (1.0)	11 (1.4)	5 (0.6)	9 (1.1)	33 (1.0)
Investigational Product Administration/Study Treatment	14 (1.7)	23 (2.9)	28 (3.5)	23 (2.9)	88 (2.8)
Study Vaccine Was Not Administered at All	0	2 (0.3)	2 (0.3)	1 (0.1)	5 (0.2)
Randomization Error	1 (0.1)	0	0	1 (0.1)	2 (0.1)
Vaccination not According to the Protocol	0	0	0	1 (0.1)	1 (0.0)
Subject Did not Comply with Study Vaccination Schedule	13 (1.6)	21 (2.6)	26 (3.3)	20 (2.5)	80 (2.5)
Disallowed Medications	6 (0.7)	11 (1.4)	6 (0.8)	14 (1.8)	37 (1.2)
Administration of Prohibited Medication(s)	3 (0.4)	5 (0.6)	2 (0.3)	8 (1.0)	18 (0.6)
Administration of Prohibited Vaccine(s)	3 (0.4)	6 (0.8)	4 (0.5)	6 (0.8)	19 (0.6)
Procedure/Tests	71 (8.8)	76 (9.5)	69 (8.7)	89 (11.2)	305 (9.5)
Serological Results Are not Available	5 (0.6)	11 (1.4)	6 (0.8)	15 (1.9)	37 (1.2)
Did not Comply with Blood Draw Schedule	62 (7.7)	65 (8.1)	59 (7.4)	73 (9.1)	259 (8.1)
Underlying Medical Condition Prohibited by the Protocol	0	0	0	1 (0.1)	1 (0.0)
Subject Did not Provide any Postvaccination Solicited Safety Data	5 (0.6)	1 (0.1)	4 (0.5)	2 (0.3)	12 (0.4)

Source: Table 14.1.1.8
Abbreviations: n=number of subjects with values in category; N=total number of subjects.
As randomized: according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received.
The same protocol deviation subcategory can be listed under multiple categories.
Percentages are based on the number of subjects in each treatment group.

- **Baseline data**

The study was conducted in the USA. The demographic and other baseline characteristics for the All Enrolled Set were well balanced between the 4 treatment groups (Table 13). The demographic and baseline characteristics of note were:

- Overall, there was a higher proportion of females than males in all treatment groups (55.3% vs. 44.7% in the active treatment groups and 54.9% vs. 45.1% in the placebo group).
- The majority of the subjects enrolled (84.0%) were White.
- Overall, 53.0% of subjects had received an influenza vaccination in the last 12 months and the proportion of these subjects was similar across the 4 treatment groups.

Table 13. Demographic and Baseline Characteristics – Overall - All Enrolled Set

Treatment Group	aH5N1c Lot #1 (N=804)	aH5N1c Lot #2 (N=799)	aH5N1c Lot #3 (N=795*)	Active Treatment Groups (N=2398*)	Placebo (N=798)	Total (N=3196*)
Age (years)						
Mean	58.1	57.5	57.5	57.7	57.7	57.7
SD	17.67	17.83	18.24	17.91	18.29	18.00
Minimum	19	18	18	18	18	18
Median	64.0	65.0	65.0	64.5	65.0	65.0
Maximum	96	94	89	96	90	96
Age Group per CBER						
18 to <65 years	403 (50.1%)	399 (49.9%)	397 (49.9%)	1199 (50.0%)	398 (49.9%)	1597 (50.0%)
≥65 years	401 (49.9%)	400 (50.1%)	398 (50.1%)	1199 (50.0%)	400 (50.1%)	1599 (50.0%)
Age Group per CHMP						
18 to <60 years	355 (44.2%)	346 (43.3%)	357 (44.9%)	1058 (44.1%)	356 (44.6%)	1414 (44.2%)
≥60 years	449 (55.8%)	453 (56.7%)	438 (55.1%)	1340 (55.9%)	442 (55.4%)	1782 (55.8%)
Sex						
Male	360 (44.8%)	365 (45.7%)	348 (43.8%)	1073 (44.7%)	360 (45.1%)	1433 (44.8%)
Female	444 (55.2%)	434 (54.3%)	447 (56.2%)	1325 (55.3%)	438 (54.9%)	1763 (55.2%)
Ethnic Origin						
Hispanic or Latino	53 (6.6%)	61 (7.6%)	64 (8.1%)	178 (7.4%)	55 (6.9%)	233 (7.3%)
Not Hispanic or Latino	746 (92.8%)	729 (91.2%)	721 (90.7%)	2196 (91.6%)	732 (91.7%)	2928 (91.6%)
Not Reported	5 (0.6%)	9 (1.1%)	10 (1.3%)	24 (1.0%)	10 (1.3%)	34 (1.1%)
Unknown	0	0	0	0	1 (0.1%)	1 (0.0%)
Race						
American Indian or Alaska Native	6 (0.7%)	4 (0.5%)	5 (0.6%)	15 (0.6%)	3 (0.4%)	18 (0.6%)
Asian	12 (1.5%)	7 (0.9%)	9 (1.1%)	28 (1.2%)	7 (0.9%)	35 (1.1%)
Black or African American	110 (13.7%)	102 (12.8%)	104 (13.1%)	316 (13.2%)	112 (14.0%)	428 (13.4%)
Native Hawaiian or Other Pacific Islander	3 (0.4%)	2 (0.3%)	1 (0.1%)	6 (0.3%)	4 (0.5%)	10 (0.3%)
White	668 (83.1%)	679 (85.0%)	674 (84.8%)	2021 (84.3%)	665 (83.3%)	2686 (84.0%)
Other	5 (0.6%)	5 (0.6%)	2 (0.3%)	12 (0.5%)	7 (0.9%)	19 (0.6%)
Weight (kg)						
Mean	78.86	80.22	78.86	79.31	79.84	79.44
SD	15.049	15.165	15.302	15.179	15.255	15.197
Minimum	42.8	42.5	40.9	40.9	41.7	40.9
Median	78.55	79.60	78.00	78.90	79.30	78.90
Maximum	124.7	127.0	129.3	129.3	127.2	129.3
Height (cm)						
Mean	169.38	169.37	169.37	169.37	169.78	169.47
SD	9.596	9.708	10.153	9.817	9.424	9.720
Minimum	139.7	144.8	144.0	139.7	144.0	139.7
Median	168.50	167.60	169.50	168.00	170.00	169.00
Maximum	195.6	196.0	195.6	196.0	195.6	196.0
Body Mass Index (kg/m^2)						
Mean	27.40	27.86	27.41	27.56	27.60	27.57
SD	4.213	4.080	4.227	4.177	4.199	4.182
Minimum	15.1	16.3	17.0	15.1	15.9	15.1
Median	27.44	27.80	27.29	27.49	27.55	27.49
Maximum	35.0	35.0	35.1	35.1	35.1	35.1
Previous Seasonal Influenza Vaccine in the Last 12 Months						
Yes	430 (53.5%)	416 (52.1%)	426 (53.6%)	1272 (53.0%)	422 (52.9%)	1694 (53.0%)
No	374 (46.5%)	383 (47.9%)	369 (46.4%)	1126 (47.0%)	376 (47.1%)	1502 (47.0%)

Source: Table 14.1.1.3
Abbreviations: CBER=Center for Biologics Evaluation and Research; CHMP=Committee for Medicinal Products for Human Use; N=total number of subjects; SD=standard deviation.
*N=794 for aH5N1C lot#3, 2397 for active treatment groups and 3195 for total for weight, height and body mass index.
Percentages are based on the number of subjects in each treatment group.

Medical History: Medical history events were reported for 2886 (90.3%) subjects in total; of whom 2153 (89.8%) subjects were in the active treatment groups and 733 (91.9%) subjects were in the placebo group. The most frequently reported medical history events in the 18 to <65 years of age group were Hypertension (17.4%), Seasonal allergy (16.4%), and Drug hypersensitivity (13.4%). The most frequently reported medical history events in the ≥65 years of age group were Hypertension (58.2%), Osteoarthritis (34.3%), and Gastrooesophageal reflux disease (29.3%).

Demographics Microneutralisation analysis set (*post hoc*):

The demographic and other baseline characteristics for this subset of 100 subjects 18 to <65 years old were well balanced between the active treatment and placebo (Table 14). The mean (standard deviation [SD]) age of the study population was 41 (13.4) years for active treatment and 41.5 (12.7) years for placebo. The gender distribution was mostly balanced between groups with slightly more female in active treatment group (aH5N1c: 60.5%; placebo: 50%). The majority of subjects were White (aH5N1c: 80.3%; placebo: 83.3%), and not Hispanic or Latino (aH5N1c: 84.2%; placebo: 91.7%). In the active treatment group 34.2% of subjects and in the placebo group 50% of subjects had received an influenza vaccination in the last 12 months.

Table 14. Demographic and Baseline Characteristics by Treatment Group – MN subset

	Active Treatment N=76	Placebo N=24
Age, years		
Mean (SD)	41.0 (13.43)	41.5 (12.66)
Gender, n (%)		
Male	30 (39.5)	12 (50.0)
Female	46 (60.5)	12 (50.0)
Ethnic Origin, n (%)		
Hispanic or Latino	12 (15.8)	2 (8.3)
Not Hispanic or Latino	64 (84.2)	22 (91.7)
Race, n (%)		
White	61 (80.3)	20 (83.3)
Black or African American	13 (17.1)	3 (12.5)
Asian	2 (2.6)	1 (4.2)
Weight, kg		
Mean (SD)	78.39 (14.747)	82.63 (17.708)
Height, cm		
Mean (SD)	168.88 (10.195)	173.59 (8.487)
Body Mass Index, kg/m^2		
Mean (SD)	27.38 (3.889)	27.30 (4.654)
Previous Seasonal Influenza Vaccine in the Last 12 Months, n (%)		
Yes	26 (34.2)	12 (50.0)
No	50 (65.8)	12 (50.0)

Source: Appendix 14, Table 1.
Abbreviations: MN = microneutralization; N/n = number of subjects; SD = standard deviation.
MN subset = randomly selected subset of subjects 18 to <65 years of age

- **Numbers analysed**

All vaccinations were administered to the subjects during study visits. The number of subjects who received each vaccination is presented in Table 15):

Table 15. Vaccination Administration by Vaccination – All Enrolled Set

Treatment Group	Active Treatment Groups (N=2398) n (%)	Placebo (N=798) n (%)	Total (N=3196) n (%)
Vaccination 1			
Vaccine given:			
Yes	2394 (99.8)	797 (99.9)	3191 (99.8)
No	4 (0.2)	1 (0.1)	5 (0.2)
Vaccination 2			
Vaccine given:			
Yes	2334 (97.3)	780 (97.7)	3114 (97.4)
No	64 (2.7)	18 (2.3)	82 (2.6)

Source: Table 14.1.1.5
Abbreviations: n=number of values in category; N=total number of subjects.
Percentages are based on the number of subjects in each treatment group.

Most subjects (97.4%) received both vaccinations. Five subjects who did not meet the study entry criteria were randomised in error, and did not receive study vaccination (either Vaccination 1 or Vaccination 2). A total of 82 (2.6%) randomised subjects did not receive their second study vaccination.

Data Sets Analysed: In total, 3196 subjects were enrolled into the study and the majority of the subjects were included in the FAS and PPS immunogenicity sets (Table 16). The most commonly reported reason for exclusion from the FAS was 'serological results not available' (193 [6.0%] subjects) and the most commonly reported reason for exclusion from the PPS was 'did not comply with blood draw schedule' (259 [8.1%] subjects)

Table 16. Overview of Datasets Analysed – Overall – As Randomised - All Enrolled Set

Treatment Group	aH5N1c Lot #1 (N=804) n (%)	aH5N1c Lot #2 (N=799) n (%)	aH5N1c Lot #3 (N=795) n (%)	Active Treatment Groups (N=2398) n (%)	Placebo (N=798) n (%)	Total (N=3196) n (%)
All Enrolled Set	804 (100.0)	799 (100.0)	795 (100.0)	2398 (100.0)	798 (100.0)	3196 (100.0)
FAS, Immunogenicity Set	787 (97.9)	783 (98.0)	775 (97.5)	2345 (97.8)	776 (97.2)	3121 (97.7)
FAS-1, Day 43	778 (96.8)	773 (96.7)	769 (96.7)	2320 (96.7)	766 (96.0)	3086 (96.6)
FAS-2, Day 22	787 (97.9)	781 (97.7)	773 (97.2)	2341 (97.6)	773 (96.9)	3114 (97.4)
FAS-3, Day 183	757 (94.2)	754 (94.4)	747 (94.0)	2258 (94.2)	754 (94.5)	3012 (94.2)
PPS, Immunogenicity Set	761 (94.7)	747 (93.5)	741 (93.2)	2249 (93.8)	739 (92.6)	2988 (93.5)
PPS-1, Day 43	729 (90.7)	710 (88.9)	717 (90.2)	2156 (89.9)	700 (87.7)	2856 (89.4)
PPS-2, Day 22	761 (94.7)	744 (93.1)	740 (93.1)	2245 (93.6)	736 (92.2)	2981 (93.3)
PPS-3, Day 183	698 (86.8)	689 (86.2)	692 (87.0)	2079 (86.7)	687 (86.1)	2766 (86.5)

Source: Table 14.1.1.1
Abbreviations: FAS=Full Analysis Set; n=number of subjects with values in category; N=total number of subjects; PPS=Per Protocol Set.
As randomized: according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received.
Percentages are based on the number of subjects in each treatment group in the All Enrolled Set.

Table 14.1.1.8.1
Exclusions from Immunogenicity Sets - As Randomized For Each Population
Overall and By Age
All Enrolled Set

Age: Overall
Type of Population: FAS

	aH5N1c Lot#1 (N=804)	aH5N1c Lot#2 (N=799)	aH5N1c Lot#3 (N=795)	Placebo (N=798)	Total (N=3196)
Total Number of Subjects with Exclusions:					
Any Exclusion	47 (5.8%)	48 (6.0%)	49 (6.2%)	49 (6.1%)	193 (6.0%)
Reasons:					
Study Vaccine Was not Administered at All	0	2 (0.3%)	2 (0.3%)	1 (0.1%)	5 (0.2%)
Serological Results are not Available	47 (5.8%)	48 (6.0%)	49 (6.2%)	49 (6.1%)	193 (6.0%)

Table 14.1.1.8.1
Exclusions from Immunogenicity Sets - As Randomized For Each Population
Overall and By Age
All Enrolled Set

Age: Overall
Type of Population: PPS

	aH5N1c Lot#1 (N=804)	aH5N1c Lot#2 (N=799)	aH5N1c Lot#3 (N=795)	Placebo (N=798)	Total (N=3196)
Total Number of Subjects with Exclusions:					
Any Exclusion	124 (15.4%)	133 (16.6%)	117 (14.7%)	141 (17.7%)	515 (16.1%)
Reasons:					
Administration of Prohibited Medication(s)	3 (0.4%)	5 (0.6%)	2 (0.3%)	8 (1.0%)	18 (0.6%)
Administration of Prohibited Vaccine(s)	3 (0.4%)	6 (0.8%)	4 (0.5%)	6 (0.8%)	19 (0.6%)
Did not Comply with Blood Draw Schedule (P)	62 (7.7%)	65 (8.1%)	59 (7.4%)	73 (9.1%)	259 (8.1%)
Randomization failure	1 (0.1%)	0	0	1 (0.1%)	2 (0.1%)
Serological Results Are not Available	5 (0.6%)	11 (1.4%)	6 (0.8%)	15 (1.9%)	37 (1.2%)
Study Vaccine Was Not Administered at All	0	2 (0.3%)	2 (0.3%)	1 (0.1%)	5 (0.2%)
Subject Did not Comply with Study Vaccination Schedule (IP)	17 (2.1%)	24 (3.0%)	30 (3.8%)	21 (2.6%)	92 (2.9%)
Subject Did not Meet Inclusion Criteria	1 (0.1%)	0	0	0	1 (0.0%)
Subject Met Exclusion Criteria	8 (1.0%)	11 (1.4%)	5 (0.6%)	9 (1.1%)	33 (1.0%)
Underlying Medical Condition Prohibited by the Protocol (P)	0	0	0	1 (0.1%)	1 (0.0%)
Vaccination not according to protocol	1 (0.1%)	0	0	1 (0.1%)	2 (0.1%)

Data Sets Analysed by Age: In total, 1597 subjects were enrolled into the study in the 18 to <65 years age group and 1599 subjects in the ≥65 years age group (17). The majority of the subjects in both age groups were included in the FAS and PPS immunogenicity sets.

As reported for the total study population, the most commonly reported reason for exclusion from the FAS for both age groups was 'serological results not available' (129 [8.1%] subjects in the 18 to <65 years of age group and 64 [4.0%] subjects in the ≥65 years of age group) and from the PPS was 'did not comply with blood draw schedule' (118 [7.4%] subjects in the 18 to <65 years of age group and 141 [8.8%] subjects in the ≥65 years of age group) (not all Tables shown in this AR).

Table 17. Overview of Datasets Analysed by Age- As Randomised – All Enrolled

Age Group	18 to <65 Years			≥65 Years		
Treatment Group	Active Treatment Groups (N=1199) n (%)	Placebo (N=398) n (%)	Total (N=1597) n (%)	Active Treatment Groups (N=1199) n (%)	Placebo (N=400) n (%)	Total (N=1599) n (%)
All Enrolled Set	1199 (100.0)	398 (100.0)	1597 (100.0)	1199 (100.0)	400 (100.0)	1599 (100.0)
FAS, Immunogenicity Set	1162 (96.9)	383 (96.2)	1545 (96.7)	1183 (98.7)	393 (98.3)	1576 (98.6)
FAS-1, Day 43	1142 (95.2)	376 (94.5)	1518 (95.1)	1178 (98.2)	390 (97.5)	1568 (98.1)
FAS-2, Day 22	1160 (96.7)	381 (95.7)	1541 (96.5)	1181 (98.5)	392 (98.0)	1573 (98.4)
FAS-3, Day 183	1102 (91.9)	370 (93.0)	1472 (92.2)	1156 (96.4)	384 (96.0)	1540 (96.3)
PPS, Immunogenicity Set	1116 (93.1)	372 (93.5)	1488 (93.2)	1133 (94.5)	367 (91.8)	1500 (93.8)
PPS-1, Day 43	1076 (89.7)	349 (87.7)	1425 (89.2)	1080 (90.1)	351 (87.8)	1431 (89.5)
PPS-2, Day 22	1115 (93.0)	370 (93.0)	1485 (93.0)	1130 (94.2)	366 (91.5)	1496 (93.6)
PPS-3, Day 183	1025 (85.5)	341 (85.7)	1366 (85.5)	1054 (87.9)	346 (86.5)	1400 (87.6)

Source: Table 14.1.1.1
Abbreviations: FAS=Full Analysis Set; n=number of subjects with values in category; N=total number of subjects; PPS=Per Protocol Set.
As randomized: according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received.
Percentages are based on the number of subjects in each treatment group in the All Enrolled Set.

Post hoc analysis (microneutralisation assay)

A listing including 200 subjects, who consented to future testing for purposes not related to the V89_18 study, with Day 1 and Day 43 serum samples containing sufficient serum, was generated in random order. Serum samples of 100 subjects (aH5N1c N=76; placebo N=24) of this listing were randomly selected for MN testing against the homologous H5N1 strain.

- **Outcomes and estimation**

Analysis of Co-Primary Objectives

The co-primary immunogenicity endpoints were based on HI antibody response to the H5N1 vaccine strain for subjects ≥18 years of age, in the aH5N1c vaccine groups only. The PPS was used as the primary analysis population for both objectives.

Because the criteria for lot-to-lot consistency were met, the CBER criterion for the percentage of subjects with HI titre ≥1:40 on Day 43 was assessed for subjects receiving any of the 3 aH5N1c lots, pooled from both age cohorts (18 to <65 years of age and ≥65 years of age).

Lot-to-lot Consistency

In Study V89_18, lot-to-lot consistency of 3 consecutively produced lots of aH5N1c vaccine was achieved, as assessed by the ratio of GMTs of HI antibody responses to the H5N1 vaccine strain at Day 43. The 2-sided 95% CIs of all the 3 pairwise comparisons of GMTs fell between the predefined equivalence ranges of 0.667 and 1.5 (Table 18).

Table 18.

Study V89_18 – GMTs and GMT Ratios Against the Homologous Strain for Lot-to-Lot Consistency – HI Assay – PPS

	Treatment Groups (95% CI)		
	aH5N1c Lot #1	**aH5N1c Lot #2**	**aH5N1c Lot #3**
	N=761	N=747	N=741
Day 1	16.1 (15.1; 17.2)	17.0 (15.9; 18.2)	17.0 (15.9; 18.2)
	N=729	N=710	N=717
Day 43	128.6 (118.9; 139.1)	127.4 (117.6; 138.0)	132.2 (122.1; 143.1)
	Treatment Group Ratios		
	aH5N1c Lot #1 vs. aH5N1c Lot #2	**aH5N1c Lot #2 vs. aH5N1c Lot #3**	**aH5N1c Lot #1 vs. aH5N1c Lot #3**
	1.01 (**0.90; 1.13**)	0.96 (**0.86; 1.08**)	0.97 (**0.87; 1.09**)

Source: CSR V89_18: Table 14.2.1.1.

Abbreviations: ANCOVA = analysis of covariance; CI = confidence interval; GMT = geometric mean titre; N = number of subjects; PPS = per protocol set.

Notes:

The comparison is done based on Log10-transformed data.

Bold shows the 2-sided 95% CI lies within the protocol-defined equivalence range of (0.667, 1.5).

Adjusted estimates of GMTs, and their associated 95% CIs at Day 43 were computed using ANCOVA with factors for lot, age groups (18 to <65 and ≥ 65 years of age), centre and a covariate for the effect defined by the log-transformed prevaccination antibody titre (Day 1).

aH5N1c versus Placebo

On Day 43, both CBER criteria were met by subjects 18 to <65 years of age in the aH5N1c full dose group. On Day 22 neither CBER criterion was met. No changes over time in the placebo group (seroconversion and HI ≥1:40) were observed (Table 19).

On Day 43, both CBER criteria were met by subjects ≥65 years of age in the aH5N1c full dose group. On Day 22 neither CBER criterion was met. No changes over time in the placebo group (seroconversion and HI ≥1:40) were observed (Table 19).

All 3 CHMP immunogenicity criteria were met at Day 43 in both subjects 18 to <60 and ≥60 years of age for the active treatment groups. At Day 22, two of 3 CHMP criteria (seroconversion and GMR) were met by subjects 18 to <60 years of age. Subjects ≥60 years of age met 1 of 3 criteria (GMR).

At Day 183 (6 months after first vaccination), none of the CBER or CHMP criteria were met by subjects 18 to <65 years, 18 to <60 years, ≥60 years and ≥65 years of age.

Table 19.

Study V89_18 – Percentages of Subjects Achieving Seroconversion and Percentages of Subjects Achieving an HI Titre ≥1:40 Against the Homologous Strain at Days 1, 22, 43, and 183 in Subjects ≥18 Years of Age – HI Assay – PPS

	18 to <65 Years (95% CI)		≥65 Years (95% CI)		≥18 Years (95% CI)	
	Full Dose	**Placebo**	**Full Dose**	**Placebo**	**Full Dose**	**Placebo**
Seroconversion						
	N=1115	N=370	N=1130	N=366	N=2245	N=736
Day 22	***40.4*** (37.6; 43.4)	1.9 (0.8; 3.9)	24.2 (21.7; 26.8)	0.3 (0.0; 1.5)	32.2 (30.3; 34.2)	1.1 (0.5; 2.1)
	N=1076	N=349	N=1080	N=351	N=2156	N=700
Day 43	***79.9*** (**77.4**; 82.3)	0.3 (0.0; 1.6)	***54.0*** (**51.0**; 57.0)	1.7 (0.6; 3.7)	***66.9*** (**64.9**; 68.9)	1.0 (0.4; 2.0)
	N=1025	N=341	N=1054	N=346	N=2079	N=687
Day 183	16.2 (14.0; 18.6)	0.3 (0.0; 1.6)	8.0 (6.4; 9.8)	1.2 (0.3; 2.9)	12.0 (10.7; 13.5)	0.7 (0.2; 1.7)
HI Titre ≥1:40						
	N=1116	N=372	N=1133	N=367	N=2249	N=739
Day 1	17.4 (15.2; 19.7)	19.6 (15.7; 24.0)	28.2 (25.6; 31.0)	25.6 (21.2; 30.4)	22.9 (21.1; 24.6)	22.6 (19.6; 25.8)
	N=1115	N=370	N=1130	N=366	N=2245	N=736
Day 22	63.0 (60.1; 65.9)	13.5 (10.2; 17.4)	55.6 (52.6; 58.5)	16.4 (12.7; 20.6)	59.3 (57.2; 61.3)	14.9 (12.4; 17.7)
	N=1076	N=349	N=1080	N=351	N=2156	N=700
Day 43	***93.1*** (**91.4**; 94.6)	10.9 (7.8; 14.6)	***83.5*** (**81.2**; 85.7)	22.8 (18.5; 27.5)	**88.3** (**86.9**; 89.6)	16.9 (14.2; 19.8)
	N=1025	N=341	N=1054	N=346	N=2079	N=687
Day 183	34.2 (31.3; 37.2)	2.1 (0.8; 4.2)	30.9 (28.1; 33.8)	8.4 (5.7; 11.8)	32.6 (30.6; 34.6)	5.2 (3.7; 7.2)

Source: CSR V89_18: Table 14.2.1.2, Table 14.2.1.2.6, Table 14.2.1.3, Table 14.2.1.3.6.

Abbreviations: CI = confidence interval; HI = hemagglutination inhibition; N = number of subjects; PPS = per protocol set; seroconversion = subjects with a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40, or, subjects with a prevaccination HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titre.

Bold = CBER criteria met (lower bound of CI); ***Bold, italic*** = former CHMP criteria met

Table 20.

Study V89_18 – GMTs and GMRs Against the Homologous Strain at Days 1, 22, 43, and 183 in Subjects ≥18 Years of Age – HI Assay – PPS

	18 to <65 Years (95% CI)		≥65 Years (95% CI)		≥18 Years (95% CI)	
	Full Dose	**Placebo**	**Full Dose**	**Placebo**	**Full Dose**	**Placebo**
	N=1116	N=372	N=1133	N=367	N=2249	N=739
GMT Day 1	13.5 (12.8; 14.2)	13.7 (12.5; 15.0)	20.5 (19.4; 21.8)	20.6 (18.6; 22.7)	16.6 (16.0; 17.3)	16.7 (15.6; 17.9)
	N=1115	N=370	N=1130	N=366	N=2245	N=736
GMT Day 22	50.6 (47.6; 53.8)	11.6 (10.4; 12.9)	42.4 (40.0; 45.0)	14.5 (13.1; 16.0)	46.4 (44.5; 48.4)	13.0 (12.1; 14.0);
GMR Day 22/Day 1	***3.81*** (3.58; 4.05)	0.87 (0.79; 0.97)	***2.14*** (2.02; 2.27)	0.73 (0.66; 0.81)	***2.86*** (2.74; 2.98)	0.80 (0.74; 0.86)
	N=1076	N=349	N=1080	N=351	N=2156	N=700
GMT Day 43	170.7 (160.5; 181.6)	11.0 (9.9; 12.2)	97.9 (92.1; 104.1)	16.7 (15.0; 18.5)	130.6 (124.8; 136.6)	13.7 (12.6; 14.8)
GMR Day 43/Day 1	***12.70*** (11.94; 13.51)	0.82 (0.73; 0.91)	***4.90*** (4.61; 5.20)	0.83 (0.75; 0.92)	***7.96*** (7.61; 8.33)	0.83 (0.77; 0.90)
	N=1025	N=341	N=1054	N=346	N=2079	N=687
GMT Day 183	20.4 (19.3; 21.6)	6.8 (6.1; 7.4)	19.3 (18.2; 20.4)	8.6 (7.8; 9.5)	20.0 (19.2; 20.8)	7.7 (7.2; 8.2)
GMR Day 1/Day 183	1.53 (1.44; 1.61)	0.51 (0.46; 0.56)	0.97 (0.91; 1.02)	0.43 (0.39; 0.47)	1.22 (1.17; 1.27)	0.47 (0.44; 0.50)

Source: CSR V89_18: Table 14.2.1.4, Table 14.2.1.4.6.

Abbreviations: CI = confidence interval; GMR = geometric mean ratio; GMT = geometric mean titre; N = number of subjects; PPS = per protocol set.

Bold, italic = former CHMP criteria met

An overview of timepoints when the CBER and former CHMP criteria were met is provided in Table 21.

Table 21.

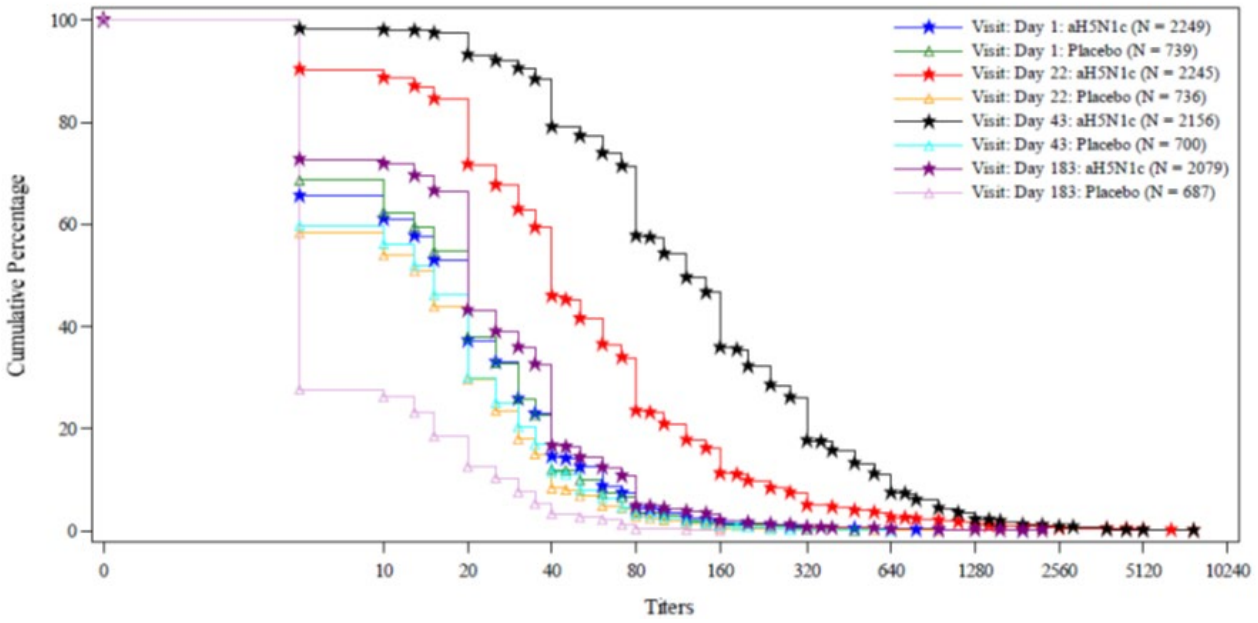
Overview of CBER and CHMP Success Criteria for Homologous Strain at Day 22 and Day 43 – Per Protocol Set

CBER Age Group	18 to <65 Years				≥65 Years			
Treatment Group	Active Treatment Groups		Placebo		Active Treatment Groups		Placebo	
	Day 22	Day 43	Day 22	Day 43	Day 22	Day 43	Day 22	Day 43
Percentage of subjects achieving an HI antibody titer ≥1:40	No	**Yes**	No	No	No	**Yes**	No	No
Percentage of subjects achieving seroconversion	No	**Yes**	No	No	No	**Yes**	No	No
CHMP Age Group	**18 to <60 Years**				**≥60 Years**			
Treatment Group	**Active Treatment Groups**		**Placebo**		**Active Treatment Groups**		**Placebo**	
	Day 22	**Day 43**	**Day 22**	**Day 43**	**Day 22**	**Day 43**	**Day 22**	**Day 43**
GMR	**Yes**	**Yes**	No	No	**Yes**	**Yes**	No	No
Percentage of subjects achieving an HI titer ≥1:40	No	**Yes**	No	No	No	**Yes**	No	No
Percentage of subjects achieving seroconversion	**Yes**	**Yes**	No	No	No	**Yes**	No	No

Source: Table 14.2.1.2.6, Table 14.2.1.2.7, Table 14.2.1.3.6, Table 14.2.1.3.7 and Table 14.2.1.4.7
Abbreviations: CBER=Center for Biologics Evaluation and Research; CHMP=Committee for Medicinal Products for Human Use, GMR=geometric mean ratio; HI=hemagglutination inhibition.
Yes shows respective criterion was met. No shows respective criterion was not met.
CBER criteria for subjects 18 to <65 years of age: The lower bound of the adjusted 2-sided 95% CI for the percentage of subjects achieving an HI antibody titer ≥1:40 should meet or exceed 70%. The lower bound of the 2-sided 95% CI for the percentage of subjects achieving seroconversion for HI antibody should meet or exceed 40%.
CBER criteria for subjects ≥65 years of age: The lower bound of the adjusted 2-sided 95% CI for the percentage of subjects achieving an HI antibody titer ≥1:40 should meet or exceed 60%. The lower bound of the 2-sided 95% CI for the percentage of subjects achieving seroconversion for HI antibody should meet or exceed 30%.
CHMP Criteria for subjects 18 to <60 years of age: The percentage of subjects with seroconversion in HI antibody was >40%, the GMR was >2.5 and the percentage of subjects achieving an HI titer ≥1:40 was >70%.
CHMP criteria for subjects ≥60 years of age: The percentage of subjects with seroconversion in HI antibody was >30%, the GMR was >2.0 and the percentage of subjects achieving an HI titer ≥1:40 was >60%.
Seroconversion=either a prevaccination (baseline) HI titer <1:10 and postvaccination HI titer ≥1:40 or a prevaccination HI titer ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titer.

The reverse cumulative distributions of HI titres at Day 22, Day 43 and Day 183 are presented in Figure 3.

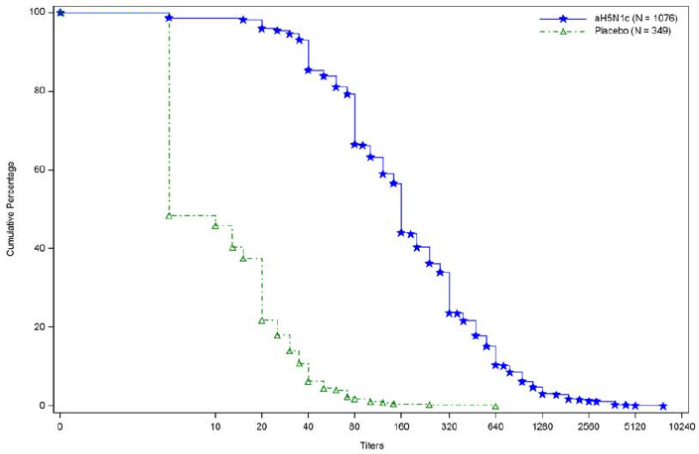
Figure 3. Reverse Cumulative Distribution of HI Titres at Day 1, 22, 43 and 183 by Pooled aH5N1c Lots and Placebo for the Overalll Population Pooled aH5N1c Lots and Placebo – Per Protocol Set



The reverse cumulative distributions of HI antibody titres at Day 43 for subjects 18 to <65 years of age and ≥65 years of age are presented in Figure 4 and Figure 5, respectively.

Figure 4.

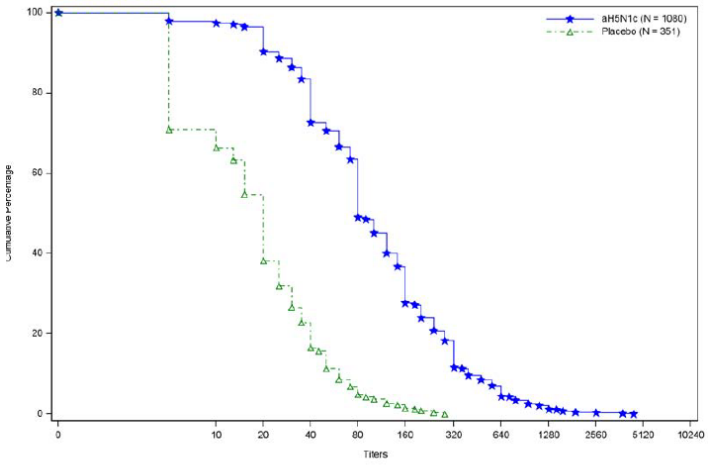
Reverse Cumulative Distributions of HI Titer at Day 43 in Subjects 18 to <65 Years of Age – Per Protocol Set



Source: Figure 14.2.2.2
Abbreviation: HI=hemagglutination inhibition.

Figure 5.

Reverse Cumulative Distributions of HI Titer at Day 43 in Subjects ≥65 Years of Age – Per Protocol Set



ɔurce: Figure 14.2.2.2
bbreviation: HI=hemagglutination inhibition.

Microneutralisation data (*post hoc* analysis):

The baseline MN GMTs were very low, bordering on the LLOQ of 10 in both the active treatment and placebo groups. At Day 43, 3 weeks after the second vaccination, increases in the MN GMTs from baseline in the active treatment groups were observed, with the GMR of 23.80. No increases in MN GMTs at Day 43 were observed in the placebo group (Table 22).

Table 22.

Immune responses (GMT and GMR) Against the Homologous Strain at Days 1 and 43 by Treatment Group – MN Assay – MN Subset

	Active Treatment N=76	Placebo N=24
Day 1 GMT (95% CI)	5.53 (5.2, 5.9)	5.30 (4.7, 6.0)
Day 43 GMT (95% CI)	130.91 (103.1, 166.3)	5.23 (3.4, 8.0)
GMR Day 43/Day 1 (95% CI)	23.80 (18.7, 30.3)	0.97 (0.6, 1.5)

Source: Appendix 14, Table 4.

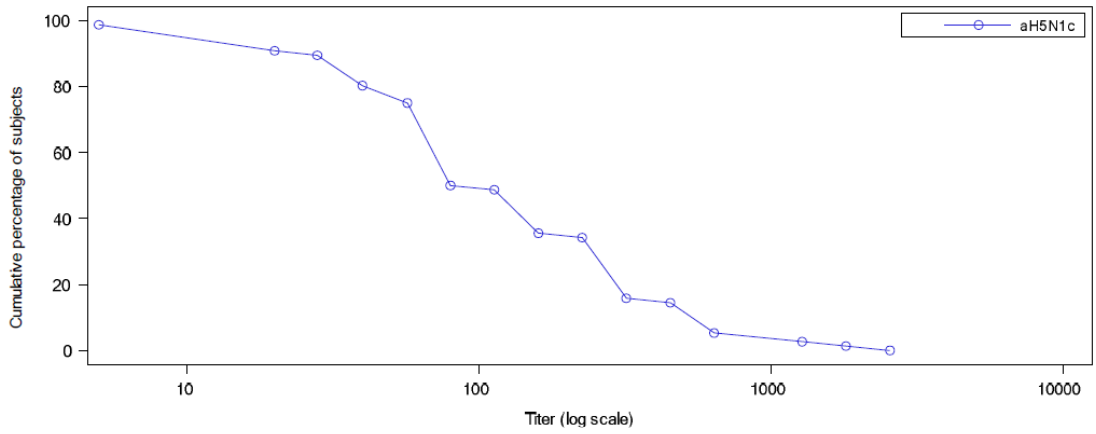
Abbreviations: CI = confidence interval; GMR = geometric mean ratio; GMT = geometric mean titer; MN = microneutralization; N = number of subjects

MN subset = randomly selected subset of subjects 18 to <65 years of age

Note 1: Adjusted GMT and GMR are presented.

GMR is defined as the within subject geometric mean of the fold increases of postvaccination antibody titer (Day 43) over the prevaccination antibody titer (Day 1).

Figure 6. Reverse Cumulative Distributions of MN Titre at Day 43



Source: Appendix 14, Figure 1.

At Day 1, the percentages of subjects with a baseline MN titre ≥1:40, ≥1:80, or ≥1:160 were similar for the active treatment group and placebo group. At Day 43, 3 weeks after the second vaccination, the percentages of subjects in the active treatment group with MN titre ≥1:40, ≥1:80, or ≥1:160 increased from baseline, whereas no increases in percentages of subjects with MN titre ≥1:40, ≥1:80, or ≥1:160 were observed in the placebo group.

The percentage of subjects achieving seroconversion in the active treatment group was 89.5% at Day 43. No seroconversion was observed in the placebo group at Day 43 (Table 23).

Table 23.

Percentage of Subjects with MN Titer Against the Homologous Strain ≥1:40, ≥1:80 and ≥1:160 at Days 1 and 43 and Seroconversion at Day 43 by Treatment Group – MN Subset

		Active Treatment N=76	Placebo N=24
MN Titer ≥1:40			
Day 1	% (95% CI)	1.3 (0.03, 7.11)	0
Day 43	% (95% CI)	89.5 (80.31, 95.34)	0
MN Titer ≥1:80			
Day 1	% (95% CI)	0	0
Day 43	% (95% CI)	75.0 (63.74, 84.23)	0
MN Titer ≥1:160			
Day 1	% (95% CI)	0	0
Day 43	% (95% CI)	48.7 (37.04, 60.43)	0
Seroconversion			
Day 43	% (95% CI)	89.5 (80.31, 95.34)	0

Source: Appendix 14, Table 2, Table 3.
Abbreviations: CI = confidence interval; MN = microneutralization; N = number of subjects
MN subset = randomly selected subset of subjects 18 to <65 years of age
Seroconversion defined as a ≥4-fold increase in post-vaccination titer in subjects with pre-vaccination titer above or equal to the lower limit of quantification (1:10), or a post-vaccination titer ≥1:40 for subjects with pre-vaccination titer below the lower limit of quantification (1:10).

- **Summary of main efficacy results**

The following table summarises the efficacy results from the main study supporting the present application (V89_18). This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 24. Summary of Efficacy for Trial V89_18

Title: Phase 3 Randomised, Observer-Blind, Multi-centre, Controlled Study to Evaluate Safety, Immunogenicity, and Lot-to-Lot Consistency of an Adjuvanted Cell Culture-Derived, H5N1 Subunit Influenza Virus Vaccine in Healthy Adult Subjects ≥18 years of Age		
Study identifier	V89_18 NCT02839330	
Design	Randomised, observer-blind, placebo controlled, lot-to-lot consistency	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	13 months not applicable not applicable
Hypothesis	Pairwise comparison of immunogenicity of 3 lots of the selected dose of aH5N1c (Full dose: 7.5 µg HA + 0.25 mL MF59), lot-to-lot consistency will be claimed if two-sided 95% confidence intervals of all the three pairwise comparisons fall within 0.667 and 1.5. After demonstration of lot-to-lot consistency, HI titre ≥1:40 on Day 43 for the pooled lots according to CBER criteria will be evaluated	
Treatments groups	**Full dose** (**aH5N1c Lot#1; aH5N1c Lot#2; aH5N1c Lot#3**)	Two vaccinations 3 weeks apart, number randomised: 2398
	Placebo (0.9% NaCl)	Two vaccinations 3 weeks apart, number randomised: 798

Endpoints and definition	Primary endpoint	D43 GMT by lot	Geometric mean HI antibody titres by aH5N1c lot
	Co-Primary endpoint	HI titre ≥1:40 D43	Percentage of subjects with a HI titre ≥1:40 on Day 43 for the pooled lots, by age cohort (18 to <65 years of age and ≥65 years of age) according to age-appropriate CBER criteria
	Secondary endpoint	HI seroconversion	Percentage of subjects achieving seroconversion on Day 22, Day 43, and Day 183 (age-appropriate CBER and CHMP criteria)
	Secondary endpoint	HI titre ≥1:40	Percentage of subjects with a HI titre ≥1:40 on Day 22, Day 43, and Day 183 (age-appropriate CBER and CHMP criteria)
	Secondary endpoint	HI GMR	Mean fold increase in HI geometric mean antibody titre, Day 22/Day 1, Day 43/Day 1 and Day 183/Day 1 (age-appropriate CHMP criteria)
Database lock	Last Subject completed: 04 Jun 2017; CSR date: 20 Sep 2018		

Results and Analysis

Analysis description	**Primary Analysis**				
Analysis population and time point description	Per Protocol set Day 43 (3 weeks after the 2nd vaccination)				
Descriptive statistics and estimate variability	Treatment group	**Full Dose aH5N1c Lot#1**	**Full Dose aH5N1c Lot#2**	**Full Dose aH5N1c Lot#3**	
	Number of subjects	761	747	741	
	D43 GMT by lot (95% confidence interval (CI))	128.6 (118.9-139.1)	127.4 (117.6-138.0)	132.2 (122.1-143.1)	
	Treatment group	**Full Dose (pooled lots)**		**Placebo**	
	Age group	18 to <65 years	≥65 years	18 to <65 years	≥65 years
	HI ≥1:40 D43 (95% CI)	95% (**93.4**, 96.2)	85.7% (**83.3,** 87.9)	8.5% (5.9, 12.1)	20.8% (16.6, 25.8)
Effect estimate per comparison	Primary endpoint D43 GMT by lot	Comparison groups		**aH5N1c Lot #1 vs. aH5N1c Lot #2**	
		GMT ratio (95% CI)		1.01 (0.90, 1.13)	
		Comparison groups		**aH5N1c Lot #2 vs. aH5N1c Lot #3**	
		Comparison of≥1:40 (95% CI)		0.96 (0.86, 1.08)	
		Comparison groups		**aH5N1c Lot #1 vs. aH5N1c Lot #3**	

		Comparison of≥1:40 (95% CI)		0.97 (0.87, 1.09)	
Notes	Lot-to-lot consistency of the 3 consecutively produced lots of aH5N1c vaccine was achieved. Three weeks after the 2nd vaccination subjects in both age groups in the aH5N1c treatment group (7.5 µg HA + 0.25 mL MF59) met the CBER criterion for HI ≥ 1:40, i.e. lower bound of 95% CI ≥70% (indicated in **bold** in table).				
Analysis description	**Secondary analysis as pre-specified**				
Analysis population and time point description	Per Protocol set Day 1, Day 22, Day 43 and Day 387				
Descriptive statistics and estimate variability	Treatment group	**Full Dose (pooled lots)**		**Placebo**	
	Age group	18 to <65	≥65 years	18 to <65	≥65 years
	Number of subjects	1115	1130	370	366
	HI seroconversion D22 (95% CI)	40.4 (37.6; 43.4)	24.2 (21.7; 26.8)	1.9 (0.8; 3.9)	0.3 (0.0; 1.5)
	HI ≥1:40 D22 (95% CI)	63.0 (60.1; 65.9)	55.6 (52.6; 58.5)	13.5 (10.2; 17.4)	16.4 (12.7; 20.6)
	HI GMR D22/D1 (95% CI)	3.81 (3.58; 4.05)	2.14 (2.02; 2.27)	0.87 (0.79; 0.97)	0.73 (0.66; 0.81)
	Number of subjects	1076	1080	349	351
	HI seroconversion D43 (95% CI)	79.9 (**77.4**; 82.3)	54.0 (**51.0**; 57.0)	0.3 (0.0; 1.6)	1.7 (0.6; 3.7)
	HI ≥1:40 D43 (95% CI)	93.1 (**91.4**; 94.6)	83.5 (**81.2**; 85.7)	10.9 (7.8; 14.6)	22.8 (18.5; 27.5)
	HI GMR D43/D1 (95% CI)	12.70 (11.94;13.51)	4.90 (4.61; 5.20)	0.82 (0.73; 0.91)	0.83 (0.75; 0.92)
	Number of subjects	1025	1054	341	346
	HI seroconversion D183 (95% CI)	16.2 (14.0; 18.6)	8.0 (6.4; 9.8)	0.3 (0.0; 1.6)	1.2 (0.3; 2.9)
	HI ≥1:40 D183 (95% CI)	34.2 (31.3; 37.2)	30.9 (28.1; 33.8)	2.1 (0.8; 4.2)	8.4 (5.7; 11.8)
	HI GMR D183/D1 (95% CI)	1.53 (1.44; 1.61)	0.97 (0.91; 1.02)	0.51 (0.46; 0.56)	0.43 (0.39; 0.47)
	Treatment group	**Full Dose (pooled lots)**		**Placebo**	
	Age group	18 to <60	≥60 years	18 to <60	≥60 years
	Number of subjects	979	1269	331	408
	HI seroconversion D22 (95% CI)	***42.2*** (39.1, 45.4)	24.6 (22.2, 27.0)	1.5 (0.5, 3.5)	0.7 (0.2, 2.1)

	HI ≥1:40 D22 (95% CI)	63.2 (60.1, 66.3)	56.2 (53.5, 59.0)	12.2 (8.8, 16.2)	17.2 (13.7, 21.2)
	HI GMR D22/D1 (95% CI)	***3.92*** (3.67, 4.18)	***2.22*** (2.10, 2.35)	0.85 (0.76, 0.96)	0.75 (0.68, 0.82)
	Treatment group	**Full Dose (pooled lots)**		**Placebo**	
	Number of subjects	947	1209	309	391
	HI seroconversion D43 (95% CI)	***81.6*** (79.0, 84.0)	***55.4*** (52.6, 58.2)	0.3 (0.0, 1.8)	1.5 (0.6, 3.3)
	HI ≥1:40 D43 (95% CI)	***93.6*** (91.8, 95.0)	***84.2*** (82.0, 86.2)	10.0 (6.9, 13.9)	22.3 (18.2, 26.7)
	HI GMR D43/D1 (95% CI)	***13.44*** (12.59, 14.35)	***5.18*** (4.88, 5.49)	0.81 (0.72, 0.90)	0.83 (0.76, 0.92)
	Number of subjects	899	1180	302	385
	HI seroconversion D183 (95% CI)	17.0 (14.6, 19.6)	8.2 (6.7, 9.9)	0.3 (0.0, 1.8)	1.0 (0.3, 2.6)
	HI ≥1:40 D183 (95% CI)	34.3 (31.2, 37.5)	31.3 (28.6, 34.0)	2.3 (0.9, 4.7)	7.5 (5.1, 10.6)
	HI GMR D183/D1 (95% CI)	1.56 (1.47, 1.66)	0.99 (0.94, 1.05)	0.51 (0.46, 0.57)	0.44 (0.40, 0.48)
Notes	Three weeks after the second vaccination (Day 43), subjects in the aH5N1c (7.5 µg HA + 0.25 mL MF59) vaccine group met all three age appropriate CBER (**bold**) and CHMP criteria (***bold italic***), whereas none of the criteria were met in the placebo group. The other secondary immunogenicity endpoints as assessed by HI assay showed higher immune responses in the aH5N1c (7.5 µg HA + 0.25 mL MF59) full dose vaccine group at Day 22, and Day 387 as compared with placebo.				

2.6.5.3. Clinical studies in special populations

Study V89_11 (paediatric population)

Methods

Design:

This phase 2, randomised, controlled, observer-blind multicentre study was designed to evaluate immunogenicity, tolerability and safety of 2 intramuscular (IM) doses of either low dose or high dose aH5N1c (A/turkey/Turkey/1/2005 NIBRG-23 strain) in healthy subjects 6 months through 17 years of age. A total of approximately 666 subjects were to be randomised at a 1:1 ratio to receive 2 vaccinations 3 weeks apart of either study vaccine (high or low dose aH5N1c). Randomisation was stratified by site and age cohort (6 through 35 months, 3 through 8 years and 9 through 17 years).

Study participants

Individuals 6 months through 17 years of age, in good health as determined by medical history, physical assessment and clinical judgment of the investigator and who have not received influenza vaccine within 60 days prior to study enrolment. The subject's parent(s)/legal guardian(s) was required to be able to understand and comply with all study procedures.

The exclusion criteria in the paediatric phase 2 trial were more extensive than in the adult phase 3 study. In addition to for example known or suspected impairment/alteration of the immune system, children with any serious chronic or progressive disease according to judgment of the investigator were also excluded. These include potential risk factors for severe influenza complications like severe asthma, autoimmune disease, or diabetes mellitus type I.

Treatments

Investigational vaccine: MF59 adjuvanted cell-culture derived subunit inactivated monovalent, A/turkey/Turkey/1/2005 (H5N1) NIBRG-23 strain2 vaccine formulated as 7.5 µg HA of H5N1 with 0.25 mL MF59 for a total of 0.5 mL extractable volume in prefilled syringe (PFS). This 0.5 mL extractable volume was the high dose vaccine. The low dose aH5N1c consisted of approximately 50% of all vaccine components of the high dose aH5N1c vaccine and was delivered in 0.25 mL volume. The PFS included a ring mark to indicate the volume for administration of the low dose (3.75 µg HA of H5N1 with 0.125 mL MF59 for a total injection volume of 0.25 mL) and was ready for use for IM administration, preferably in the nondominant arm or in the anterolateral thigh as necessary for younger children.

Blood was drawn from each subject for immunogenicity assessments before each vaccination (day 1 and day 22) and also drawn at clinic visits on day 43 and day 387. Sera were evaluated for antibody responses as measured by HI antibody responses and also MN antibody responses.

Objectives

Primary Immunogenicity Objective: To select the vaccine (low dose or high dose aH5N1c) to be tested in phase 3 based on achievement of Centre for Biologics Evaluation and Research (CBER) criteria 3 weeks after the second vaccine administration as measured by strain-specific hemagglutination inhibition (HI) assays.

Secondary Immunogenicity Objectives:

- For each aH5N1c vaccine (low dose or high dose), to evaluate achievement of all Committee for Medicinal Products for Human Use (CHMP) criteria 3 weeks after the second vaccine administration as measured by strain-specific HI assay.
- For each aH5N1c vaccine (low dose or high dose), to evaluate achievement of CBER and CHMP criteria 3 weeks after the first vaccine administration as measured by strain-specific HI assay.
- To evaluate the immunogenicity of each aH5N1c vaccine (low dose or high dose) 12 months after the primary 2-dose course with respect to CBER and CHMP criteria, as measured by strain-specific HI assays.

Exploratory Objectives:

Exploratory objectives were to be analysed if the corresponding assays are available:

- For each aH5N1c vaccine (low dose or high dose), to evaluate the antibody responses against heterologous influenza strain(s) as measured by HI assay.
- For each aH5N1c vaccine (low dose or high dose), to evaluate the antibody responses against heterologous and homologous influenza strain(s) as measured by microneutralisation (MN) assay.

Outcomes/endpoints

Primary Endpoints

The primary measures of immunogenicity, as determined by the HI assays, against the H5N1 homologous strain, included the following:

- Percentage of subjects achieving seroconversion on day 43.
- Percentage of subjects with a HI ≥1:40 on day 43.

The vaccines immunogenicity was evaluated following measurements according to the current CBER criteria for the adult population (Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines) of May 2007):

- The lower bound of the adjusted 2-sided 95% confidence interval (CI) for the percentage of subjects achieving seroconversion for HI antibody should meet or exceed 40%.
- The lower bound of the adjusted 2-sided 95% CI for the percentage of subjects achieving an HI antibody ≥1:40 should meet or exceed 70%.

All criteria as assessed at day 43 were to be met to fulfil regulatory requirements.

Secondary Endpoints

The secondary measures of immunogenicity, as determined by the HI assays, against the H5N1 homologous strain, included the following:

- Geometric mean HI titre (GMT) on day 1, day 22, day 43 and day 387.
- Day 22/day 1, day 43/day 1 and day 387/day 1 geometric mean ratio (GMR) of HI.
- Percentage of subjects achieving seroconversion on day 22, day 43 and day 387.
- Percentage of subjects with a HI ≥1:40 on day 1, day 22 and day 43 and day 387.

Immunogenicity, as determined by HI, was also assessed according to CHMP criteria (CPMP/BWP/214/96). As there are no established CHMP criteria for evaluation of immunogenicity in paediatric populations, the criteria defined for adult populations were used.

- The percentage of subjects with seroconversion or significant increase in HI antibody is >40%;
- The GMR is >2.5;
- The percentage of subjects achieving an HI ≥ 1:40 is >70%.

All 3 criteria (seroconversion1/significant increase, HI ≥ 1:40, and GMR) were to be fulfilled to establish immunogenicity and were assessed for day 43.

Exploratory Endpoints

The same measures of immunogenicity, as determined by the HI assay, were also applied to heterologous strain(s). MN as well as additional tests to further characterise the immune response was to be performed for exploratory purposes.

Sample size

Regarding the hypothesis on the proportion of subjects with HI ≥1:40, a one group χ^2 test with a 2.5% 2-sided significance level was calculated to have 84% power to detect the difference between the null hypothesis proportion, π_0, of 70% and the alternative proportion, π_A, of 84% when the sample size was 100 evaluable children per vaccination group and age cohort. With this sample size the power to

achieve the criterion on seroconversion with an assumed proportion of at least 84% (and a null hypothesis proportion of 40%), was calculated to be > 99%. This led to a calculated overall power of approximately 83%. In total, 600 evaluable children were needed (100 per vaccination group and age cohort), i.e., approximately 666 enrolled Subjects (111 per group and age cohort) assuming a drop-out rate of 10%.

Randomisation and blinding (masking)

Enrolled subjects were randomly assigned to the study groups (low dose or high dose aH5N1c) in a pre-specified ratio of 1:1 by web-based randomisation. The list of randomisation assignments was produced by a validated system used by the Novartis Vaccines and Diagnostics Biostatistics and Clinical Data Management department. At randomisation, and the subject was automatically assigned a unique Subject ID. Randomisation was stratified by site and equally by age group (Cohort 1: 6 to 35 months, Cohort 2: 3 to 8 years and Cohort 3: 9 to 17 years).

The trial was designed as an observer-blind study. During the treatment phase of the study (day 1 through day 43), designated unblinded nurse(s) or physician(s) were responsible for administering the study vaccines to the subjects. They were instructed not to reveal the identity of the study vaccines either to the subject and/or parents/legal guardian(s) or the investigative site personnel involved in the monitoring of conduct of the trial, except in an emergency up until completion of the trial and final data review. The designated unblinded nurse(s) or physician(s) were not to take part in evaluating the subject(s) for safety or collect study data after the vaccinations. If the study vaccine allocation was supplied to the investigator in the event of an emergency, the Novartis Vaccines and Diagnostics medical monitor or his/her designee (i.e., site monitor) was to be notified immediately by the investigator.

Primary safety and immunogenicity analysis was planned to be performed when Day 43 HI data were available. Analysis was to be done on group unblinded data by the Sponsor, i.e. the unblinding was to be restricted to only selected individuals of the Sponsor who had been entitled to get access to the randomisation for the purpose of preparing the day 43 analysis.

Statistical methods

Analysis sets

All Enrolled Set: All screened subjects who provided informed consent and provide demographic and/or other baseline screening measurements, regardless of the subject's randomisation and treatment status in the trial.

Exposed Set: All subjects in the All Enrolled Set who received a study vaccination.

Full Analysis Set (FAS) Immunogenicity

All subjects in the All Enrolled Set who:

- Received at least one study vaccination and provided immunogenicity data at Day 1 (baseline) and Day 43 (FAS Day 43) – primary;
- Received at least one study vaccination and provided immunogenicity data at Day 1 (baseline) and Day 22 (FAS Day 22) – secondary;
- Received at least one study vaccination and provided immunogenicity data at Day 1 (baseline) and Day 387 (FAS Day 387) - secondary.

FAS was analysed "as randomised" (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).

Per Protocol Set (PPS), Immunogenicity

All subjects in the FAS Immunogenicity who:

- Correctly received the vaccine to which the subjects is randomised and at the scheduled time points.
- Had no major protocol deviations leading to exclusion as defined prior to unblinding/analysis.
- Were not excluded due to other reasons defined prior to unblinding or analysis.

Three Per Protocol Sets – at Day 22, 43 and 387 – were used.

Safety Set

Safety Set (solicited AEs): All subjects in the Exposed Set with solicited (local/systemic) AE data.

Safety Set (Unsolicited AEs): All subjects in the Exposed Set with unsolicited AE data.

Safety Set (Overall): All subjects in the Exposed Set who had either postvaccination or solicited AE data.

Subjects were analysed according to the vaccine a subject received.

Subjects providing only 30 minutes postvaccination safety data were to be reported separately in a 30 minutes postvaccination safety analysis and excluded from all other safety analysis.

Analysis of Demographic and Baseline Characteristics

Descriptive statistics (mean, standard deviation, median, minimum and maximum) for age, height and weight at enrolment were calculated by overall and by vaccine group. Distributions of subjects by sex, ethnicity, race and previous influenza vaccination (in the past 12 months) were summarised overall and by vaccination group. The frequencies and percentages of subjects with medical history were to be presented overall and by vaccine group, age cohort, and country (or centre), when applicable.

Analysis of the immunogenicity endpoints

The primary immunogenicity endpoints were the seroconversion and an HI antibody titre ≥ 1:40 in subjects at Day 43. A high and a low dose was tested and a dose was deemed successful/protective if for both endpoints the CBER criteria were fulfilled.

For the following, group L denotes the vaccination group receiving low dose aH5N1c vaccine and group H the one receiving high dose aH5N1c vaccine and SC denotes seroconversion. Assuming that Y_{ik}, i= L, H; k = 1,....n, n denotes number of subjects; are identical and independent Bernoulli-distributed random variables with π_i representing the unknown seroconversion proportion and with τ_i the unknown proportion of subjects with a HI ≥1:40 in group i.

CBER requirements can be translated into following hypotheses:

$H_{0i}^{(SC)}: \pi_i - \pi_0 \leq 0$ vs. $H_{1i}^{(SC)}: \pi_i - \pi_0 > 0$

$H_{0i}^{(titer \geq 1:40)}: \tau_i - \tau_0 \leq 0$ vs. $H_{1i}^{(titer \geq 1:40)}: \tau_i - \tau_0 > 0$

π_0 denotes the threshold for seroconversion (π_0 = 0.4) and τ_0 the threshold for proportion of subjects with a HI ≥1:40 (τ_0 = 0.7).

The primary analysis population for testing the null hypotheses below was the FAS and in terms of robustness, the analysis of the primary objectives was repeated in the PPS. All other analyses were performed in the FAS and additionally in the PPS if there was a >10% difference between the FAS and the PPS.

Proportions of subjects with seroconversion and with HI titre ≥1:40 were calculated unadjusted, and corresponding 2-sided Clopper-Pearson confidence intervals were computed.

All statistical analyses for HI concerning secondary endpoints were to be performed on the logarithmically (base 10) transformed values. Individual HI titres below detection limit were set to half that limit.

For immunogenicity endpoints regarding CBER analyses, the log10-transformed antibodies were to be modelled using ANCOVA with a factor for dose group, age cohort, baseline titre and centre. Additionally as secondary analysis the log10-transformed antibodies were to be modelled by vaccine group using ANOVA with a factor for age cohort, race, gender and centre. Geometric means, geometric mean ratios and pertaining 2-sided adjusted 95% confidence intervals were to be calculated based on these models. Summary statistics were to be completed by providing minimum, maximum and median titres for the different groups.

Subgroup analyses of the primary and secondary objectives were performed by baseline titre (HI titre ≤1:10 vs. >1:10, HI titre ≤1:40 vs. >1:40), by seasonal influenza vaccine status (subjects with and without seasonal influenza vaccine in last 12 months), by centre, by sex, by race, by ethnicity, by country and by age (6 to <36 months, 3 to <9 years, 9 to <18 years) based on the FAS.

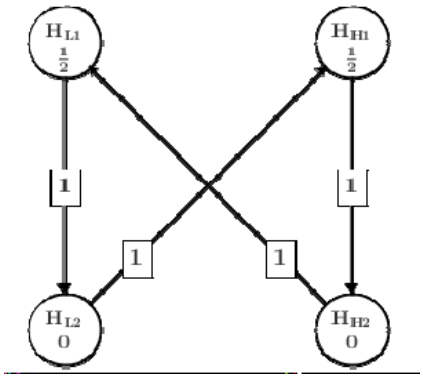
Missing value imputation

Missing immunogenicity values were considered 'missing completely at random' (MCAR) and where therefore not supposed to contain information that impacted the result of the analysis. Imputation methods were therefore not used.

Multiplicity correction in the primary analysis

For each of the two dose groups both null hypotheses associated with the primary objective regarding CBER had to be rejected simultaneously. Multiplicity has been accounted for by adjusting the significance levels of the statistical tests used for testing the hypotheses related to the primary objectives. The overall error rate has been fixed at a 1-sided alpha of 2.5%.

The graph below visualizes the initial state of the multiple test procedure to be used. The nodes H_{L1} and H_{L2} denote the first family of hypotheses containing the two hypotheses expressing CBER criteria for dose group L and H_{H1} and H_{H2} denote the family for dose group H respectively.



Initially the significance level for each family was set to ½ α, i.e. 2.5%. The two hypotheses within a family were to be tested according intersection-union test, i.e. both hypotheses had to be rejected each tested by using the respective α of the family. As soon as all the hypotheses of one of the families could successfully be rejected, the α (2.5%) of the respective family could be transferred to the remaining family, which then could be tested on the full α of 5% according to weighted Bonferroni test procedure. The possibility to spend the alpha of one node to the other is visualised by the edges with weight 1. In the case that the hypotheses of one family could not be rejected, the alpha was spent and nothing could be transferred to the remaining family, which then had to be tested on 2.5%.

Interim analysis

Primary safety and immunogenicity analysis were to be performed when Day 43 HI data are available. Analysis were to be done on restricted group unblinded data by the Sponsor, i.e. the unblinding will be restricted to only selected individuals of the Sponsor who will get access to the randomisation for the purpose of preparing the Day 43 analysis. If both formulations achieve all CBER criteria, the formulation selected for the Phase III were to be the one with the lower antigen/adjuvant content and an acceptable safety profile. The final analysis was to be performed when all data up to the study end (Day 387) are available.

Results

Participant flow

A total of 662 subjects were enrolled and were randomised in 1:1 ratio (330 subjects in low dose group and 332 subjects in high dose group) to receive 2 vaccinations 3 weeks apart of either high or low dose aH5N1c. Out of total population enrolled, 99% subjects (329 subjects in each group) received study vaccine and 94% subjects completed the study. One subject in low dose group and 3 subjects in high dose group did not receive any study vaccine (Table 25).

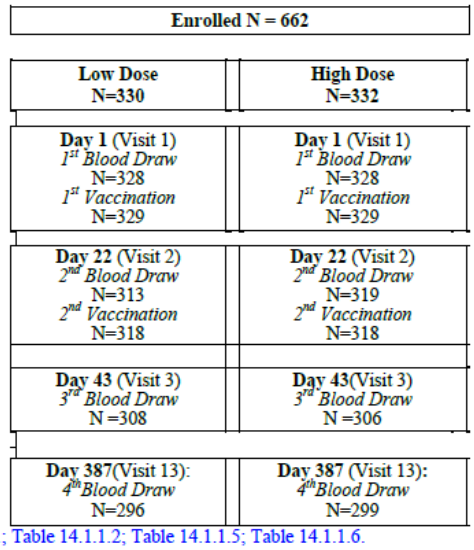
In total 40 (6%) subjects were prematurely withdrawn from the study, most often because they were lost to follow-up (15 subjects) or withdrew consent (11 subjects). One subject in the high dose group was prematurely withdrawn from the study due to an AE. One subject in the high dose group was excluded due to protocol deviation or violation (Table 25).

Table 25. Summary of Study Terminations – All Enrolled Set

Vaccine Groups	Low Dose N=330	High Dose N=332	Total N=662
Enrolled	330 (100%)	332 (100%)	662 (100%)
Exposed	329 (100%)	329 (99%)	658 (99%)
Completed study	307 (93%)	315 (95%)	622 (94%)
Premature withdrawals[a]	23 (7%)	17 (5%)	40 (6%)
Adverse event	0	1 (<1%)	1 (<1%)
Withdrew consent	8 (2%)	3 (<1%)	11 (2%)
Lost to follow-up	9 (3%)	6 (2%)	15 (2%)
Administrative reason	5 (2%)	2 (<1%)	7 (1%)
Protocol deviation/violation	0	1 (<1%)	1 (<1%)
Other	1 (<1%)	4 (1%)	5 (<1%)

Source: Table 14.1.1.2.
[a] primary reason.

Figure 7. Subject Completion Flowchart



Source: Table 14.1.1.1; Table 14.1.1.2; Table 14.1.1.5; Table 14.1.1.6.

Recruitment

Date of first enrolment: 31 Jan 2013

Date of last visit: 16 Jun 2014

Study Centres: Ten centres in the USA and 2 centres in Thailand.

It has to be noted that the majority of participants were recruited at the 2 sites in Thailand (country of enrolment: 73% Thailand, 27% USA).

Conduct of the study

Amendments: There were 5 protocol amendments and 4 of them were introduced prior to first enrolment. One major change was the removal of an initially planned booster dose at day 366 and the subsequent safety follow-up. Additional exclusion criteria (e.g., recent history of Guillain-Barré disease or individuals with any disorder in growth) and different exploratory/subgroup analyses were included.

Protocol deviations: In total, 26% subjects reported protocol deviations (25% subjects in low dose group and 28% subjects in high dose group) that led to exclusion of the subject or part of the subject's data from at least one analysis set. The majority of these subjects had missing serology data. An overview of subjects reporting protocol deviations is presented in Table 26. These exclusions did not concern all, but only certain visits.

Table 26. Number (%) of Subjects with Protocol Deviation(s)

Treatment Group	Low Dose N=330	High Dose N=332	Total N=662
Subjects with at least one protocol deviation:			
Protocol deviations	82 (25%)	92 (28%)	174 (26%)
Reasons:			
Acute illness prior to second vaccination	1 (<1%)	0	1 (<1%)
Blood sample could not be obtained, visit 1	0	1 (<1%)	1 (<1%)
Body temperature 38.0°C on visit 2, but 2nd vaccination given	1 (<1%)	0	1 (<1%)
Eligibility criteria not met	1 (<1%)	2 (<1%)	3 (<1%)
Exclusion criterion 21: Failure to thrive	0	1 (<1%)	1 (<1%)
History of latex allergy	0	1 (<1%)	1 (<1%)
No Solicited AE safety data	1 (<1%)	3 (<1%)	4 (<1%)
No unsolicited AE safety data	6 (2%)	6 (2%)	12 (2%)
No vaccination, injection 1 & 2	1 (<1%)	3 (<1%)	4 (<1%)
No vaccination, injection 2	11 (3%)	11 (3%)	22 (3%)
Parental ICF signed by grandmother, not legal representative	0	1 (<1%)	1 (<1%)
Patient was randomized into wrong cohort due to wrong date of birth	0	2 (<1%)	2 (<1%)
Prohibited medications/vaccines	5 (2%)	3 (<1%)	8 (1%)
Randomized into wrong Age group	0	1 (<1%)	1 (<1%)
Severe reaction recorded, but 2nd vaccination given	0	1 (<1%)	1 (<1%)
Subject experienced severe reactions, but received 2nd vaccination	2 (<1%)	3 (<1%)	5 (<1%)
Subjects with missing serology result at visit 1	29 (9%)	25 (8%)	54 (8%)
Subjects with missing serology result at visit 1 – HI	29 (9%)	25 (8%)	54 (8%)
Subjects with missing serology result at visit 2	23 (7%)	29 (9%)	52 (8%)
Subjects with missing serology result at visit 2 – HI	23 (7%)	29 (9%)	52 (8%)
Subjects with missing serology result at visit 3	7 (2%)	7 (2%)	14 (2%)
Subjects with missing serology result at visit 3 – HI	7 (2%)	7 (2%)	14 (2%)
Subjects with missing serology result at visit 3 – MN	0	1 (<1%)	1 (<1%)
Subjects with missing serology result at visit 13	21 (6%)	24 (7%)	45 (7%)
Subjects with missing serology result at visit 13 – HI	21 (6%)	24 (7%)	45 (7%)
Subjects without blood draw at visit 2	9 (3%)	9 (3%)	18 (3%)
Subjects without blood draw at visit 2 – HI	9 (3%)	9 (3%)	18 (3%)
Subjects without blood draw at visit 3	12 (4%)	14 (4%)	26 (4%)
Subjects without blood draw at visit 3 – HI	12 (4%)	14 (4%)	26 (4%)
Subjects without blood draw at visit 13	28 (8%)	18 (5%)	46 (7%)
Subjects without blood draw at visit 13 – HI	28 (8%)	18 (5%)	46 (7%)
Took prednisolone before 2nd vaccination	0	1 (<1%)	1 (<1%)
Visit 1 vaccination not completely administered	0	1 (<1%)	1 (<1%)
Visit 2 Outside the window	7 (2%)	3 (<1%)	10 (2%)
Visit 3 Outside the window	7 (2%)	9 (3%)	16 (2%)
Visit 13 Outside the window	4 (1%)	10 (3%)	14 (2%)

Source: Table 14.1.1.8. Abbreviations: AE, adverse event; HI, hemagglutination inhibition; ICF, informed consent form; MN, microneutrilization.

Baseline data

The demographic and other baseline characteristics of subjects enrolled into the study were well balanced between the low dose and high dose groups. Majority of subjects included in the study were Asians (73%) followed by Whites (21%) and the average age of the subjects was 78.4±55.7 months. Out of the total enrolled subjects, 125 (19%) subjects had received previous influenza vaccination. Among the subjects who received previous influenza vaccination, 70 subjects received vaccine within last 12 months of study initiation (Table 27).

Table 27. Demography and Other Baseline Characteristics – All Enrolled Set

Treatment Group	Low Dose N=330	High Dose N=332	Total N=662
Age (Months): Mean±Std	78.1±55.6	78.7±55.9	78.4±55.7
Sex:			
Male	166 (50%)	180 (54%)	346 (52%)
Female	164 (50%)	152 (46%)	316 (48%)
Race[1]:			
American Indian or Alaska native	0	2 (<1%)	2 (<1%)
Asian	240 (73%)	240 (72%)	480 (73%)
Black or African American	18 (5%)	13 (4%)	31 (5%)
Other	6 (2%)	5 (2%)	11 (2%)
White	66 (20%)	72 (22%)	138 (21%)
Ethnic Origin:			
Hispanic or Latino	15 (5%)	13 (4%)	28 (4%)
Not Hispanic or Latino	314 (95%)	319 (96%)	633 (96%)
Weight (kg): Mean±Std	26.07±17.60	26.09±17.29	26.08±17.43
Height (cm): Mean±Std	115.7±29.6	115.8±30.1	115.8±29.9
BMI (kg/m^2): Mean±Std	17.4±3.6	17.4±3.4	17.4±3.5
Previous influenza vaccination?			
No	268 (81%)	269 (81%)	537 (81%)
Yes	62 (19%)	63 (19%)	125 (19%)
Previous vaccine within last (12 months)			
No	9 (3%)	10 (3%)	19 (3%)
Yes	38 (12%)	32 (10%)	70 (11%)
Met eligibility criteria			
No	1 (<1%)	2 (<1%)	3 (<1%)
Yes	329 (100%)	330 (99%)	659 (100%)

Source: Table 14.1.1.3.
Categorical parameters: N (%), non-categorical parameters: Mean±Std.
Abbreviations: Std., standard deviation; BMI, body mass index.
Note: As treated: according to the vaccine a subject receives, rather than the vaccine to which the subject is randomized.

Numbers analysed

The FAS was the primary analysis population for immunogenicity, and the same primary analysis was repeated for PPS to check robustness of the immunogenicity results.

FAS day 43 was considered to analyse the primary objectives of the study, which included 87% of subjects from the enrolled set who received at least one study vaccination and provided immunogenicity data at day 1 (baseline) and day 43 (Table 28).

Table 28. Overview of Datasets Analysed for Immunogenicity – As Randomised

Vaccine Group	Low Dose (N=330)	High Dose (N=332)
Enrolled Dataset:	330 (100%)	332 (100%)
Full Analysis Set (FAS)		
Day 1	300 (91%)	304 (92%)
Day 22 (Secondary)	287 (87%)	283 (85%)
Day 43 (Primary)	288 (87%)	289 (87%)
Day 387 (Secondary)	271 (82%)	271 (82%)
Per Protocol Set (PPS)		
Day 1	**299** (91%)	**302 (91%)**
Day 22	272 (82%)	270 (81%)
Day 43	269 (82%)	270 (81%)
Day 387	251 (76%)	248 (75%)

Source: Table 14.1.1.1 issued on 14 AUG 2015.
Abbreviations: FAS, full analysis set; PPS, per protocol set.
Bold: Changes from results presented in Table 1 above.

Of note, the numbers analysed at the different time points deviate based on availability of immunogenicity on the relevant day (HI titre ≥1:40), or on the relevant day AND at baseline (seroconversion, GMR). Therefore, the N values in the result tables below do slightly differ between study visits and type of analysis.

Outcomes and estimation

Percentage of Subjects with Seroconversion: After 3 weeks of first vaccination (day 22), 38% of subjects in low dose group and 52% of subjects in high dose group achieved seroconversion against the homologous strain (Influenza A/H5N1/Turkey/2005 CC Ab). On day 43, the percentage of subjects achieving seroconversion had increased to 86% in low dose group and 96% in high dose group. However, 12 months following the second vaccination (day 387), the percentages decreased to 31% in the low dose group and 47% in the high dose group. At all timepoints, higher percentages of subjects achieved seroconversion in high dose group than low dose group (Table 29).

Table 29.

Percentage of Subjects (97.5% CI) with Seroconversion[a] Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) – HI Assay – FAS

	Low Dose	High Dose
	N=288	N=281
Day 22	38% (31%-44%) N=287	52% (45%-58%)
Day 43	86% (81%-90%)	96% (93%-98%) N=279
Day 387	31% (25%-38%) N=271	47% (40%-54%) N=264

Source: Table 14.2.1.2.
Abbreviations: CI, confidence interval, HI, hemagglutination inhibition, FAS, full analysis set.
[a] Seroconversion is defined as the percentage of subjects with either a prevaccination HI < 1:10 and a postvaccination HI ≥ 1:40 or a prevaccination HI ≥ 1:10 and a minimum four-fold rise in postvaccination HI antibody titer.

Percentage of Subjects with HI ≥ 1:40: At baseline no or negligible percentages of subjects in the low and high dose groups had HI ≥1:40 against homologous strain (Influenza A/H5N1/Turkey/2005 CC Ab). On day 22, 3 weeks after the first vaccination, 38% of subjects in the low dose group and 51% of

subjects in the high dose group achieved HI ≥1:40. There was a marked increase in immune response after second vaccination on day 43, with 86% of subjects in the low dose group and 96% of subjects in the high dose group achieving an HI ≥ 1:40. Following 12 months after second vaccination on day 387, 31% of subjects in the low dose group and 47% of subjects in the high dose group achieved an HI ≥ 1:40. In comparison to the low dose group, higher percentages of subjects achieved an HI ≥ 1:40 in the high dose group at all time- points (Table 30).

Table 30.

Percentage of Subjects (97.5% CI) with HI ≥ 1:40 Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) – HI Assay – FAS

Vaccine Group	Low Dose	High Dose
	N=300	N=294
Day 1	0% (0.0042%-2%)	1% (0%-3%)
Day 22	38% (32%-45%) N=287	51% (44%-58%) N=283
Day 43	86% (81%-90%) N=288	96% (92%-98%) N=287
Day 387	31% (25%-38%) N=271	47% (40%-54%) N=271

Source: Table 14.2.1.1.
Abbreviations: CI, confidence interval; HI, hemagglutination inhibition; FAS, full analysis set.

GMTs and GMRs: Baseline titres against Influenza A/ H5N1/ Turkey/2005 CC Ab among the vaccine groups bordered the limit of detection for the HI serology assay (titres below the limit of detection of the HI assay (< 1:10) were imputed as 5). On day 22, 3 weeks after the first vaccination, GMRs increased over baseline in both low dose (6.63-fold) and high dose (13-fold) groups. Three weeks after the second vaccination (day 43), the magnitude of increase from day 1 was higher in high dose group (262-fold) than low dose group (84-fold; Table 11.4.1-3). On day 387, 12 months after the second vaccination, there was a decline in GMTs, which remained elevated over baseline in both low dose (5.62-fold) and high dose (12-fold) groups (Table 31).

Table 31.

Geometric Mean HI Titers and Geometric Mean Ratios (97.5% CI) Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) – HI Assay – FAS

Vaccine Group	Low Dose	High Dose
	N= 300	N = 294
Day 1	5.15 (4.91-5.39)	5.23 (5-5.48)
Day 22	34 (24-48) N=287	64 (46-90) N=283
Day 22/Day 1	6.63 (4.71-9.34) N=287	13 (9-18) N=281
Day 43	431 (312-595) N=288	1356 (985-1866) N=287
Day 43/Day 1	84 (61-116) N=288	262 (190-361) N=279
Day 387	29 (21-40) N=271	62 (45-86) N=271
Day 387/Day 1	5.62 (4.05-7.81) N=271	12 (8.76-17) N=264

Source: Table 14.2.1.3.
Abbreviations: CI, confidence interval; HI, hemagglutination inhibition; FAS, full analysis set.

Primary Objective

To select the vaccine (low dose or high dose aH5N1c) to be tested in phase 3 based on achievement of CBER criteria 3 weeks after the second vaccine administration as measured by strain-specific HI assays. The primary population used for testing study hypotheses was FAS; nevertheless the immunogenicity analysis was also repeated by PPS which demonstrated results similar to FAS for all study endpoints. For homologous strain (Influenza A/H5N1/Turkey/2005 CC Ab), both low dose and high dose groups satisfied the CBER criteria of seroconversion and HI ≥ 1:40 (Table 32).

Table 32.

Overview of CBER Criteria Achievement on Day 43 for Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) – FAS - HI Assay

Vaccine Group	Low Dose	High Dose
	N=288	N=287
Lower bound of 97.5% CI for percentage of subjects achieving seroconversion[a] ≥ 40%	+	+ N=279
Lower bound of 97.5% CI for percentage of subjects achieving HI ≥ 1:40 ≥ 70%	+	+

Source: Table 14.2.1.1; Table 14.2.1.2.
Abbreviations: CBER, Committee for Biologics Evaluation and Research; CI, confidence interval, HI, hemagglutination inhibition; FAS, full analysis set.
[a] Seroconversion is defined as the percentage of subjects with either a prevaccination HI < 1:10 and a postvaccination HI ≥ 1:40 or a prevaccination HI ≥ 1:10 and a minimum 4-fold rise in postvaccination HI antibody titer.

Secondary Objectives

First secondary objective: For each aH5N1c vaccine (low dose or high dose), to evaluate achievement of all CHMP criteria 3 weeks after the second vaccine administration as measured by strain-specific HI assay. Three weeks following the second vaccination (day 43), all 3 CHMP criteria were met in the both low dose and high dose groups (

Table **33**).

Table 33.

Overview of CHMP Criteria Achievement on Day 43 for Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) – FAS - HI Assay

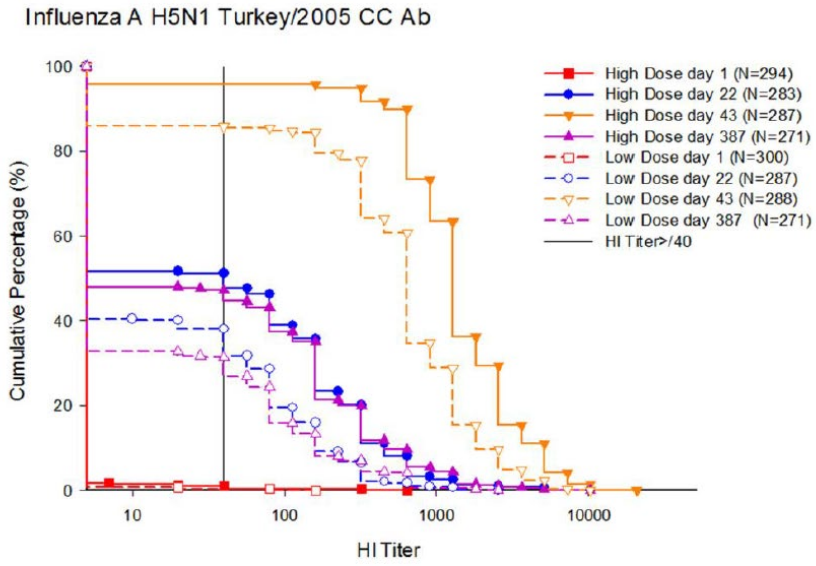
Vaccine Group	Low Dose	High Dose
	N = 288	N = 287
Percentage of subjects with seroconversion[a] is > 40%	+	+ N=279
GMR > 2.5	+	+ N=279
Percentage of subjects achieving an HI ≥ 1:40 is > 70%	+	+

Source: Table 14.2.1.1; Table 14.2.1.2; Table 14.2.1.3.
Abbreviations: CHMP, Committee for Medicinal Products for Human Use; HI, hemagglutination inhibition; FAS, full analysis set; GMR, geometric mean ratio.
[a] Seroconversion is defined as the percentage of subjects with either a prevaccination HI titer < 1:10 and a postvaccination HI ≥ 1:40 or a prevaccination HI ≥ 1:10 and a minimum 4-fold rise in postvaccination HI antibody titer (also referred to as seroconversion or significant increase).

RCDF curves of HI titres for low and high doses against the Influenza A H5N1 Turkey/2005 CC Ab strain at baseline (day 1), 3 weeks following the first vaccination (day 22), 3 weeks following the second vaccination (day 43) and 12 months following the second vaccination (day 387) are presented in Figure 8. The Figure illustrates that the immune response on day 43, i.e., 3 weeks after the second vaccination was remarkably higher than baseline in both low and high dose groups. The cumulative frequency curves of day 22 GMTs and day 387 GMTs overlapped in both the dose groups depicting the fall in GMTs on day 387, after 12 months following the second vaccination to approximate day 22 GMTs i.e., post first vaccination levels.

Figure 8.

Reverse Cumulative Distribution Frequency of HI Titers Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) at Day 1, Day 22, Day 43 and Day 387 – HI Assay – FAS



Source: Figure 14.2.3.3.9, Figure 14.2.3.3.10, Figure 14.2.3.3.13.

HI titres by paediatric subgroups (full dose vs. half dose)

Geometric Mean Titre (GMT), Geometric Mean Ratio (GMR) and Percentage of Subjects with HI titre ≥1:40 and achieving Seroconversion (97.5% CI) Against Homologous Strain at Day 1, 22, 43 and 387 by Age - Study V89_11– Full Analysis Set

Age Group	6–36 Months		3–9 Years		9–18 Years	
Treatment Group	**Full dose**	**Half dose**	**Full dose**	**Half dose**	**Full dose**	**Half dose**
Day 1	**N=93**	**N=90**	**N=98**	**N=101**	**N=103**	**N=109**
GMT (97.5% CI)*	5.66 (5.2, 6.15)	5.39 (4.94, 5.88)	5 (5, 5)	5 (5, 5)	5.1 (4.6, 5.66)	5.08 (4.6, 5.61)
% HI titre ≥1:40 (97.5% CI)	1% (0, 7)	0% (0, 5)	0% (0, 4)	0% (0, 4)	2% (0, 8)	1% (0, 6)
Day 22	**N=85**	**N=86**	**N=96**	**N=98**	**N=102**	**N=103**
GMT (97.5% CI)*	58.73 (33, 104.5)	23.01 (12.97, 40.83)	49.03 (27.7, 86.79)	41.11 (22.43, 75.35)	79.72 (42.02, 151.21)	35.04 (18.86, 65.08
Day 22/1 GMR (97.5% CI)	11.79 (6.78, 20.48)	4.56 (2.59, 8.02	9.81 (5.54, 17.36)	8.22 (4.49, 15.07)	15.33 (8.1, 29.01)	6.74 (3.64, 12.49)
% HI titre ≥1:40 (97.5% CI)	58% (45, 70)	36% (25, 49)	43% (31, 55)	41% (30, 53)	54% (42, 65)	37% (26, 48)
% seroconversion (97.5% CI)	58% (**46**, 70)	36% (25, 49)	43% (32, 55)	41% (30, 53)	54% (**42**, 65)	37% (26, 47)
Day 43	**N=91**	**N=85**	**N=94**	**N=98**	**N=102**	**N=105**
GMT (97.5% CI)*	1842.09 (1064.9, 3186.49)	673.53 (397.9, 1140.09)	1244 (714.19, 2166.84)	362.7 (202.67, 649.11)	961.14 (501.39, 1842.46)	300.12 (159.28, 565.52)
Day 43/ 1 GMR (97.5% CI)	302.39 (179.94, 508.17)	115.71 (69.31, 193.16)	248.8 (142.84, 433.37)	72.54 (40.53, 129.82)	186.11 (97.13, 356.61)	58.24 (30.93, 109.67)
% HI titre ≥1:40 (97.5% CI)	98% (**91**, 100)	94% (**86**, 98)	98% (**92**, 100)	86% (**76**, 93)	92% (**84**, 97)	79% (69, 87)
% seroconversion (97.5% CI)	99% (**93**, 100)	94% (**86**, 98)	98% (**92**, 100)	86% (**76**, 93)	92% (**84**, 97)	79% (**69**, 87)
Day 387	**N=84**	**N=77**	**N=90**	**N=94**	**N=97**	**N=100**
GMT (97.5% CI)*	258.08 (113.05, 589.14)	93.13 (42.02, 206.4)	57.29 (34.31, 95.65)	23.1 (13.66, 39.05)	21.18 (13.64, 32.9)	13.82 (9.06, 21.09)
Day 387/1 GMR (97.5% CI)	51.62 (22.61, 117.83)	18.63 (8.4, 41.28)	11.46 (6.86, 19.13)	4.62 (2.73, 7.81	4.05 (2.61, 6.28)	2.64 (1.73, 4.02)
% HI titre ≥1:40 (97.5% CI)	71% (59, 82)	61% (48, 73)	44% (33, 57)	22% (13, 34)	29% (19, 40)	17 % (9, 27)
% seroconversion (97.5% CI)	73% (**60**, 84)	61% (**48**, 73)	45% (33, 57)	22% (13, 34)	29% (19, 40)	16 % (9, 26)

Source: V89_11 Tables: Table 14.2.1.1c; Table 14.2.1.2c; Table 14.2.1.3c
Bold: CBER criteria met

There were no notable differences in immunogenicity by sex or race.

Study V89_13 (population ≥65 years of age)

Methods

Design

This was a phase 2, randomised, observer-blind, multicentre study to evaluate the immunogenicity and safety of 2 formulations (high dose and low dose) of the aH5N1c vaccine (A/turkey/Turkey/1/2005 NIBRG-23 strain) in a healthy elderly population, ≥ 65 years of age. Approximately 1388 subjects were planned to be randomised at a 1:1 ratio, stratified by site, to receive either low dose or high dose aH5N1c vaccine.

Study participants

Individual' s ≥ 65 years of age, in good health as determined by medical history, physical assessment and clinical judgment of the investigator had never received prior H5N1 influenza vaccine and had not received seasonal influenza vaccine within 60 days prior to study enrolment. The subjects were required to be mentally competent, able to understand and comply with all study procedures, to be contacted and available for study visits.

Frail patients were not studied. As described above in more detail for the phase 3 trial, there was a relatively extensive list of exclusion criteria for this phase 2 study (not listed in this AR). Some important exclusion criteria were serious chronic or progressive disease (e.g., diabetes mellitus type 1, severe asthma, COPD grade 3 and 4), known or suspected impairment/alteration of immune function, medically significant cancer, or a history of Guillain-Barré syndrome. The purpose of this phase 2 study was to evaluate an optimal dose (out of two options) for the general population ≥ 65 years of age.

Treatments

Investigational vaccine: MF59 adjuvanted cell-culture derived subunit inactivated monovalent, A/turkey/Turkey/1/2005 (H5N1) NIBRG-231 strain vaccine formulated as 7.5 µg haemagglutinin (HA) of H5N1 with 0.25 mL MF59 for a total of 0.5 mL extractable volume in prefilled syringe (PFS). The low dose aH5N1c consisted of approximately 50% of all vaccine components of the high dose aH5N1c vaccine and was delivered in 0.25 mL volume. The PFS included a ring mark to indicate the volume for administration of the low dose and was ready for use for intramuscular (IM) administration, preferably in the non-dominant arm.

Blood was drawn from each subject for immunogenicity assessments before each vaccination (day 1 and day 22) and also drawn at clinic visits on day 43 and day 387. Blood drawn was evaluated for antibody responses as measured by HI antibody responses and also MN antibody responses.

Objectives

Primary Immunogenicity Objective: To select the vaccine (low dose or high dose aH5N1c) to be tested in phase 3 based on achievement of CBER criteria 3 weeks after the second vaccine administration as measured by strain-specific HI assays.

Secondary Immunogenicity Objectives:

- For each aH5N1c vaccine (low dose or high dose), to evaluate achievement of all Committee for Medicinal Products for Human use (CHMP) criteria 3 weeks after the second vaccine administration as measured by strain-specific HI assay.
- For each aH5N1c vaccine (low dose or high dose), to evaluate achievement of CBER and CHMP criteria 3 weeks after the first vaccine administration as measured by strain-specific HI assay.

- To evaluate the immunogenicity of each aH5N1c vaccine (low dose or high dose) 12 months after the primary 2-dose course with respect to CBER and CHMP criteria, as measured by strain-specific HI assays.

Exploratory Objectives:

Exploratory objectives were to be analysed if the corresponding assays were available:

- For each aH5N1c vaccine (low dose or high dose), to evaluate the antibody responses against heterologous influenza strain(s) as measured by HI assay.
- For each aH5N1c vaccine (low dose or high dose), to evaluate the antibody responses against heterologous and homologous influenza strain(s) as measured by microneutralisation (MN) assay.

Outcomes/endpoints

Primary Endpoints

The primary measures of immunogenicity, as determined by the HI assays, against the H5N1 homologous strain, included the following:

- Percentage of subjects achieving seroconversion on day 43.
- Percentage of subjects with a HI $\geq$ 1:40 on day 43.

The vaccines immunogenicity was evaluated following measurements according to the current CBER criteria for the adult population aged 65 years or older (Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines) of May 2007):

- The lower bound of the 2-sided 95% confidence interval (CI) for the percentage of subjects achieving seroconversion for HI antibody should meet or exceed 30%;
- The lower bound of the 2-sided 95% CI for the percentage of subjects achieving an HI antibody $\geq$ 1:40 should meet or exceed 60%.

All criteria as assessed at day 43 were to be met to fulfil regulatory requirements.

Secondary Endpoints

The secondary measures of immunogenicity, as determined by the HI assays, against the H5N1 homologous strain, included the following:

- Geometric mean HI titre on day 1, day 22, day 43 and day 387. In absence of regulatory requirement, results collected at day 22 and day 387 were also evaluated against the CBER criteria.
- Day 22/day 1, day 43/day 1 and day 387/day 1 GMR of HI.
- Percentage of subjects achieving seroconversion on day 22, day 43 and day 387.
- Percentage of subjects with a HI $\geq$ 1:40 on day 1, day 22 and day 43 and day 387.

Immunogenicity, as determined by HI, was also assessed according to age-appropriate CHMP criteria (CPMP/BWP/214/96)

- The percentage of subjects with seroconversion or significant increase in HI antibody is > 30%;
- The GMR is > 2;
- The percentage of subjects achieving an HI $\geq$ 1:40 is > 60%.

All 3 criteria (seroconversion/significant increase, HI ≥ 1:40, and GMR) were to be fulfilled to establish immunogenicity and were assessed for day 43.

Exploratory Endpoints

The same measures of immunogenicity, as determined by the HI assay, were also applied to heterologous strain(s), if the assays were performed. Microneutralization, as well as additional tests to further characterise the immune response was to be performed for exploratory purposes, if available.

Sample size

Regarding the hypothesis on the proportion of Subjects with HI ≥ 1:40 a one group χ2 test with a 2.5% two-sided significance level would have 80% power to detect the difference between the null hypothesis proportion, π0 , of 60% and the alternative proportion, πA , of 66% when the sample size is 624 evaluable elderly per vaccination group. With this sample size the power to achieve the criterion on seroconversion with an assumed proportion of at least 50% (and a null hypothesis proportion of 30%) was 99%. This leads to an overall power of approximately 80%. In total, 1248 evaluable elderly subjects were needed (624 per vaccination group), i.e. approximately 1388 enrolled Subjects (694 per group) with assuming a dropout rate of 10%.

Randomisation and blinding (masking)

Randomisation and blinding were analogously performed like in Study V89_11 but randomisation was not stratified in V89_13.

Statistical methods

Analysis sets

The analysis sets, All Enrolled set, Exposed Set, FAS, PPS, and Safety Set were analogously defined to Study V89_11.

Analysis of Demographic and Baseline Characteristics

Descriptive statistics (mean, standard deviation, median, minimum and maximum) for age, height and weight at enrolment were calculated by overall and by vaccine group. Distributions of subjects by sex, ethnicity, race and previous influenza vaccination (in the past 12 months) were summarised overall and by vaccination group. The frequencies and percentages of subjects with medical history will be presented overall and by vaccine group, and country (or centre), when applicable.

Analysis of the immunogenicity endpoints

The primary immunogenicity endpoints were the seroconversion and an HI antibody titre ≥ 1:40 in subjects at Day 43. A high and a low dose was tested and a dose was deemed successful/protective if for both endpoints the CBER criteria were fulfilled.

For the following, group L denotes the vaccination group receiving low dose aH5N1c vaccine and group H the one receiving high dose aH5N1c vaccine and SC denotes seroconversion. Assuming that Y_{ik}, i= L, H; k = 1,....n, n denotes number of subjects; are identical and independent Bernoulli-distributed random variables with π_i representing the unknown seroconversion proportion and with τ_i the unknown proportion of subjects with a HI ≥1:40 in group i.

CBER requirements can be translated into following hypotheses:

$H_{0i}^{(SC)}: \pi_i - \pi_0 \leq 0$ vs. $H_{1i}^{(SC)}: \pi_i - \pi_0 > 0$

$H_{0i}^{(titer \geq 1:40)}: \tau_i - \tau_0 \leq 0$ vs. $H_{1i}^{(titer \geq 1:40)}: \tau_i - \tau_0 > 0$

π_0 denotes the threshold for seroconversion (π_0 = 0.3) and τ_0 the threshold for proportion of subjects with a HI ≥1:40 (τ_0 = 0.6).

The primary analysis population for immunogenicity was the FAS and in terms of robustness the analysis was repeated in the PPS. Secondary and exploratory analyses were performed on the FAS and repeated on the PPS if these sets differ by more than 10% regarding the size.

Proportions of subjects with seroconversion and with HI titre ≥1:40 were calculated unadjusted, and corresponding 2-sided Clopper-Pearson confidence intervals were computed.

All statistical analyses for HI concerning secondary endpoints were to be performed on the logarithmically (base 10) transformed values. Individual HI titres below detection limit were set to half that limit.

For immunogenicity endpoints regarding CBER analyses, the log10-transformed antibodies were to be modelled using ANCOVA with a factor for dose group, baseline titre and centre. Additionally as secondary analysis the log10-transformed antibodies were modelled by vaccine group using ANOVA with a factor for race, gender and centre. Geometric means, geometric mean ratios and pertaining 2-sided adjusted 95% confidence intervals were calculated based on these models. Summary statistics will be completed by providing minimum, maximum and median titres for the different groups.

Subgroup analyses of the primary and key secondary objectives were performed by baseline titre (HI ≤1:10 vs. >1:10, HI ≤1:40 vs. >1:40), by seasonal influenza vaccine status (subjects with and without seasonal influenza vaccine in last 12 months), by centre, by sex, by race, by ethnicity and by country based on the FAS.

Missing value imputation and multiplicity correction in the primary analysis were analogously defined and executed like in Study V89_11.

Interim analysis

The interim analysis was analogously planned like Study V89_11.

Results

Participant flow

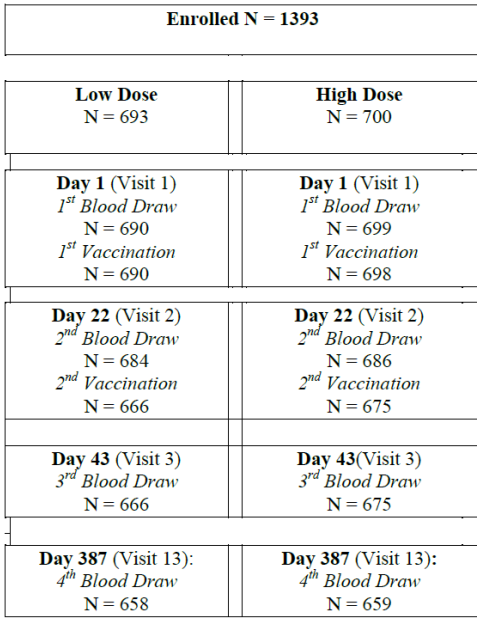
In total, 1393 subjects were enrolled into the study. The sample size of the subjects exposed into the 2 vaccine groups was balanced (690 and 698 subjects in low dose and high dose, respectively). In the low dose and high dose groups, 98% and 97% of subjects completed study, respectively (Table 34). There were 2% of subjects in low dose group who withdrew prematurely and 3% of subjects in high dose group. One subject in the low dose group and 1 subject in the high dose group were withdrawn prematurely due to death.

Table 34. Summary of Study Terminations – Enrolled Dataset

Vaccine Group	Low Dose	High Dose	Total
	N = 693	N = 700	N = 1393
Enrolled	693 (100%)	700 (100%)	1393 (100%)
Exposed	690 (100%)	698 (100%)	1388 (100%)
Completed study	676 (98%)	676 (97%)	1352 (97%)
Premature withdrawals[a]	17 (2%)	24 (3%)	41 (3%)
Death	1 (< 1%)	1 (< 1%)	2 (< 1%)
Withdrew consent	5 (< 1%)	14 (2%)	19 (1%)
Lost to follow-up	3 (< 1%)	1 (< 1%)	4 (< 1%)
Administrative reason	5 (< 1%)	5 (< 1%)	10 (< 1%)
Protocol violation	1 (< 1%)	1 (< 1%)	2 (< 1%)
Other	2 (< 1%)	2 (< 1%)	4 (< 1%)

Source: Table 14.1.1.2.
[a]Primary reason.

Figure 9. Subject Disposition Flowchart



Source: Table 14.1.1.1, Table 14.1.1.5, Table 14.1.1.6.

Recruitment

Study Initiation Date: 14 Jan 2013 (first subject enrolled)

Study Completion Date: 30 Jun 2014 (last subject completed)

Date of the Report: 04 Mar 15

Study Centres: Four centres in Thailand, 5 centres in Australia, 2 centres in New Zealand and 12 centres in the USA.

Conduct of the study

Amendments: There were 5 protocol amendments (4 of them were introduced prior to first enrolment). One major change was the removal of an initially planned booster dose at day 366 and the subsequent safety follow-up. The exclusion criteria were amended (e.g., recent history of Guillain-Barré disease

instead of current disease) and different exploratory/subgroup analyses were included (by age group, by seasonal influenza vaccination status).

Protocol deviations: Overall, 314 subjects had reported protocol deviations. There was no substantial difference in the percentage of subjects reporting protocol deviations between the low dose and high dose groups (24% and 21% of subjects, respectively). The extensive table of protocol deviations is not shown in this report, but 6% of the participants had a protocol deviation due to receipt of a tetanus and diphtheria vaccine within 4 weeks of study vaccination, which was an exclusion criterion of the study. Other protocol deviations did concern single visits (e.g., visits outside of the specified window = most common deviation, missing blood draws at certain visits, both issues mostly at the Day 387 visit).

Baseline data

Demographic and other baseline characteristics were balanced between the 2 vaccine groups. The mean age of the subjects was 71 years in both vaccine groups. In total, 1393 subjects enrolled of which 98% of subjects were aged 65 years through 84 years and 2% of subjects aged ≥ 85 years). Most subjects were of white origin, 64% of subjects in each group (Table 35). Across the 2 vaccine groups, the most of the subjects had received previous influenza vaccination (60% of subjects in the low dose and 61% of subjects in the high dose groups).

Table 35. Demography and Other Baseline Characteristics – All Enrolled Set

Vaccine Group	Low Dose N = 693	High Dose N = 700	Total N = 1393
Age (Years)	70.7±4.7	71.2±5.1	71.0±4.9
Age Group:			
65 through 84 years	684 (99%)	687 (98%)	1371 (98%)
≥ 85 years	9 (1%)	13 (2%)	22 (2%)
Gender:			
Male	275 (40%)	293 (42%)	568 (41%)
Female	418 (60%)	407 (58%)	825 (59%)
Race:			
American Indian or Alaska Native	0	2 (< 1%)	2 (< 1%)
Asian	237 (34%)	240 (34%)	477 (34%)
Black or African American	10 (1%)	10 (1%)	20 (1%)
White	444 (64%)	445 (64%)	889 (64%)
Other	2 (< 1%)	3 (< 1%)	5 (< 1%)
Ethnicity:			
Hispanic or Latino	15 (2%)	17 (2%)	32 (2%)
Not Hispanic or Latino	678 (98%)	683 (98%)	1361 (98%)
Weight (kg)	71.42 ± 16.20	71.01 ± 15.91 N = 699	71.21 ± 16.05 N = 1392
Height (cm)	163.5 ± 10.9	164.1 ± 10.7 N = 699	163.8 ± 10.8 N = 1392
Body Mass Index: (kg/m²)	26.5 ± 4.2	26.1 ± 4.0 N = 699	26.3 ± 4.1 N = 1392
Previous Influenza Vaccination	419 (60%)	429 (61%)	848 (61%)
Met Entry Criteria	679 (98%)	689 (98%)	1368 (98%)

Source: Table 14.1.1.3.
Categorical parameters: number of subjects (%); non-categorical parameters: mean ± standard deviation.

Numbers analysed

An overview of subjects considered for immunogenicity evaluation is presented in Table 36. The primary analysis population for immunogenicity was FAS and in terms of robustness the analysis of primary objectives was repeated in the PPS. In total, 1393 subjects were enrolled into the study and the majority of the subjects were included in PPS and FAS. The most common reason for exclusion from the primary FAS analysis was that the blood draw at visit 3 was not done (3% of subjects in each group).

Table 36. Overview of Datasets Analysed for Immunogenicity – As Randomised

Vaccine Group	Low Dose N = 693	High Dose N = 700
Enrolled Dataset	693 (100%)	700 (100%)
FAS		
Day 22 (Secondary Objective)	673 (97%)	681 (97%)
Day 43 (Primary Objective)	664 (96%)	673 (96%)
Day 387 (Secondary Objective)	651 (94%)	658 (94%)
PPS		
Day 22	585 (84%)	605 (86%)
Day 43	569 (82%)	594 (85%)
Day 387	533 (77%)	554 (79%)

Source: Table 14.1.1.1.
Abbreviations: FAS, full analysis set; PPS, per protocol set.

Of note, the numbers analysed at the different time points deviate based on availability of immunogenicity on the relevant day (HI titre ≥1:40), or on the relevant day AND at baseline (seroconversion, GMR). Therefore, the N values in the result tables below do slightly differ between study visits and type of analysis.

Outcomes and estimation

Percentage of Subjects with Seroconversion: Three weeks after the first vaccination (day 22), a higher percentage of subjects in the high dose group (36%) showed seroconversion against Influenza A H5N1 Turkey/2005 CC Ab strain than in the low dose group (21%), and similar trends were observed following 3 weeks after second vaccination (day 43; Table 37). Twelve months after the second vaccination (day 387), there was a decrease in the percentage of subjects with seroconversion in both vaccine groups, but it was still higher in the high dose group (23%) than in the low dose group (10%).

Table 37.

Number (%) of Subjects (97.5% CI) with Seroconversion[a] Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) Strain – HI Assay – FAS

	Low Dose N = 673	High Dose N = 681
Day 22	144 (21%) (18%-25%)	245 (36%) (32%-40%)
Day 43	345 (52%) (48%-56%) N = 664	495 (74%) (70%-77%) N = 673
Day 387	64 (10%) (7%-13%) N = 651	154 (23%) (20%-27%) N = 658

Source: Table 14.2.1.2.
Abbreviation: CBER, Center for Biologics Evaluation and Research CI, confidence interval; FAS, full analysis set; HI, hemagglutination inhibition.
[a]Seroconversion according to CBER is defined as either a prevaccination HI < 1:10 and a postvaccination HI ≥ 1:40, or a prevaccination HI ≥ 1:10 and a minimum 4-fold rise in postvaccination HI antibody titer.

Percentage of Subjects with HI ≥ 1:40: Baseline titres among the vaccine groups were low (10% and 12% of subjects in low dose and high dose groups, respectively) against Influenza A H5N1 Turkey/2005 CC Ab strain. Three weeks following the first vaccination (day 22), there was an increase in the percentages of subjects achieving HI ≥ 1:40 over baseline in the low dose and high dose groups, including almost half of the subjects in the high dose group (49% of subjects). Similar trends were observed following 3 weeks after second vaccination (day 43; Table 38). Twelve months following the

second vaccination (day 387), there was a decrease in percentages of subjects achieving HI ≥ 1:40 in both vaccine groups, but it was still higher in the high dose group (35% of subjects) than low dose group (16% of subjects) and higher than the baseline values on day 1.

Table 38.

Number (%) (97.5% CI) of Subjects with HI ≥ 1:40 Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) – HI Assay – FAS

Vaccine Group	Low Dose	High Dose
	N = 683	N = 693
Day 1	68 (10%) (8%-13%)	86 (12%) (10%-15%)
Day 22	213 (32%) (28%-36%) N = 673	331 (49%) (44%-53%) N = 681
Day 43	415 (63%) (58%-67%) N = 664	544 (81%) (77%-84%) N = 673
Day 387	104 (16%) (13%-19%) N = 651	231 (35%) (31%-39%) N = 658

Source: Table 14.2.1.1.
Abbreviations: CI, confidence interval; FAS, full analysis set; HI, hemagglutination inhibition.

GMTs and GMRs: Baseline titres against Influenza A H5N1 Turkey/2005 CC Ab strain among the vaccine groups bordered the limit of detection for the HI serology assay (titres below the limit of detection of the HI assay [< 1:10] were imputed as 5).

Three weeks following the first vaccination (day 22), titres (GMRs) were increased over baseline in the low dose (2.01-fold) and high dose (3.21-fold) groups. Three weeks after the second vaccination (day 43), titres (GMRs) were increased over baseline in the low dose (5.72-fold) and high dose (16-fold) groups (Table 39). There was a decline in titres at 12 months following the second vaccination (day 387) in the high dose and low dose groups but remained elevated over baseline levels.

Table 39.

Geometric Mean Titers and Geometric Mean Ratios (97.5% CI) Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) – HI Assay – FAS

Vaccine Group	Low Dose	High Dose
	N = 683	N = 693
GMT Day 1	7.67 (7-8.4)	8.29 (7.57-9.08)
GMT Day 22	16 (14-18) N = 673	26 (23-30) N = 681
GMR Day 22/Day 1	2.01 (1.75-2.3) N = 673	3.21 (2.8-3.68) N = 681
GMT Day 43	45 (38-53) N = 664	129 (110-152) N = 673
GMR Day 43/Day 1	5.72 (4.83-6.78) N = 664	16 (13-19) N = 673
GMT Day 387	10 (9.29-12) N=651	16 (14-18) N=658
GMR Day 387/Day 1	1.3 (1.16-1.46) N = 651	1.97 (1.76-2.2) N = 658

Source: Table 14.2.1.3.
Abbreviations: CI, confidence interval; HI, hemagglutination inhibition; FAS, full analysis set; GMT, geometric mean titer; GMR, geometric mean ratio.

Primary Objective

To select the vaccine (low dose or high dose aH5N1c) to be tested in phase 3 based on achievement of CBER criteria 3 weeks after the second vaccine administration as measured by strain-specific HI assays. The primary population used for testing study hypotheses was FAS; nevertheless the immunogenicity analysis was also repeated by PPS which demonstrated results similar to FAS for all study endpoints. Three weeks following the second vaccination (day 43), CBER criteria for seroconversion were met against homologous strain (Influenza A H5N1 Turkey/2005 CC Ab) in both vaccine groups whereas CBER criteria for HI $\geq$ 1:40 was met only in the high dose group (Table 40).

Table 40.

Overview of CBER Criteria Achievement Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) at Day 43 – HI Assay – FAS

	Number (%) of Subjects (97.5% CI)	
Vaccine Group	**Low Dose**	**High Dose**
CBER Criteria	**N = 664**	**N = 673**
Lower bound of 97.5% CI for percentage of subjects achieving seroconversion[a] ≥ 30%	345 (52%) (**48%**-56%)	495 (74%) (**70%**-77%)
Lower bound of 97.5% CI for percentage of subjects achieving HI ≥ 1:40 ≥ 60%	415 (63%) (58%-67%)	544 (81%) (**77%**-84%)

Source: Table 14.2.1.1; Table 14.2.1.2.
Abbreviation: CBER, Center for Biologics Evaluation and Research; CI, confidence interval; FAS, full analysis set; HI, hemagglutination inhibition.
[a]Seroconversion is defined as either a prevaccination HI < 1:10 and a postvaccination HI ≥ 1:40, or a prevaccination HI ≥ 1:10 and a minimum 4-fold rise in postvaccination HI antibody titer.
Bold indicates CBER MAY 2007 criteria (listed above) met.

Secondary Objectives

First secondary objective: For each aH5N1c vaccine (low dose or high dose), to evaluate achievement of all CHMP criteria 3 weeks after the second vaccine administration as measured by strain-specific HI assay. Three weeks following the second vaccination (day 43), all 3 CHMP criteria were met against homologous strain (Influenza A H5N1 Turkey/2005 CC Ab) in both the vaccine groups (Table 41).

Table 41.

Overview of CHMP Criteria Achievement Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) at Day 43 – HI Assay – FAS

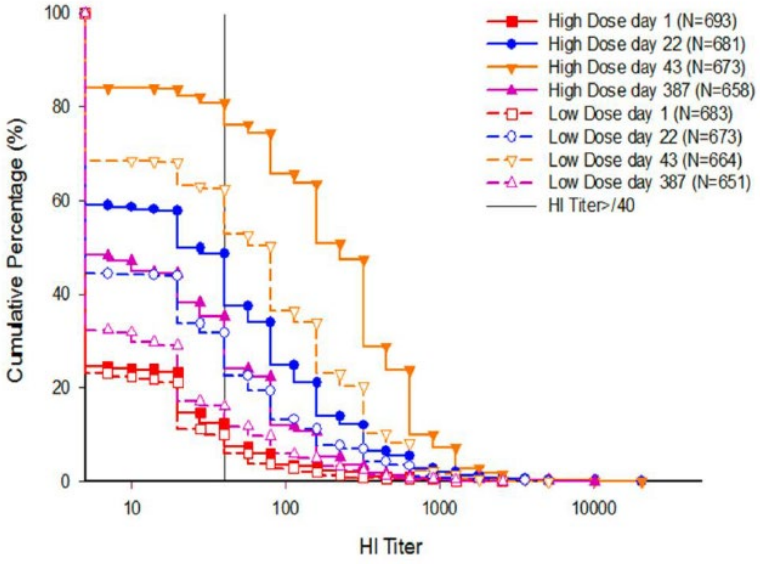
	Number (%) of Subjects (97.5% CI)	
Vaccine Group	**Low Dose**	**High Dose**
CHMP Criteria	**N = 664**	**N = 673**
Percentage of subjects with seroconversion[a] of > 30%	345 (**52%**) (48%-56%)	495 (**74%**) (70%-77%)
GMR > 2	**5.72** (4.83-6.78)	**16** (13-19)
Percentage of subjects achieving an HI ≥ 1:40 of > 60%	415 (**63%**) (58%-67%)	544 (**81%**) (77%-84%)

Source: Table 14.2.1.1; Table 14.2.1.2; Table 14.2.1.3.
Abbreviations: CHMP, Center for Medicinal Products for Human Use; CI, confidence interval; FAS, full analysis set; GMR, geometric mean ratio; HI, hemagglutination inhibition.
[a]Seroconversion is defined as either a prevaccination HI titer < 1:10 and a postvaccination HI ≥ 1:40, or a prevaccination HI ≥ 1:10 and a minimum 4-fold rise in postvaccination HI antibody titer (also referred to as seroconversion or significant increase).
Bold indicates CHMP (CPMP/BWP/214/96) criteria (listed above) met.

RCDF curves of HI titres for low dose and high dose groups against Influenza A H5N1 Turkey/2005 CC Ab strain at baseline (day 1), 3 weeks following the first vaccination (day 22), 3 weeks following the second vaccination (day 43) and 12 months following the second vaccination (day 387) are presented in Figure 10.

Figure 10.

Reverse Cumulative Distribution Frequency of HI Titers Against Homologous Strain (Influenza A H5N1 Turkey/2005 CC Ab) at Day 1, Day 22, Day 43 and Day 387 – HI Assay – FAS



Source: Figure 14.2.1.1.14; Figure 14.2.1.1.17.
Abbreviations: H5N1c, monovalent H1N1 influenza virus vaccine; FAS, full analysis set.

Overview of the available immunogenicity data from elderly individuals (65 years of age and above) by age subgroups:

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trial **V89_18** (aH5N1c, Placebo)	aH5N1c: 830/3191 Placebo: 267/796	aH5N1c: 329/3191 Placebo: 118/796	aH5N1c: 38/3191 Placebo: 13/796
Controlled trial **V89_13** (aH5N1c full dose, aH5N1c half dose)	aH5N1c full dose: 535/700 aH5N1c half dose: 557/963	aH5N1c full dose: 152/700 aH5N1c half dose: 127/693	aH5N1c full dose: 13/700 aH5N1c half dose: 9/693

The numbers presented in this table are based on the All Exposed Set for Study V89_18 and the All Enrolled Set for Study V89_13.

Table 42.

GMT, GMR and Percentage of Subjects with HI titre ≥1:40 and achieving Seroconversion (95% CI) Against the Homologous Strain at Day 1, 22, 43 and 183 for adults (≥65 years of age) by age cohort Study V89_18 – Full Analysis Set

Age Group	65-74 years		75-84 years		≥85 years	
Treatment Group	**Pooled aH5N1c**	**Placebo**	**Pooled aH5N1c**	**Placebo**	**Pooled aH5N1c**	**Placebo**
Day 1	**N=822**	**N=263**	**N=324**	**N=117**	**N=37**	**N=13**
GMT (95% CI)*	20.4 (19.0, 21.8)	20.1 (17.9, 22.5)	20.1 (17.8, 22.7)	20.8 (17.1, 25.2)	28.3 (20.9, 38.3)	17.8 (10.3, 30.9)
% HI titre ≥1:40 (95% CI)	28.8% (25.8, 32.1)	25.1% (20.0, 30.8)	25.9 (21.2, 31.1)	26.5% (18.8, 35.5)	40.5% (24.8, 57.9)	23.1% (5.0, 53.8)
Day 22	**N=822**	**N=262**	**N=322**	**N=117**	**N=37**	**N=13**
GMT (95% CI)*	44.1 (41.2, 47.2)	13.9 (12.3, 15.6)	38.7 (34.1, 43.9)	16.1 (13.2, 19.6)	30.7 (21.1, 44.4)	13.3 (6.9, 25.6)
Day 22/1 GMR (95% CI)	2.26 (2.11, 2.41)	0.71 (0.63, 0.80)	1.95 (1.72, 2.21)	0.81 (0.67, 0.99)	1.35 (0.93, 1.94)	0.58 (0.30, 1.12)
% HI titre ≥1:40 (95% CI)	58.5% (55.1, 61.9)	14.1% (10.1, 18.9)	48.8% (43.2, 54.4)	20.5% (13.6, 29.0)	40.5% (24.8, 57.9)	23.1% (5.0, 53.8)
% seroconversion (95% CI)	26.6% (23.6, 29.8)	0.4% (0.0, 2.1)	18.6% (14.5, 23.3)	0.9% (0.0, 4.7)	8.1% (1.7, 21.9)	--
Day 43	**N=817**	**N=261**	**N=324**	**N=116**	**N=37**	**N=13**
GMT (95% CI)*	106.4 (99.3, 114.1)	16.2 (14.4, 18.3)	86.8 (77.0, 97.7)	17.1 (14.1, 20.6)	50.7 (32.7, 78.5)	16.0 (7.3, 34.9)
Day 43/ 1 GMR (95% CI)	5.42 (5.05, 5.81)	0.83 (0.73, 0.93)	4.37 (3.88, 4.92)	0.86 (0.71, 1.04)	2.22 (1.43, 3.44)	0.70 (0.32, 1.53)
% HI titre ≥1:40 (95% CI)	85.6% (**83.0**, 87.9)	22.2% (17.3, 27.8)	79.9% (**75.2**, 84.2)	22.4% (15.2, 31.1)	70.3% (**53**, 84.1)	23.1% (5.0, 53.8)
% seroconversion (95% CI)	58.4% (**54.9**, 61.8)	2.7% (1.1, 5.4)	48.8% (**43.2**, 54.4)	0.9% (0.0, 4.7)	18.9% (8.0, 35.2)	--
Day 183	**N=804**	**N=256**	**N=316**	**N=115**	**N=36**	**N=13**
GMT (95% CI)*	20.0 (18.8, 21.3)	8.4 (7.5, 9.4)	17.3 (15.4, 19.4)	8.9 (7.4, 10.7)	16.1 (11.9, 22.0)	8.4 (4.9, 14.5)
Day 183/1 GMR (95% CI)	1.02 (0.96, 1.09)	0.43 (0.38, 0.48)	0.87 (0.78, 0.98)	0.45 (0.37, 0.54)	0.72 (0.53, 0.98)	0.38 (0.22, 0.65)
% HI titre ≥1:40 (95% CI)	32.5% (29.2, 35.8)	7.8% (4.8, 11.8)	28.8% (23.9, 34.1)	8.7% (4.2, 15.4)	16.7% (6.4, 32.8)	7.7% (0.2, 36.0)
% seroconversion (95% CI)	8.1% (6.3, 10.2)	1.2% (0.2, 3.4)	9.2% (6.2, 12.9)	0.9% (0.0, 4.7)	2.8% (0.1, 14.5)	--

Source: V89_18 Tables: Table 14.2.1.2.77.99; Table 14.2.1.3.77.99; Table 14.2.1.4.77.99
*Adjusted GMT and 95% CI are analyzed using analysis of covariance with factors for treatment, center, and a covariate for the effect defined by the log-transformed baseline pre-vaccination antibody titer (except for Day 1).
Bold indicates CBER criterion is met

Table 43.

GMT, GMR and Percentage of Subjects with HI titre ≥1:40 and achieving Seroconversion (97.5% CI) Against Homologous Strain at Day 1, 22, 43 and 183 for adults (≥65 years of age) by age cohort Study V89_13 – Full Analysis Set

Age Group	65-74 years		75-84 years		≥85 years	
Treatment Group	**Full dose**	**Half dose**	**Full dose**	**Half dose**	**Full dose**	**Half dose**
Day 1	**N=530**	**N=547**	**N=150**	**N=127**	**N=13**	**N=9**
GMT (97.5% CI)	7.75 (7.01, 8.57)	7.46 (6.76, 8.24)	9.68 (7.57, 12.38)	8.24 (6.31, 10.76)	12.7 (6.09, 26.51)	7.01 (2.1, 23.46)
% HI titre ≥1:40 (97.5% CI)	11% (8, 14)	10% (7, 13)	18% (12, 26)	12% (6, 20)	15% (1, 49)	11% (0, 53)
Day 22	**N=520**	**N=539**	**N=148**	**N=125**	**N=13**	**N=9**
GMT (97.5% CI)	26.63 (22.83, 31.05)	14.76 (12.71, 17.14)	23.12 (16.8, 31.81)	20.79 (14.72, 29.35)	32.6 (3.21, 330.69)	31.93 (0.94, 1083.08)
Day 22/1 GMR (97.5% CI)	3.44 (2.95, 4.02)	1.93 (1.66, 2.25)	2.43 (1.75, 3.35)	2.28 (1.61, 3.23)	2.65 (0.34, 20.8)	4.46 (0.15, 130.99)
% HI titre ≥1:40 (97.5% CI)	50% (45, 55)	30% (26, 35)	43% (34, 53)	37% (27, 47)	46% (17, 78)	33% (6, 74)
% seroconversion (97.5% CI)	39% (34, 44)	20% (17, 25)	26% (19, 35)	26% (17, 35)	14% (0, 63)	25% (2, 69)
Day 43	**N=514**	**N=530**	**N=147**	**N=125**	**N=12**	**N=9**
GMT (97.5% CI)	144.48 (120.12, 173.77)	44.53 (37.18, 53.34)	98.03 (67.64, 142.09)	50.38 (33.75, 75.2)	42.07 (4.15, 426.07)	215.98 (8.16, 5718.11)
Day 43/ 1 GMR (97.5% CI)	18.51 (15.25, 22.46)	5.82 (4.82, 7.04)	10.23 (6.95, 15.04)	5.68 (3.75, 8.61)	4.79 (0.81, 28.21)	29.14 (1.64, 518.76)
% HI titre ≥1:40 (97.5% CI)	83% (**79**, 86)	64% (**59**, 69)	74% (**65**, 82)	55 (45, 65)	75% (39, 96)	67% (26, 94)
% seroconversion (97.5% CI)	77% (**73**, 81)	53% (**48**, 58)	61% (**52**, 70)	46% (**35**, 56)	58% (24, 87)	56% (18, 89)
Day 387	**N=503**	**N=520**	**N=144**	**N=122**	**N=11**	**N=9**
GMT (97.5% CI)	16.41 (14.55, 18.52)	9.47 (8.4, 10.67)	14.72 (11.27, 19.24)	13.73 (10.29, 18.33	11.12 (2.35, 52.68)	21.69 (3.04, 154.75)
Day 387/1 GMR (97.5% CI)	2.09 (1.85, 2.38)	1.23 (1.09, 1.4)	1.56 (1.19, 2.05)	1.51 (1.12, 2.02)	1.37 (0.43, 4.34)	2.67 (0.47, 15.24)
% HI titre ≥1:40 (97.5% CI)	35% (30, 40)	13% (10, 17)	35% (27, 45)	25% (17, 35)	27% (5, 65)	44% (11, 82)
% seroconversion (97.5% CI)	24% (20, 29)	8% (6, 12)	20% (13, 29)	14% (8, 22)	18% (2, 56)	33% (6, 74)

Source: V89_13 Tables: Table 14.2.1.1.67; Table 14.2.1.2.67; Table 14.2.1.3.67
Bold indicates CBER criterion is met

2.6.5.4. Analysis performed across trials (pooled analyses and meta-analysis)

Of note, the analyses presented in this section are not pooled, but provide a comparison of independent data across trials.

Haemagglutination inhibition (HI) Analysis Homologous Strain

Table 44.

Studies V89_11, V89P1, V89_04, V89_13 and V89_18 – Percentages of Subjects Achieving Seroconversion and Percentages of Subjects Achieving an HI Titre ≥1:40 Against the Homologous Strain at Days 1, 22 and 43 in the Full Dose Group – HI Assay – FAS (V89_04, V89_11, V89_013), PPS (V89_18) and MPPS (V89P1)

	6 Months to ≤17 Years	18 to ≤40 Years	18 to <65 Years		18 to <60 Years		≥65 Years		≥60 Years
	V89_11 (97.5% CI)	V89P1[a] (95%CI)	V89_04 (97.5% CI)	V89_18 (95% CI)	V89_04 (97.5% CI)	V89_18 (95% CI)	V89_13 (97.5% CI)	V89_18 (95% CI)	V89_18 (95% CI)
Seroconversion									
	N=281	N=49	N=464	N=1115	N=434	N=979	N=681	N=1130	N=1266
Day 22	***52*** **(45; 58)**	18 (9; 32)	***48*** **(43; 53)**	***40.4*** (37.6; 43.4)	***49*** (44; 54)	***42.2*** (39.1; 45.4)	***36*** **(32; 40)**	24.2 (21.7; 26.8)	24.6 (22.2; 27.0)
	N=279	N=50	N=451	N=1076	N=423	N=947	N=673	N=1080	N=1209
Day 43	***96*** **(93; 98)**	***78*** **(64; 88)**	***83*** **(78; 87)**	***79.9*** **(77.4; 82.3)**	***83*** (80; 87)	***81.6*** (79.0; 84.0)	***74*** **(70; 77)**	***54.0*** **(51.0; 57.0)**	***55.4*** (52.6; 58.2)
HI Titre ≥1:40									
	N=294	N=50	N=478	N=1116	N=448	N=980	N=693	N=1133	N=1269
Day 1	1 (0; 3)	0 (0; 7)	4 (2; 7)	13.0 (10.7; 15.6)	4 (2; 6)	17.1 (14.8; 19.7)	12 (10; 15)	28.2 (25.6; 31.0)	27.3 (24.8; 29.8)
	N=283	N=49	N=464	N=1115	N=434	N=979	N=681	N=1130	N=1266
Day 22	51 (44; 58)	18 (9; 32)	52 (46; 57)	65.7 (62.6; 68.7)	52 (47; 57)	63.2 (60.1; 66.3)	49 (44; 53)	55.6 (52.6; 58.5)	56.2 (53.5; 59.0)
	N=287	N=50	N=451	N=1076	N=423	N=947	N=673	N=1080	N=1209
Day 43	***96*** **(92; 98)**	***78*** **(64; 88)**	***85*** **(81; 88)**	***95.0*** **(93.4; 96.2)**	***85*** (81; 88)	***93.6*** (91.8; 95.0)	***81*** **(77; 84)**	***83.5*** **(81.2; 85.7)**	***84.2*** (82.0; 86.2)

Source: CSR V89_11: Table 14.2.1.1, Table 14.2.1.2; CSR V89P1: Table 14.2.1.1.3.2, Table 14.2.1.2.3.2; CSR V89_04: Table 14.2.1.1, Table 14.2.1.2, Table 14.2.1.1.12, 14.2.1.2.10; CSR V89_18: Table 14.2.1.2.6.1, Table 14.2.1.3.6, Table 14.2.1.3.7; CSR V89_13: Table 14.2.1.1, Table 14.2.1.2.

Abbreviations: CBER = Center for Biologics Evaluation and Research; CHMP = Committee for Medicinal Products for Human Use; CI = confidence interval; FAS = full analysis set; HI = hemagglutination inhibition; MPPS = modified per protocol set; N = number of subjects; PPS = per protocol set; seroconversion = subjects with a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40, or, subjects with a prevaccination HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titre.

[a] Corresponds to the 7.5 µg HA + 0. 25 mL MF59 group in Table 8 of Section 2.7.2.

Bold = CBER criteria met (lower bound of CI); ***bold, italic*** = former CHMP criteria met

Table 45.

Studies V89_11, V89P1, V89_04, V89_13, and V89_18 – GMTs and GMRs Against the Homologous St at Days 1, 22, and 43 in the Full Dose Group - HI Assay – FAS (V89_04, V89_11 and V89_13), PPS (V89_18) and MPPS (V89P1)

	6 Months to ≤17 Years	18 to ≤40 Years	18 to <65 Years		18 to <60 Years		≥65 Years		≥60 Years
	V89_11 (97.5% CI)	V89P1[a] (95% CI)	V89_04 (95% CI)	V89_18 (95% CI)	V89_04 (95% CI)	V89_18 (95% CI)	V89_13 (97.5% CI)	V89_18 (95% CI)	V89_18 (95% CI)
	N=294	N=50	N=478	N=1116	N=448	N=980	N=693	N=1133	N=1269
GMT Day 1	5.23 (5; 5.48)	5 (4.73; 5.29)	6.11 (5.78; 6.46)	13.5 (12.8; 14.2)	6 (5.67; 6.35)	13.0 (12.3; 13.8)	8.29 (7.57; 9.08)	20.5 (19.4; 21.8)	20.1 (19.1; 21.3)
	N=283	N=49	N=464	N=1115	N=434	N=979	N=681	N=1130	N=1266
GMT Day 22	64 (46; 90)	8.93 (6.89; 12)	33 (28; 39)	50.6 (47.6; 53.8)	32 (27; 38)	51.1 (47.9; 54.6)	26 (23; 30)	42.4 (40.0; 45.0)	42.9 (40.5; 45.3)
GMR Day 22/ Day 1	***13*** (9; 18)[b]	1.79 (1.37; 2.32)	***5.37*** (4.6; 6.27)	***3.81*** (3.58; 4.05)	***5.36*** (4.55; 6.31)	***3.92*** (3.67; 4.18)	***3.21*** (2.8; 3.68)	***2.14*** (2.02; 2.27)	***2.22*** (2.10; 2.35)
	N=287	N=50	N=451	N=1076	N=423	N=947	N=673	N=1080	N=1209
GMT Day 43	1356 (985; 1866)	131 (82; 209)	250 (208; 302)	170.7 (160.5; 181.6)	265 (218; 322)	177.4 (166.2; 189.4)	129 (110; 152)	97.9 (92.1; 104.1)	100.7 (95.0; 106.7)
GMR Day 43/ Day 1	***262*** (190; 361)[c]	***26*** (16; 42)	***41*** (34; 49)	***12.70*** (11.94; 13.51)	***44*** (36; 53)	***13.44*** (12.59; 14.35)	***16*** 913; 19)	***4.90*** (4.61; 5.20)	***5.18*** (4.88; 5.49)

Source: CSR V89_11: Table 14.2.1.3; CSR V89P1: Table 14.2.1.3.3.2; CSR V89_04: Table 14.2.1.3, Table 14.2.1.3.10; CSR V89_18: Table 14.2.1.4.6, Table 14.2.1.4.7; CSR V89_13: Table 14.2.1.3.

Abbreviations: CHMP = Committee for Medicinal Products for Human Use; CI = confidence interval; FAS = full analysis set; GMR = geometric mean ratio; GMT = geometric mean titre; MPPS = modified per protocol set; N = number of subjects; PPS = per protocol set.

[a] Corresponds to the 7.5 µg HA + 0. 25 mL MF59 group in Table 9 of Section 2.7.2.

[b] N=281, [c] N=279

Bold, italic = former CHMP criterion met

Microneutralisation (MN) Analysis Homologous Strain

Table 46.

Percentages of Subjects Achieving an at Least 4-Fold Increase in MN Titre and Percentages of Subject Achieving MN Titres of ≥1:40, ≥1:80 and ≥1:160 Against the Homologous Strain at Days 1 and 43 in the Full Dose Group – MN Assay – FAS (Studies V89_11, V89_04 and V89_13), MPPS (Study V89P1), MN Subset (V89_18)

	V89_11	V89P1	V89_04[a]	V89_18[a,b]	V89_13
	6 Months to ≤17 Years (95% CI)	18 to ≤40 Years (95% CI)	18 to <65 Years (95% CI)	18 to <65 Years (95% CI)	≥65 Years (97.5% CI)
	N=69	N=51	N=69	N=76	N=35
≥4-fold increase in MN Titre					
Day 43	100 (95; 100)	94 (84; 99)	94 (86; 98)	89.5 (80.31; 95.34)	77 (60; 90)
MN Titre ≥1:40					
Day 1	0 (0; 5)	4 (0; 13)	4 (1; 12)	1.3 (0.03; 7.11)	6 (1; 19)
Day 43	100 (95; 100)	94 (84; 99)	99 (92; 100)	89.5 (80.31; 95.34)	86 (70; 95)
MN Titre ≥1:80					
Day 1	0 (0; 5)	2 (0.05; 10)	1 (0.04; 8)	0.0 (-)	0 (0; 10)
Day 43	100 (95; 100)	92 (81; 98)	91 (82; 97)	75.0 (63.74; 84.23)	74 (57; 88)
MN Titre ≥1:160					
Day 1	0 (0; 10)	-	1 (0.04; 8)	0.0 (-)	0 (0; 5)
Day 43	99 (92; 100)	-	81 (70; 80)	48.7 (37.04; 60.43)	54 (37; 71)

Source: CSR V89_11: Table 14.2.1.1.11, Table 14.2.1.1.11.1, Table 14.2.1.1.11.2, Table 14.2.1.2.11; CSR V89P1: Table 14.2.1.5.3.2, Table 14.2.1.6.3.2; CSR V89_04: Table 14.2.1.1.9, Table 14.2.1.1.10, Table 14.2.1.1.11; CSR V89_13: Table 14.2.1.2.11, Table 14.2.1.2.11.1 and Table 14.2.1.2.11.2; CSR V89_18 Addendum 1 Section 14: Table 2 and Table 3.

Abbreviations: CI = confidence interval; FAS = full analysis set; MN = microneutralisation; N = number of subjects; MPPS = modified per protocol set.

[a] In Studies V89_04 and V89_18, the category '≥4-fold increase in MN titre' represents subjects with either a prevaccination MN <1:10 and a postvaccination MN ≥ 1: 40 or a prevaccination MN ≥ 1:10 and a minimum 4-fold rise in postvaccination MN antibody titre.

[b] In Study V89_18, post hoc MN analysis was done in subset of subjects 18 to <65 years of age.

Table 47.

GMTs and GMRs Against the Homologous Strain at Days 1 and 43 in the Full Dose Group – MN Assay – FAS (Studies V89_11, V89_04 and V89_13), MPPS (Study V89P1), and MN Subset (V89_18)

	V89_11	V89P1	V89_04	V89_18[c]	V89_13
	6 Months to ≤17 Years (95% CI)	18 to ≤40 Years (95% CI)	18 to <65 Years (95% CI)	18 to <65 Years (95% CI)	≥65 Years (95% CI)
	N=69	N=51	N=69	N=76	N=35
GMT Day 1	5 (5; 5)[a]	11 (10; 13)	6.7 (5.62; 7.98)	5.53 (5.2; 5.9)[b]	7.49 (5.87; 9.56)
GMT Day 43	1284 (1046;1576)	645 (459;906)	410 (313;537)	130.91 (103.1; 166.3)[b]	169 (107;268)
GMR Day 43/Day 1	257 (209; 315)	57 (39; 83)	61 (44; 86)	23.80 (18.7; 30.3)[b]	23 (13; 39)

Source: CSR V89_11: Table 14.2.1.4; CSR V89P1: Table 14.2.1.8.3.2; CSR V89_04: Table 14.2.1.3.9; CSR V89_13: Table 14.2.1.3.11; CSR V89_18 Addendum 1 Section 14: Table 4.

Abbreviations: CI = confidence interval; FAS = full analysis set; GMR = geometric mean ratio; GMT = geometric mean titre; MN = microneutralisation; N = number of subjects; MPPS = modified per protocol set.

[a] N=70 [b] Adjusted values

[c] In Study V89_18, post hoc MN analysis was done in subset of subjects 18 to <65 years of age.

Heterologous Strain Analysis (HI assay)

Table 48.

Studies V89_11, V89_04 and V89_13 – Percentages of Subjects Achieving Seroconversion and Percentages of Subjects Achieving an HI Titre ≥1:40 Against Heterologous Strains at Days 1 and 43 in the Full Dose Group – HI Assay – FAS

	Percentages of Subjects with Seroconversion				Percentages of Subjects with HI Titre ≥1:40			
	V89_11	V89_04		V89_13	V89_11	V89_04		V89_13
	6 M to ≤17 Y (97.5% CI)	18 to <65 Y (97.5% CI)	18 to <60 Y (95% CI)	≥65 Y (97.5% CI)	6 M to ≤17 Y (97.5% CI)	18 to <65 Y (97.5% CI)	18 to <60 Y (95% CI)	≥65 Y (97.5% CI)
	N=69	N=69	N=61	N=35	N=69	N=69	N=61	N=35
A/Anhui/1/2005								
Day 1	-	-	-	-	0 (0; 6)	0 (0; 6)	0 (0; 6)	0 (0; 12)
Day 43	32 (20; 46)	28 (16; 41)	28 (17; 41)	17 (6; 36)	32 (20; 46)	28 (16; 41)	28 (17; 41)	17 (6; 36)
A/Egypt/N03072/2010								
Day 1	-	-	-	-	0 (0; 6)	4 (1; 13)	5 (1; 14)	9 (1; 25)
Day 43	***72*** (**59**; 84)	***55*** (**41**; 69)	***59*** (**46**; 71)	***43*** (24; 63)	***72*** (59; 84)	58 (44; 71)	62 (49; 74)	49 (29; 68)
A/Hubei/1/2010								
Day 1	-	-	-	-	0 (0; 6)	13 (5; 25)	13 (6; 24)	11 (3; 29)
Day 43	***54*** (**40**; 67)	***55*** (**41**; 69)	***56*** (**42**; 68)	***46*** (27; 66)	54 (40; 67)	64 (50; 76)	66 (52; 77)	57 (37; 76)
A/Indonesia/5/2005								
Day 1	-	-	-	-	0 (0; 6)	0 (0; 6)	0 (0; 6)	0 (0; 12)
Day 43	36 (24; 50)	35 (22; 49)	36 (24; 49)	26 (11; 46)	36 (24; 50)	35 (22; 49)	36 (24; 49)	26 (11; 46)
A/Vietnam/1203/2004								
Day 1	-	-	-	-	0 (0; 6)	7 (2; 17)	8 (3; 18)	11 (3; 29)
Day 43	***54*** (**40**; 68)	***52*** (38; 66)	***54*** (**41**; 67)	***43*** (24; 63)	54 (40; 68)	54 (40; 67)	56 (42; 68)	51 (32; 71)

Source: CSR V89_11: Table 14.2.1.1, Table 14.2.1.2; V89_13: Table 14.2.1.1, Table 14.2.1.2; CSR V89_04: Table 14.2.1.1, Table 14.2.1.2, Table 14.2.1.2.10, Table 14.2.1.1.12.

Abbreviations: CI = confidence interval; FAS = full analysis set; HI = hemagglutination inhibition; N = number of subjects; seroconversion = subjects with a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40, or, subjects with a prevaccination HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titre; Y = years.

Bold = CBER criteria met (lower bound of CI); ***bold, italic*** = former CHMP criteria met

Table 49.

Studies V89_11, V89_04 and V89_13 – GMTs and GMRs Against Heterologous Strains at Days 1 and 43 in the Full Dose Group – HI Assay – FAS

	V89_11	V89_04		V89_13
	6 Months to ≤17 Years (97.5% CI)	18 to <65 Years (95% CI)	18 to <60 Years (95% CI)	≥65 Years (97.5% CI)
	N=69	N=69	N=61	N=35
A/Anhui/1/2005				
GMT Day 1	5 (5; 5)	5.02 (4.87; 5.18)	5.03 (4.86; 5.2)	5 (5; 5)
GMT Day 43	42 (20; 87)	11 (6.64; 17)	10 (6.23; 17)	7.67 (4.55; 13)
GMR Day 43/Day 1	***8.39*** (4.02; 17)	2.09 (1.29; 3.37)	1.98 (1.21; 3.23)	1.53 (0.91; 2.58)
A/Egypt/N03072/2010				
GMT Day 1	5 (5; 5)	5.87 (4.51; 7.63)	5.81 (4.39; 7.69)	7.67 (5.13; 11)
GMT Day 43	201 (74; 544)	39 (22; 71)	40 (21; 75)	25 (11; 58)
GMR Day 43/Day 1	***40*** (15; 109)	***6.52*** (3.62; 12)	***6.64*** (3.52; 13)	***3.62*** (1.61; 8.17)
A/Hubei/1/2010				
GMT Day 1	5 (5; 5)	7.12 (5.01; 10)	7 (4.82; 10)	8.2 (5.33; 13)
GMT Day 43	172 (57; 523)	56 (31; 102)	65 (35; 121)	36 (16; 78)
GMR Day 43/Day 1	***34*** (11; 105)	***7.3*** (4.01; 13)	***8.33*** (4.45; 16)	***4.77*** (2.25; 10)
A/Indonesia/5/2005				
GMT Day 1	5 (5; 5)	5.02 (4.87; 5.18)	5.03 (4.86; 5.2)	5 (5; 5)
GMT Day 43	55 (24; 125)	16 (9.17; 28)	15 (8.5; 27)	10 (5.72; 19)
GMR Day 43/Day 1	***11*** (4.87; 25)	***3.12*** (1.81; 5.39)	***2.97*** (1.67; 5.27)	***2.09*** (1.14; 3.82)
A/Vietnam/1203/2004				
GMT Day 1	5 (5; 5)	5.97 (4.41; 8.06)	6.22 (4.46; 8.66)	8.48 (5.12; 14)
GMT Day 43	113 (42; 300)[a]	44 (24; 81)	46 (24; 85)	34 (15; 75)
GMR Day 43/Day 1	***23*** (8.64; 60)[a]	***6.99*** (3.84; 13)	***6.98*** (3.74; 13)	***4.26*** (1.98; 9.2)

Source: CSR V89_11; Table 14.2.1.3; CSR V89_04: Table 14.2.1.3.10; CSR V89_13: Table 14.2.1.3.

Abbreviations: FAS = full analysis set; GMR = geometric mean ratio; GMT = geometric mean titre; N = number of subjects.

[a] N=68

Bold, italic = former CHMP criteria met

Heterologous Strain Analysis (MN assay)

Table 50.

Studies V89_11, V89_04 and V89_13 – Percentages of Subjects Achieving an at least 4-Fold Increase in MN Titre and Percentages of Subjects Achieving MN Titres of ≥1:40, ≥1:80 and ≥1:160 Against the Heterologous Strain at Days 1 and 43 in the Full Dose Group – MN Assay – FAS

		≥4-fold Increase in MN Titre			MN Titre ≥1:40			MN Titre ≥1:80			MN Titre ≥1:160		
		V89_11	V89_04[a]	V89_13	V89_11	V89_04	V89_13	V89_11	V89_04	V89_13	V89_11	V89_04	V89_13
		6 M to ≤17 Y (95% CI)	18 to <65 Y (95% CI)	≥65 Y (95% CI)	6 M to ≤17 Y (95% CI)	18 to <65 Y (95% CI)	≥65 Y (95% CI)	6 M to ≤17 Y (95% CI)	18 to <65 Y (95% CI)	≥65 Y (95% CI)	6 M to ≤17 Y (95% CI)	18 to <65 Y (95% CI)	≥65 Y (95% CI)
		N=69	N=69	N=35	N=70[b]	N=69	N=35	N=70[b]	N=69	N=35	N=70[b]	N=69	N=35
A/Anhui /1/2005	Day 1	-	-	-	0 (0; 5)	28 (17; 40)	49 (31; 66)	0 (0; 5)	9 (3; 18)	9 (2; 23)	0 (0; 5)	1 (0.037; 8)	3 (0.072; 15)
	Day 43	99 (92; 100)	74 (62-84)	40 (24; 58)	99 (92; 100)	97 (90; 100)	91 (77; 98)	97 (90; 100)	86 (75; 93)	83 (66; 93)	80 (68; 88)	42 (30; 55)	34 (19; 52)
A/Egypt /N03072/ 2010	Day 1	-	-	-	16 (8; 26)	23 (14; 35)	40 (24; 58)	3 (0; 10)	14 (7; 25)	17 (7; 34)	1 (0.036; 8)	9 (3; 18)	9 (2; 23)
	Day 43	100 (95; 100)	88 (78-95)	74 (57; 88)	100 (95; 100)	100 (95; 100)	97 (85; 100)	100 (95; 100)	96 (88; 99)	89 (73; 97)	100 (95; 100)	81 (70; 90)	83 (66; 93)
A/Hubei /1/2010	Day 1	-	-	-	1 (0.036; 8)	6 (2; 14)	20 (8; 37)	0 (0; 5)	0 (0; 5)	0 (0; 10)	0 (0; 5)	0 (0; 5)	0 (0; 10)
	Day 43	93 (84; 98)	55 (43-67)	31 (17; 49)	94 (86; 98)	68 (56; 79)	49 (31; 66)	67 (54; 78)	28 (17; 40)	17 (7; 34)	20 (12; 32)	12 (5; 22)	9 (2; 23)
A/Indonesia /5/2005	Day 1	-	-	-	6 (2; 14)	12 (5; 22)	31 (17; 49)	1 (0.036; 8)	3 (0; 10)	9 (2; 23)	1 (0.036; 8)	0 (0; 5)	0 (0; 10)
	Day 43	94 (86; 98)	72 (60-83)	49 (31; 66)	99 (92; 100)	86 (75; 93)	89 (73; 97)	96 (88; 99)	64 (51; 75)	57 (39; 74)	77 (65; 86)	29 (19; 41)	23 (10; 40)
A/Vietnam /1203/2004	Day 1	-	-	-	0 (0; 5)	3 (0; 10)	9 (2; 23)	0 (0; 5)	1 (0.037; 8)	3 (0.072; 15)	0 (0; 5)	0 (0; 5)	3 (0.072; 15)
	Day 43	83 (72; 91)	94 (86-98)	26 (12; 43)	84 (73; 92)	38 (26; 50)	37 (21; 55)	39 (28; 52)	13 (6; 23)	6 (1; 19)	7 (2; 16)	4 (1; 12)	6 (1; 19)

Source: CSR V89_11: Table 14.2.1.2.12, Table 14.2.1.1.12, Table 14.2.1.1.12.1, Table 14.2.1.1.12.2; CSR V89_04: Table 14.2.1.1.9, Table 14.2.1.1.10, Table 14.2.1.1.11, Table 14.2.1.2.9; CSR V89_13: Table 14.2.1.2.12, Table 14.2.1.1.12, Table 14.2.1.1.12.1, Table 14.2.1.1.12.2.

Abbreviations: CI = confidence interval; FAS = full analysis set; M = months; MN = microneutralisation; N = number of subjects, Y = years.

[a] In Study V89_04, the category '≥4-fold increase in MN titre' represents subjects with either a prevaccination MN <1:10 and a postvaccination MN ≥ 1: 40 or a prevaccination MN ≥ 1:10 and a minimum 4-fold rise in postvaccination MN antibody titre.

[b] Day 43, N=69

Table 51.

Studies V89_11, V89_04 and V89_13 – GMTs and GMRs Against the Heterologous Strain at Days 1 and 43 in the Full Dose Group – MN Assay – FAS

		V89_11	V89_04	V89_13
		6 Months to ≤17 Years (95% CI)	18 to <65 Years (95% CI)	≥65 Years (95% CI)
		Day 1: N=70[a]	N=69	N=35
A/Anhui /1/2005	GMT Day 1	6.01 (5.37; 6.73)	16 (12; 21)	28 (20; 41)
	GMT Day 43	240 (205; 281)	156 (131; 186)	128 (99; 165)
	GMR Day 43/Day 1	40 (33; 49)	9.93 (7.47; 13)	4.52 (2.93; 6.97)
A/Egypt /N03072/2010	GMT Day 1	9.71 (7.65; 12)	15 (11; 22)	25 (15; 41)
	GMT Day 43	1566 (1332; 1842)	522 (398; 685)	303 (206; 447)
	GMR Day 43/Day 1	160 (120; 212)	34 (23; 49)	12 (6.69; 22)
A/Hubei /1/2010	GMT Day 1	5.16 (4.84; 5.51)	7.2 (6; 8.63)	11 (7.98; 16)
	GMT Day 43	104 (88; 122)	51 (41; 63)	42 (31; 58)
	GMR Day 43/Day 1	20 (17; 24)	7.07 (5.56; 8.98)	3.75 (2.6; 5.4)
A/Indonesia /5/2005	GMT Day 1	6.18 (5.25; 7.28)	8.79 (7; 11)	18 (13; 27)
	GMT Day 43	236 (200; 278)	114 (89; 146)	87 (63; 120)
	GMR Day 43/Day 1	38 (30; 49)	13 (9.42; 18)	4.77 (3.1; 7.34)
A/Vietnam /1203/2004	GMT Day 1	5.12 (4.88; 5.37)	5.59 (4.98; 6.27)	7.32 (5.39; 9.95)
	GMT Day 43	67 (57; 79)	27 (21; 34)	27 (19; 39)
	GMR Day 43/Day 1	13 (11; 15)	4.76 (3.74; 6.06)	3.68 (2.62; 5.17)

Source: CSR V89_11: Table 14.2.1.4.1; CSR V89_04: Table 14.2.1.3.9; CSR V89_13: Table 14.2.1.3.12.
Abbreviations: CI = confidence interval; FAS = full analysis set; GMR = geometric mean ratio; GMT = geometric mean titre; N = number of subjects.
[a] Day 43, N=69

Analyses of HI and MN titres against heterologous strains are only available for the phase II studies.

Vaccination with Seasonal Influenza Vaccine Within the Past 12 Months

Table 52.

Studies V89_11, V89_04, V89_13 and V89_18 – Percentages of Subjects Achieving Seroconversion and Percentages of Subjects Achieving an HI Titre ≥1:40 Against the Homologous Strain by Previous Vaccination with Seasonal Influenza Vaccine Within the Last 12 Months at Days 1, 22 and 43 in the Full Dose Group - HI Assay – FAS (V89_11, V89_04, V89_13) and PPS (V89_18)

	6 Months to ≤17 Years (95% CI)		18 to <65 Years (95% CI)		≥65 Years (95% CI)		≥18 Years (95% CI)	
	V89_11		V89_04		V89_13		V89_18	
	Prev. Vacc.	No Prev. Vacc.	Prev. Vacc.	No Prev. Vacc.	Prev. Vacc.	No Prev. Vacc.	Prev. Vacc.	No Prev. Vacc.
Seroconversion								
	N=568	N=225	N=114	N=350	N=420	N=261	N=1194	N=1051
Day 22	***70*** **(56; 81)**	***47*** **(40; 54)**	***47*** (38; 57)	***49*** **(44; 54)**	***37*** **(33; 42)**	***34*** (28; 40)	29.0 (26.4; 31.6)	36.0 (33.1; 39.0)
	N=54	N=225	N=108	N=343	N=414	N=259	N=1154	N=1002
Day 43	***100*** **(93; 100)**	***95*** **(91; 98)**	***79*** (70; 86)	***84*** **(80; 88)**	***72*** **(68; 77)**	***75*** (70; 80)	***60.4*** **(57.5; 63.2)**	***74.5*** **(71.6; 77.1)**
HI Titre ≥1:40								
	N=60	N=234	N=117	N=361	N=427	N=266	N=1197	N=1052
Day 1	0 (0; 6)	1 (0; 4)	9 (5; 16)	2 (1; 5)	12 (9; 15)	13 (9; 18)	23.6 (21.3; 26.2)	22.0 (19.5; 24.6)
	N=57	N=226	N=114	N=350	N=420	N=261	N=1194	N=1051
Day 22	68 (55; 80)	47 (40; 54)	54 (44; 63)	51 (45; 56)	49 (44; 53)	49 (42; 55)	56.5 (53.7; 59.4)	62.4 (59.4; 65.4)
	N=56	N=231	N=108	N=343	N=414	N=259	N=1154	N=1002
Day 43	***98*** **(90; 100)**	***95*** **(92; 98)**	***85*** (77; 91)	***85*** **(80; 88)**	***80*** **(76; 84)**	***82*** (77; 86)	***85.7*** **(83.5; 87.7)**	***91.3*** **(89.4; 93.0)**

Source: CSR V89_11: Table 14.2.1.1.7, Table 14.2.1.2.7; CSR V89_04: Table 14.2.1.1.7, Table 14.2.1.2.7; CSR V89_13: Table 14.2.1.1.7, Table 14.2.1.2.7; CSR V89_18: Table 14.2.1.3.2, Table 14.2.1.2.2.

Abbreviations: CBER = Center for Biologics Evaluation and Research; CI = confidence interval; FAS = full analysis set; HI = hemagglutination inhibition; N = number of subjects; PPS = per protocol set; No Prev. Vacc = no previous vaccination with a seasonal influenza vaccine within the past 12 months; Prev. Vacc = previous vaccination with a seasonal influenza vaccine within the past 12 months; seroconversion = subjects with a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40, or, subjects with a prevaccination HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titre.

Bold = CBER criteria met (lower bound of CI), ***bold, italic*** = former CHMP criteria met

Table 53.

Studies V89_11, V89_04, V89_13 and V89_18 – GMTs and GMRs Against the Homologous Strain by Previous Vaccination with Seasonal Influenza Vaccine Within the Last 12 Months at Days 1, 22 and 43 in the Full Dose Group – HI Assay – FAS (V89_11, V89_04, V89_13) and PPS (V89_18)

	6 Months to ≤17 Years (95% CI)		18 to <65 Years (95% CI)		≥65 Years (95% CI)		≥18 Years (95% CI)	
	V89_11		V89_04		V89_13		V89_18	
	Prev. Vacc.	No Prev. Vacc.	Prev. Vacc.	No Prev. Vacc.	Prev. Vacc.	No Prev. Vacc.	Prev. Vacc.	No Prev. Vacc.
	N=60	N=232	N=117	N=361	N=427	N=266	N=1197	N=1052
GMT Day 1	4.96 (4.75; 5.18)	5.29 (4.95; 5.66)	6.34 (5.01; 8.03)	5.96 (5.59; 6.35)	8.26 (7.49; 9.1)	7.51 (6.32; 8.93)	18.1 (16.9; 19.3)	15.2 (14.3; 16.1)
	N=52	N=218	N=114	N=350	N=420	N=261	N=1194	N=1051
GMT Day 22	80 (41; 156)	63 (39; 101)	28 (17; 45)	36 (30; 44)	28 (24; 33)	26 (20; 32)	43.4 (40.5; 46.5)	50.3 (47.2; 53.7)
GMR Day 22/Day 1	***16*** (8.37; 31)[a]	***12*** (7.66; 20)[b]	***4.17*** (2.59; 6.72)	***6.08*** (4.99; 7.4)	***3.52*** (3.01; 4.12)	***3.14*** (2.49; 3.95)	***2.53*** (2.36; 2.71)	***3.29*** (3.09; 3.52)
	N=49	N=219	N=108	N=343	N=414	N=259	N=1154	N=1002
GMT Day 43	1330 (932; 1897)	1425 (856; 2371)	195 (110; 345)	302 (239; 382)	124 (103; 148)	156 (118; 207)	108.2 (100.7; 116.3)	162.5 (151.7; 174.0)
GMR Day 43/Day 1	***277*** (191; 403)[c]	***281*** (169; 468)[d]	***29*** (16; 53)	***51*** (40; 64)	***15*** (13; 18)	***20*** (15; 26)	***6.28*** (5.84; 6.75)	***10.48*** (9.78; 11.22)

Source: CSR V89_11: Table 14.2.3.3.7; CSR V89_04: Table 14.2.1.3.7; CSR V89_13: Table 14.2.1.3.7; CSR V89_18: Table 14.2.1.4.2.

Abbreviations: CI = confidence interval; FAS = full analysis set; HI = hemagglutination inhibition; GMR = geometric mean ratio; GMT = geometric mean titre; N = number of subjects; PPS = per protocol set.

[a] N=51, [b] N=217, [c] N=47, [d] N=213

Bold, italic = former CHMP criteria met

Immunogenicity by Age groups

Table 54.

Studies V89_11, V89_13 and V89_18 – Percentages of Subjects Achieving Seroconversion and Percentages of Subjects Achieving an HI Titre ≥1:40 Against the Homologous Strain by Age at Days 1, 22 and 43 in the Full Dose Group – HI Assay – FAS (V89_11, V89_13) and PPS (V89_18)

	V89_11 (95% CI)			V89_18 (95% CI)		V89_13 (95% CI)	V89_18 (95% CI)	V89_13 (95% CI)	V89_18 (95% CI)
	6 to <36 Months	3 to <9 Years	9 to <18 Years	18 to <50 Years	50 to <65 Years	65 to <75 Years		≥75 Years	
Seroconversion									
Day 22	N=83 ***58*** (**46**; 69)	N=95 ***43*** (33; 54)	N=1023 ***54*** (**44**; 64)	N=651 ***46.2*** (**42.4**; 50.2)	N=464 32.3 (28.1; 36.8)	N=520 **39** (**35**; 43)	N=785 26.9 (23.8; 30.1)	N=161 27 (20; 34)	N=345 18.0 (14.1; 22.4)
Day 43	N=84 ***99*** (**94**; 100)	N=93 ***98*** (**92**; 100)	N=102 ***92*** (**85**; 97)	N=625 ***85.4*** (**82.4**; 88.1)	N=451 ***72.3*** (**67.9**; 76.4)	N=514 ***77*** (**74**; 81)	N=745 ***57.6*** (**53.9**; 61.2)	N=159 ***61*** (**53**; 69)	N=335 ***46.0*** (**40.5**; 51.5)
HI Titre ≥1:40									
Day 1	N=93 1 (0.027; 6)	N=98 0 (0; 4)	N=103 2 (0; 7)	N=652 16.7 (13.9; 19.8)	N=464 18.3 (14.9; 22.1)	N=530 11 (8; 14)	N=785 28.8 (25.6; 32.1)	N=163 18 (12; 25)	N=348 27.0 (22.4; 32.0)
Day 22	N=85 58 (46; 68)	N=96 43 (33; 53)	N=102 54 (44; 64)	N=651 66.7 (62.9; 70.3)	N=464 58.0 (53.3; 62.5)	N=520 50 (46; 55)	N=785 58.7 (55.2; 62.2)	N=161 43 (36; 52)	N=345 48.4 (43.0; 53.8)
Day 43	N=91 ***98*** (**92**; 100)	N=94 ***98*** (**93**; 100)	N=102 ***92*** (**85**; 97)	N=625 ***95.7*** (**93.8**; 97.1)	N=451 ***89.6*** (**86.4**; 92.2)	N=514 ***83*** (**79**; 86)	N=745 ***85.4*** (**82.6**; 87.8)	N=159 ***74*** (**67**; 81)	N=335 ***79.4*** (**74.7**; 83.6)

Source: CSR V89_11: Table 14.2.1.2.8, Table 14.2.1.1.8; CSR V89_13: Table 14.2.1.2.8, Table 14.2.1.1.8; CSR V89_18: Table 14.2.1.2.8, Table 14.2.1.3.8.

Abbreviations: CBER = Center for Biologics Evaluation and Research; CI = confidence interval; FAS = full analysis set; HI = hemagglutination inhibition; N = number of subjects; PPS = per protocol set; seroconversion = subjects with a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40, or, subjects with a prevaccination HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titre.

Bold = CBER criteria met (lower bound of CI), ***bold, italic*** = former CHMP criteria met

Table 55.

Studies V89_11, V89_13 and V89_18 – GMTs and GMRs Against the Homologous Strain by Age at Days 1, 22 and 43 in the Full Dose Group - HI Assay – FAS (V89_11, V89_13) and PPS (V89_18)

	V89_11 (95% CI)			V89_18 (95% CI)		V89_13 (95% CI)	V89_18 (95% CI)	V89_13 (95% CI)	V89_18 (95% CI)
	6 to <36 Months	3 to <9 Years	9 to <18 Years	18 to <50 Years	50 to <65 Years	65 to <75 Years		≥75 Years	
GMT Day 1	N=93 5.66 (5.26; 6.09)	N=98 5 (5; 5)	N=103 5.1 (4.66; 5.58)	N=652 12.7 (11.9; 13.6)	N=464 14.8 (13.6; 16.2)	N=530 7.75 (7.1; 8.47)	N=785 20.4 (19.0; 21.8)	N=163 10 (8.18; 12)	N=348 20.6 (18.3; 23.2)
GMT Day 22	N=85 59 (36; 97)	N=96 49 (30; 81)	N=102 80 (46; 139)	N=651 55.4 (51.0; 60.2)	N=464 44.2 (40.0; 48.9)	N=520 27 (23; 30)	N=785 44.6 (41.6; 47.8)	N=161 24 (18; 31)	N=345 38.5 (34.0; 43.5)
GMR Day 22 /Day 1	***12*** (7.28; 19)[a]	***9.81*** (5.96; 16)[b]	***15*** (8.78; 27)	***4.22*** (3.88; 4.59)	***3.27*** (2.95; 3.62)	***3.44*** (3.01; 3.94)	***2.26*** (2.11; 2.42)	***2.46*** (1.86; 3.24)	1.91 (1.69; 2.16)
GMT Day 43	N=91 1842 (1142; 2972)	N=94 1244 (766; 2019)	N=102 961 (545; 1696)	N=625 206.5 (190.4; 224.0)	N=451 134.4 (121.1; 149.1)	N=514 144 (123; 170)	N=745 105.4 (98.1; 113.3)	N=159 95 (70; 130)	N=335 82.5 (72.9; 93.2)
GMR Day 43 /Day 1	***302*** (192; 476)[c]	***249*** (153; 404)[d]	***186*** (105; 328)	***15.53*** (14.32; 16.85)	***9.85*** (8.88; 10.93)	***19*** (16; 22)	***5.30*** (4.93; 5.70)	***9.92*** 7.19; 14)	***4.07*** (3.60; 4.60)

Source: CSR V89_11: Table 14.2.1.3.8; CSR V89_18: Table 14.2.1.4.8; CSR V89_13: Table 14.2.1.3.8.

Abbreviations: CI = confidence interval; FAS = full analysis set; GMR = geometric mean ratio; GMT = geometric mean titre; N = number of subjects; PPS = per protocol set.

Bold, italic = former CHMP criteria met

[a] N=84, [b] N=95, [c] N=84, [d] N=93

Antibody persistence against homologous strains

Table 56.

Studies V89_11, V89P1, V89_04, V89_13, V89_18 and V89P1 – Percentages of Subjects Achieving Seroconversion and Percentages of Subjects Achieving an HI Titre ≥1:40 at Days 1, 22, 43, 183, 366 or 387 in the Full Dose Group – HI Assay – FAS (V89_04, V89_11, V89_13), PPS (V89_18) and MPPS (V89P1)

	6 Months to ≤17 Years	18 to ≤40 Years	18 to <65 Years		18 to <60 Years		≥65 Years		≥60 Years
	V89_11 (97.5% CI)	V89P1 (95% CI)	V89_04 (97.5% CI)	V89_18 (95% CI)	V89_04 (95% CI)	V89_18 (95% CI)	V89_13 (97.5% CI)	V89_18 (95% CI)	V89_18 (95% CI)
Seroconversion									
	N=281	N=40	N=464	N=1115	N=434	N=979	N=681	N=1130	N=1266
Day 22	***52*** **(45**; 58)	20 (9; 36)	***48*** **(43**; 54)	***40.4*** (37.6; 43.4)	***49*** (44; 54)	***42.2*** (39.1; 45.4)	***36*** **(32**; 40)	24.2 (21.7; 26.8)	24.6 (22.2; 27.0)
	N=279	N=41	N=451	N=1076	N=423	N=947	N=673	N=1080	N=1209
Day 43	***96*** **(93**; 98)	***80*** **(65**; 91)	***83*** **(78**; 87)	***79.9*** **(77.4**; 82.3)	***83*** (80; 87)	***81.6*** (79.0; 84.0)	***74*** (70; 77)	***54.0*** **(51.0**; 57.0)	***55.4*** (52.6; 58.2)
	N=264	N=41	N=411	N=1025	N=386	N=899	N=658	N=1054	N=1180
Day 183	-	-	-	16.2 (14.0; 18.6)	-	17.0 (14.6; 19.6)	-	8.0 (6.4; 9.8)	8.2 (6.7; 9.9)
Day 366	-	7 (2; 20)	-	-	-	-	-	-	-
Day 387	***47*** **(40**; 54)	-	22 (17; 27)	-	22 (18;26)	-	23 (20; 27)	-	-
HI Titre ≥1:40									
	N=294	N=41	N=478	N=1116	N=448	N=980	N=693	N=1133	N=1269
Day 1	1 (0; 3)	0 (0; 9)	4 (2; 7)	13.0 (10.7; 15.6)	4 (2; 6)	17.1 (14.8; 19.7)	12 (10; 15)	27.8 (24.9; 30.9)	27.3 (24.8; 29.8)
	N=283	N=40	N=464	N=1115	N=434	N=979	N=681	N=1130	N=1266
Day 22	51 (44; 58)	20 (9; 36)	52 (46; 57)	65.7 (62.6; 68.7)	52 (47; 57)	63.2 (60.1; 66.3)	49 (44; 53)	57.3 (54.0; 60.4)	56.2 (53.5; 59.0)
	N=287	N=41	N=451	N=1076	N=423	N=947	N=673	N=1080	N=1209
Day 43	***96*** **(92**; 98)	***80*** (65; 91)	***85*** **(81**; 88)	***95.0*** **(93.4**; 96.2)	***85*** (81; 88)	***93.6*** (91.8; 95.0)	***81*** (77; 84)	***85.7*** **(83.3**; 87.9)	***84.2*** (82.0; 86.2)
	N=271	N=41	N=411	N=1025	N=386	N=899	N=658	N=1054	N=1180
Day 183	-	-	-	33.6 (30.6; 36.8)	-	34.3 (31.2; 37.5)	-	29.2 (26.2; 32.4)	31.3 (28.6; 34.0)
Day 366	-	7 (2;20)	-	-	-	-	-	-	-
Day 387	47 (40; 54)	-	27 (22; 32)	-	26 (22;31)	-	35 (31; 39)	-	-

Source: CSR V89P1: Table 14.2.1.1.5.2, Table 14.2.1.2.5.2; CSR V89_04: Table 14.2.1.1, Table 14.2.1.1.12, Table 14.2.1.2, Table 14.2.1.2.10; CSR V89_11: Table 14.2.1.1, Table 14.2.1.2; CSR V89_13: Table 14.2.1.1, Table 14.2.1.2, Table 14.2.1.2.10; CSR V89_18: Table 14.2.1.3.6, Table 14.2.1.3.7, Table 14.2.1.2.6.1, Table 14.2.1.2.7.

Abbreviations: CBER = Center for Biologics Evaluation and Research; CI = confidence interval; FAS = full analysis set; HI = hemagglutination inhibition; MPPS = modified per protocol set; N = number of subjects; PPS = per protocol set; seroconversion = subjects with a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40, or, subjects with a prevaccination HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titre.

Bold = CBER criteria met (lower bound of CI), ***bold, italic*** = former CHMP criteria met

Table 57.

Studies V89_11, V89P1, V89_04, V89_13 and V89_18 – GMTs and GMRs Against the Homologous Strain at Days 1, 22, 43, 183, 366 or 387 in the Full Dose Group – HI Assay – FAS (V89_04, V89_11, V89_13), PPS (V89_18) and MPPS (V89P1)

	6 Months to ≤17 Years	18 to ≤40 Years	18 to <65 Years		18 to <60 Years		≥65 Years		≥60 Years
	V89_11 (97.5% CI)	V89P1 (95% CI)	V89_04 (95% CI)	V89_18 (95% CI)	V89_04 (95% CI)	V89_18 (95% CI)	V89_13 (97.5% CI)	V89_18 (95% CI)	V89_18 (95% CI)
	N=294	N=41	N=478	N=1116	N=448	N=980	N=693	N=1133	N=1269
GMT Day 1	5.23 (5; 5.48)	5 (4.66; 5.36)	6.11 (5.78; 6.46)	13.5 (12.8; 14.2)	6 (5.67;6.35)	13.0 (12.3; 13.8)	8.29 (7.57; 9.08)	20.5 (19.4; 21.8)	20.1 (19.1; 21.3)
	N=283	N=40	N=464	N=1115	N=434	N=979	N=681	N=1130	N=1266
GMT Day 22	64 (46; 90)	9.17 (7.08; 12)	33 (28; 39)	50.6 (47.6; 53.8)	32 (27; 38)	51.1 (47.9; 54.6)	26 (23; 30)	42.4 (40.0; 45.0)	42.9 (40.5; 45.3)
GMR Day 22/Day 1	***13*** (9; 18)[a]	1.83 (1.41; 2.39)	***5.37*** (4.6; 6.27)	***3.81*** (3.58; 4.05)	**5.36** (4.55; 6.31)	**3.92** (3.67; 4.18)	***3.21*** (2.8; 3.68)	***2.14*** (2.02; 2.27)	***2.22*** (2.10; 2.35)
	N=287	N=41	N=451	N=1076	N=423	N=947	N=673	N=1080	N=1209
GMT Day 43	1356 (985; 1866)	143 (86; 238)	250 (208; 302)	170.7 (160.5; 181.6)	265 (218; 322)	177.4 (166.2; 189.4)	129 (110; 152)	97.9 (92.1; 104.1)	100.7 (95.0; 106.7)
GMR Day 43/Day 1	***262*** (190; 361)[b]	***29*** (17; 48)	***41*** (34; 49)	***12.70*** (11.94; 13.51)	***44*** (36; 53)	***13.44*** (12.59; 14.35)	***16*** (13; 19)	***4.90*** (4.61; 5.20)	**5.18** (4.88; 5.49)

	6 Months to ≤17 Years	18 to ≤40 Years	18 to <65 Years		18 to <60 Years		≥65 Years		≥60 Years
	V89_11 (97.5% CI)	V89P1 (95% CI)	V89_04 (95% CI)	V89_18 (95% CI)	V89_04 (95% CI)	V89_18 (95% CI)	V89_13 (97.5% CI)	V89_18 (95% CI)	V89_18 (95% CI)
	N=271	N=41	N=411	N=1025	N=386	N=899	N=658	N=1054	N=1180
GMT Day 183	-	-	-	20.4 (19.3; 21.6)	-	20.4 (19.3; 21.7)	-	19.3 (18.2; 20.4)	19.3 (18.3; 20.4)
GMR Day 183/Day 1	-	-	-	1.53 (1.44; 161)	-	1.56 (1.47; 1.66)	-	0.97 (0.91; 1.02)	0.99 (0.94; 1.05)
Day 366	-	6.28 (5.21; 7.57)	-	-	-	-	-	-	-
Day 366/Day 1	-	1.26 (1.03; 1.53)	-	-	-	-	-	-	-
Day 387	62 (45; 86)	-	12 (11; 14)	-	12 (10;13)	-	16 (14; 18)	-	-
Day 387/Day 1	***12*** (8.76; 17)[c]	-	1.95 (1.73; 2.19)	-	1.93 (1.71; 2.17)	-	1.97 (1.76; 2.2)	-	-

Source: CSR V89P1: Table 14.2.1.3.5.2; CSR V89_11: Table 14.2.1.3; CSR V89_04: Table 14.2.1.3, Table 14.2.1.3.10; CSR V89_13: Table 14.2.1.3; CSR V89_18: Table 14.2.1.4.6, Table 14.2.1.4.7.

Abbreviations: CI = confidence interval; FAS = full analysis set; GMR = geometric mean ratio; GMT = geometric mean titre; MPPS = modified per protocol set; N = number of subjects; PPS = per protocol set.

[a] N=281, [b] N=279, [c] N=264

Bold, italic = former CHMP criteria met

There were no consistent or remarkable differences in immunogenicity by sex or race (see the Clinical AR for detailed tables).

2.6.5.5. Supportive studies

Supportive Paediatric H1N1 Study V110_04

Study V110_04 was a Phase 3, randomised, observer-blind, dose-ranging, multicentre study conducted to evaluate immunogenicity, safety and tolerability of different formulations of MF59 adjuvanted and non-adjuvanted cell culture-derived, inactivated novel swine origin A/H1N1 monovalent subunit influenza vaccine (H1N1c) in paediatric subjects 6 months to ≤17 years of age.

Subjects were randomly assigned (1:1 or 1:1:1) to receive a series of 2 IM injections administered 3 weeks apart, of H1N1c containing either 3.75 µg of the H1N1 (A/California/7/2009 strain) influenza antigen with 0.125 mL MF59 (half dose), 7.5 µg of the H1N1 influenza antigen with 0.25 mL MF59 (full

dose), or 15 µg of the H1N1 influenza antigen without MF59 (the latter non-adjuvanted formulation only for subjects aged 3 to 8 years or 12 to 35 months). Subjects were stratified by age (9 to 17 years, 3 to 8 years, 12 to 35 months, and 6 to 11 months). A total of 660 healthy paediatric subjects were vaccinated, of whom 290 received the half dose (3.75 µg HA+0.125 mL MF59), 298 received the full dose (7.5 µg HA+0.25 mL MF59), and 72 received 15 µg HA without MF59.

Criteria for evaluation: The measures of immunogenicity included seroconversion rate, the percentage of subjects achieving an HI titre ≥1:40 and GMTs/GMRs. In addition, antibody responses against the homologous influenza strain were evaluated using the MN assay. Results were analysed according to CHMP criteria, CBER criteria were also applied to the results *post hoc*. Of note, CBER criteria were not part of the original study analyses and were applied for the purpose of this MAA.

Demographic and other baseline characteristics of the enrolled subjects were balanced between the vaccine groups within all age cohorts.

Immunogenicity results: This study demonstrated that a two-dose regimen of either the full or half dose of adjuvanted H1N1c achieved the desired immunogenicity titre and seroconversion rates for both CHMP and CBER criteria in a paediatric population (6 months to 17 years of age).

At Day 43, in all age cohorts, all 3 CHMP criteria were met in both the half and full dose group as well as the non-adjuvanted dose group. At Day 22, in all age cohorts, all 3 CHMP criteria were met in both the half and full dose group except for the half dose group in the 6 to 11 months cohort where 57% of the subjects had an HI titre ≥ 40 (see Table 58 and Table 59).

At Day 43, in all age cohorts, both CBER criteria were met in both the half and full dose group as well as the non-adjuvanted dose group (Table 58). At Day 22, the CBER criterion for seroconversion was met in the half dose and the full dose group in all age cohorts. At Day 22, the CBER criterion for HI titre ≥40 was met in the 9 to 17 years cohort (half and full dose) and in the 3 to 8 years cohort (full dose only).

Prevaccination HI titres on Day 1 were negligible in all age cohorts and dose groups. At Day 22, HI titres were increased over baseline in all age cohorts and dose groups and a further increase over baseline was observed at Day 43 (Table 59).

The MN assay results were consistent with the immunogenicity results of the HI assay.

Table 58.

Study V110_04 - Percentages of Subjects Achieving Seroconversion (95% CI) and Percentages of Subjects Achieving an HI Titre ≥1:40 (95% CI) Against the Homologous Strain per Vaccine Group at Days 1, 22 and 43 (Without Site 3) – PPS Day 43, HI Assay

	Cohort 9 to 17 Years		Cohort 3 to 8 Years			Cohort 12 to 35 Months			Cohort 6 to 11 Months	
	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59	15 µg HA	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59	15 µg HA	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59
	N=72	N=71	N=58	N=60	N=31	N=51	N=53	N=25	N=58	N=54
Seroconversion										
Day 22	***78*** **(66; 87)**	***89*** **(79; 95)**	***79*** **(67; 89)**	***83*** **(71; 92)**	***65*** **(45; 81)**	***73*** **(58; 84)**	***77*** **(64; 88)**	32 (15; 54)	***57*** **(43; 70)**	***74*** **(60; 85)**
Day 43	***99*** **(93; 100)**	***100*** **(95; 100)**	***100*** **(94; 100)**	***100*** **(94; 100)**	***97*** **(83; 100)**	***98*** **(90; 100)**	***100*** **(93; 100)**	***84*** **(64; 95)**	***98*** **(91; 100)**	***100*** **(93; 100)**
HI Titre ≥1:40										
Day 1	6 (2; 14)	3 (0; 10)	0 (0; 6)	2 (0.042; 9)	0 (0.042; 7)	12 (4; 24)	13 (5; 25)	20 (7; 41)	17 (9; 29)	17 (8; 29)
Day 22	***82*** **(71; 90)**	***90*** **(81; 96)**	***79*** (67; 89)	***83*** **(71; 92)**	65 (45; 81	***73*** (58; 84)	***77*** (64; 88)	32 (15; 54)	57 (43; 70)	***74*** (60; 85)
Day 43	***100*** **(95; 100)**	***100*** **(95; 100)**	***100*** **(94; 100)**	***100*** **(94; 100)**	***97*** **(83; 100)**	***100*** **(93; 100)**	***100*** **(93; 100)**	***88*** (69; 97)	***100*** **(94; 100)**	***100*** **(93; 100)**

Source: CSR V110_04 Addendum 1: Table 14.2.1.1.1, Table 14.2.1.1.1.1, Table 14.2.1.1.1.2, Table 14.2.1.1.1.3, Table 14.2.1.1.4, Table 14.2.1.1.4.1, Table 14.2.1.1.4.2, Table 14.2.1.1.4.3.

Abbreviations: CBER = Center for Biologics Evaluation and Research; CHMP = Committee for Medicinal Products for Human Use; CI = confidence interval; HA = hemagglutinin; HI = hemagglutination inhibition; MPPS = modified per protocol set; N = total number of subjects; HI titre ≥1:40 = percentages of subjects with HI titre ≥1:40; Seroconversion = subjects with a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40, or, subjects with a prevaccination HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titre.

Note: CBER criteria were not part of the original study analyses, and were applied for the purpose of this document; **bold** = CBER criteria met; ***bold, italic*** = former CHMP criteria met.

Table 59.

Study V110_04 - GMTs and GMRs (95% CI) Against the Homologous Strain per Vaccine Group at Days 1, 22 and 43 (Without Site 3) – PPS, HI Assay

	Cohort 9 to 17 Years		Cohort 3 to 8 Years			Cohort 12 to 35 Months			Cohort 6 to 11 Months	
	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59	15 µg HA	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59	15 µg HA	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59
	N=72	N=71	N=58	N=60	N=31	N=51	N=53	N=25	N=58	N=54
GMT Day 1	6.72 (5.58; 8.1)	5.86 (4.89; 7.01)	5.65 (5.1; 6.26)	5.39 (4.9; 5.94)	5.38 (4.75; 6.08)	7.33 (4.08; 13)	8.52 (5.01; 14)	12 (5.79; 23)	8.35 (3.37; 21)	8.33 (3.35; 21)
GMT Day 22	90 (61; 131)	140 (97; 203)	61 (41; 90)	73 (50; 107)	35 (21; 56)	43 (21; 91)	63 (32; 123)	22 (9.26; 54)	43 (13; 148)	82 (24; 282)
GMR Day 22/ Day 1	***13*** (9.09; 20)	***24*** (17; 35	***11*** (7.29; 16)	***14*** (9.46; 20)	***6.46*** (4.07; 10)	***5.93*** (3.53; 9.97)	***7.36*** (4.6; 12)	1.92 (1.04; 3.56)	***5.19*** (2.63; 10)	***9.89*** (5.01; 20)
GMT Day 43	314 (254; 387)	466 (380; 572)	487 (379; 626)	622 (492; 788)	151 (112; 204)	650 (419; 1009)	917 (616; 1366)	131 (77; 221)	770 (431; 1376)	1074 (600; 1921)
GMR Day 43/ Day 1	***47*** (36; 61)	***80*** (61; 103)	***86*** (67; 111)	***115*** (91; 147)	***28*** (21; 38)	***89*** (54; 145)	***108*** (69; 169)	***11*** (6.23; 20)	***92*** (51; 166)	***129*** (72; 232)

Source: CSR V110_04 Addendum 1: Table 14.2.1.1.6, Table 14.2.1.1.6.1, Table 14.2.1.1.6.2, Table 14.2.1.1.6.3.

Abbreviations: CI = confidence interval; GMR = geometric mean ratio; GMT = geometric mean titre; HA = hemagglutinin; HI = hemagglutination inhibition; PPS = per protocol set; N = total number of subjects.

Note: ***bold, italic*** = former CHMP criterion met.

Supportive H3N2 Study V129_01

Study V129_01 was a Phase 1, randomised, observer-blind, dose-ranging, multicentre study conducted to evaluate immunogenicity, safety and tolerability of different formulations of MF59 adjuvanted and non-adjuvanted cell culture-derived, inactivated novel swine origin A/H3N2 monovalent subunit influenza vaccine (H3N2c) in paediatric subjects 3 to<18 years, adults 18 to <65 years, and elderly ≥ 65 years.

Subjects were randomly assigned (1:1:1) to receive a series of 2 IM injections administered 3 weeks apart, of H3N2c containing either 3.75 µg of the H3N2 (A/Minnesota/11/2010 strain) influenza antigen with 0.125 mL MF59 (half dose), 7.5 µg of the H3N2 influenza antigen with 0.25 mL MF59 (full dose),

or 15 µg of the H3N2 influenza antigen without MF59. Subjects were stratified by age (3 to <9 years, 9 to <18 years, 18 to <65 years, and ≥65 years). A total of 624 healthy subjects were vaccinated, of whom 211 received the half dose (3.75 µg HA+0.125 mL MF59), 210 received the full dose (7.5 µg HA+0.25 mL MF59), and 203 received 15 µg HA without MF59.

Criteria for evaluation: The measures of immunogenicity included seroconversion rate, the percentage of subjects achieving an HI titre ≥1:40 and GMTs/GMRs. Results were also evaluated according to CBER and CHMP criteria.

Demographic and other baseline characteristics of the enrolled subjects were balanced between the vaccine groups within all age groups.

Immunogenicity results: This study demonstrated that after the first and the second vaccination, adequate antibody response can be achieved using adjuvanted and non-adjuvanted H3N2c vaccine meeting both CBER and CHMP criteria across all age groups (3 to <9 years, 9 to <18 years, 18 to <65 years and ≥65 years). After both the first and the second vaccination, increases in GMTs were higher in subjects receiving adjuvanted vaccine than in subjects receiving non-adjuvanted vaccine.

At Day 22 and Day 43, CBER criteria for seroconversion and HI titre ≥40 were met in both the half and full dose group as well as the non-adjuvanted dose group across all age groups, except for subjects 3 to <9 years of age in the non-adjuvanted dose group who did not meet the CBER criterion for HI titre ≥ 40 at Day 22 and required a second dose of non-adjuvanted vaccine to achieve both CBER criteria. (see Table 60, Table 61, Table 62 and Table 63).

At Day 22 and Day 43, CHMP criteria for seroconversion, HI titre ≥40, and GMR were met in both the half and full dose group as well as the non-adjuvanted dose group across all age groups (see Table 60,

Table 61, Table 62 and Table 63).

Prevaccination HI titres on Day 1 were low across all age and dose groups. At Day 22, HI titres were increased over baseline across all age cohorts and dose groups and this increase was maintained at Day 43 (Table 61 and Table 63).

Table 60.

Study V129_01 - Percentages of Subjects >18 Years of Age Achieving Seroconversion (95% CI) and Percentages of Subjects Achieving an HI Titre ≥1:40 (95% CI) Against the Homologous Strain per Vaccine and Age Group at Days 1, 22 and 43 – FAS

	18 to <65 Years			≥65 Years		
	3.75 µg HA + 0.125 mL MF59	**7.5 µg HA + 0.25 mL MF59**	**15 µg HA**	**3.75 µg HA + 0.125 mL MF59**	**7.5 µg HA + 0.25 mL MF59**	**15 µg HA**
Seroconversion						
Day 22	N=52 ***73*** (**59**; 84.4)	N=54 ***89*** (**77.4**; 95.8)	N=48 ***67*** (**51.6**; 79.6)	N=49 ***57*** (**42.2**; 71.2)	N=49 ***71*** (**56.7**; 83.4)	N=51 ***61*** (**46.1**; 74.2)
Day 43	N=51 ***82*** (**69.1**; 91.6)	N=52 ***92*** (**81.5**; 97.9)	N=48 ***71*** (**55.9**; 83)	N=48 ***58*** (**43.2**; 72.4)	N=49 ***80*** (**65.7**; 89.8)	N=50 ***64*** (**49.2**; 77.1)
HI Titre ≥1:40						
Day 1	N=53 57 (42.3; 70.2)	N=55 67 (53.3; 79.3)	N=48 67 (51.6; 79.6)	N=50 56 (41.3; 70)	N=50 52 (37.4; 66.3)	N=52 46 (32.2; 60.5)
Day 22	N=52 ***96*** (**86.8**; 99.53)	N=54 ***100*** (**93.4**; 100)	N=48 ***88*** (**74.8**; 95.3)	N=49 ***94*** (**83.1**; 98.7)	N=49 ***100*** (**92.7**; 100)	N=51 ***96*** (**86.5**; 99.52)
Day 43	N=51 ***96*** (**86.5**; 99.52)	N=52 ***100*** (**93.2**; 100)	N=48 ***98*** (**88.9**; 99.95)	N=48 ***98*** (**88.9**; 99.95)	N=49 ***100*** (**92.7**; 100)	N=50 ***96*** (**86.3**; 99.51)

Source: CSR V129_01: Table 14.2.1.1; Table 14.2.1.2; Table 14.2.1.1.7 and Table 14.2.1.2.7.
Abbreviations: CBER = Center for Biologics Evaluation and Research; CHMP = Committee for Medicinal Products for Human Use; CI = confidence interval; FAS = full analysis set; HA = hemagglutinin; HI = hemagglutination inhibition; N = total number of subjects; HI titre ≥1:40 = percentages of subjects with HI titre ≥1:40; Seroconversion = subjects with a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40, or, subjects with a prevaccination HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titre.
Note: **bold** = CBER criteria met; ***bold, italic*** = former CHMP criteria met.

Table 61.

Study V129_01 - GMTs and GMRs (95% CI) Against the Homologous Strain in Subjects >18 Years of Age per Vaccine and Age Group at Days 1, 22 and 43 – FAS, HI Assay

	18 to <65 Years			≥65 Years		
	3.75 µg HA + 0.125 mL MF59	**7.5 µg HA + 0.25 mL MF59**	**15 µg HA**	**3.75 µg HA + 0.125 mL MF59**	**7.5 µg HA + 0.25 mL MF59**	**15 µg HA**
GMT Day 1	N=53 34 (26; 44)	N=55 52 (40; 68)	N=48 42 (32; 56)	N=50 36 (28; 47)	N=50 32 (25; 42)	N=52 26 (20; 34)
GMT Day 22	N=52 300 (217; 415)	N=54 504 (367; 692)	N=48 226 (162; 315)	N=49 135 (103; 177)	N=49 206 (157; 270)	N=51 140 (107; 183)
GMR Day 22/ Day 1	N=52 ***7.54*** (5.41; 11)	N=54 ***11*** (8.16; 16)	N=48 ***5.35*** (3.80; 7.55)	N=49 ***3.79*** (2.71; 5.29)	N=49 ***6.64*** (4.75; 9.28)	N=51 ***5.13*** (3.70; 7.13)
GMT Day 43	N=51 276 (208; 365)	N=52 427 (324; 564)	N=48 261 (196; 347)	N=48 131 (102; 170)	N=49 202 (157; 261)	N=50 163 (126; 210)
GMR Day 43/ Day 1	N=51 ***7.16*** (5.34; 9.61)	N=52 ***9.58*** (7.17; 13)	N=48 ***6.24*** (4.62; 8.44)	N=48 ***3.82*** (2.80; 5.21)	N=49 ***6.55*** (4.81; 8.91)	N=50 ***5.94*** (4.38; 8.07)

Source: CSR V129_01: Table 14.2.1.3 and Table 14.2.1.3.7.
Abbreviations: CI = confidence interval; FAS = full analysis set; GMR = geometric mean ratio; GMT = geometric mean titre; HA = haemagglutinin; HI = haemagglutination inhibition; N = total number of subjects.
Note: ***bold, italic*** = former CHMP criterion met.

Table 62.

Study V129_01 - Percentages of Subjects 3 to <18 Years Achieving Seroconversion (95% CI) and Percentages of Subjects Achieving an HI Titre ≥1:40 (95% CI) Against the Homologous Strain per Vaccine and Age Group at Days 1, 22 and 43 – FAS

	3 to <9 Years			9 to <18 Years		
	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59	15 µg HA	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59	15 µg HA
Seroconversion						
Day 22	N=47 ***96*** (**85.5**; 99.5)	N=54 ***98*** (**90.1**; 99.95)	N=49 ***71*** (**56.7**; 83.4)	N=52 ***88*** (**76.6**; 95.6)	N=48 ***98*** (**88.9**; 99.95)	N=53 ***85*** (**72.4**; 93.3)
Day 43	N=43 ***100*** (**91.8**; 100)	N=53 ***100*** (**93.3**; 100)	N=48 ***92*** (**80**; 97.7)	N=52 ***94*** (**84.1**; 98.8)	N=49 ***98*** (**89.1**; 99.95)	N=53 ***91*** (**79.3**; 96.9)
HI Titre ≥1:40						
Day 1	N=53 8 (2.1; 18.2)	N=55 7 (2; 17.6)	N=50 14 (5.8; 26.7)	N=53 45 (31.6; 59.6)	N=51 59 (44.2; 72.4)	N=53 49 (35.1; 63.2)
Day 22	N=47 ***96*** (**85.5**; 99.5)	N=54 ***98*** (**90.1**; 99.95)	N=49 ***78*** (63.4; 88.2)	N=52 ***100*** (**93.2**; 100)	N=48 ***100*** (**92.6**; 100)	N=53 ***98*** (**89.9**; 99.95)
Day 43	N=43 ***100*** (**91.8**; 100)	N=53 ***100*** (**93.3**; 100)	N=48 ***96*** (**85.7**; 99.5)	N=52 ***100*** (**93.2**; 100)	N=49 ***100*** (**92.7**; 100)	N=53 ***100*** (**93.3**; 100)

Source: CSR V129_01: Table 14.2.1.1.7, Table 14.2.1.2.7.

Abbreviations: CBER = Center for Biologics Evaluation and Research; CHMP = Committee for Medicinal Products for Human Use; CI = confidence interval; FAS = full analysis set; HA = hemagglutinin; HI = hemagglutination inhibition; N = total number of subjects; HI titre ≥1:40 = percentages of subjects with HI titre ≥1:40; Seroconversion = subjects with a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40, or, subjects with a prevaccination HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titre.

Note: **bold** = CBER criteria met; ***bold, italic*** = former CHMP criteria met.

Table 63.

Study V129_01 - GMTs and GMRs (95% CI) Against the Homologous Strain in Subjects 3 to <18 Years per Vaccine and Age Group at Days 1, 22 and 43 – FAS, HI Assay

	3 to <9 Years			9 to <18 Years		
	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59	15 µg HA	3.75 µg HA + 0.125 mL MF59	7.5 µg HA + 0.25 mL MF59	15 µg HA
GMT Day 1	N=53 8.48 (6.89; 10)	N=55 8.26 (6.74; 10)	N=50 8.80 (7.10; 11)	N=53 28 (21; 37)	N=51 29 (22; 39)	N=53 27 (20; 35)
GMT Day 22	N=47 192 (134; 275)	N=54 277 (198; 387)	N=49 93 (65; 132)	N=52 386 (293; 510)	N=48 456 (341; 609)	N=53 343 (261; 452)
GMR Day 22/ Day 1	N=47 ***22*** (15; 32)	N=54 ***33*** (23; 47)	N=49 ***11*** (7.51; 16)	N=52 ***14*** (9.58; 19)	N=48 ***15*** (10; 22)	N=53 ***13*** (8.91; 18)
GMT Day 43	N=43 524 (396; 694)	N=53 679 (527; 874)	N=48 198 (152; 258)	N=52 486 (379; 623)	N=49 656 (508; 849)	N=53 389 (304; 498)
GMR Day 43/ Day 1	N=43 ***59*** (43; 81)	N=53 ***82*** (62; 109)	N=48 ***23*** (17; 31)	N=52 ***18*** (13; 25)	N=49 ***21*** (15; 30)	N=53 ***14*** (10; 20)

Source: CSR V129_01: Table 14.2.1.3.7.

Abbreviations: CI = confidence interval; FAS = full analysis set; GMR = geometric mean ratio; GMT = geometric mean titre; HA = hemagglutinin; HI = hemagglutination inhibition; N = total number of subjects.

Note: ***bold, italic*** = former CHMP criterion met.

Supportive H7N9 Study V131_01

Study V131_01 was a Phase 1 randomised, observer-blind, dose-ranging multicentre study conducted to evaluate immunogenicity and safety of different formulations of MF59 adjuvanted and non-adjuvanted cell culture-derived, inactivated A/H7N9 monovalent subunit influenza vaccine (H7N9c) in healthy subjects 18 to <65 years of age. This study is considered a supportive study within the MAA.

Subjects were randomly assigned (1:1:1:1) to receive a series of 2 IM injections administered 3 weeks apart, of H7N9c containing either 3.75 µg of the H7N9 (A/Shanghai/2/2013 CC Ab strain) influenza antigen with 0.125 mL MF59 (half dose), 7.5 µg of the H7N9 influenza antigen with 0.25 mL MF59 (full dose-7.5), 15 µg of the H7N9 influenza antigen with 0.25 mL MF59 (full dose-15) and 15 µg of the H7N9 influenza antigen without MF59. Subjects were stratified by centre. A total of 401 healthy subjects were vaccinated, of whom 98 received the half dose (3.75 µg HA+0.125 mL MF59), 103 received the full dose-7.5 (7.5 µg HA+0.25 mL MF59), 98 received the full dose-15 (15 µg HA+0.25 mL MF59), and 102 received 15 µg HA without MF59.

Criteria for evaluation: The measures of immunogenicity included seroconversion rate, the percentage of subjects achieving HI titres ≥1:40 and GMTs. In addition, antibody responses against heterologous and homologous influenza strain(s) were evaluated using the MN assay.

CBER and CHMP criteria were applied to the results post hoc. The original statistical analyses for immunogenicity only included descriptive statistics using point estimates and CIs.

Immunogenicity results: The immunogenicity analysis performed using HI assay showed no appreciable response in any group after the first vaccination. Three weeks after the second vaccination substantial immune responses were observed in subjects receiving adjuvanted vaccines as compared with subjects who received non-adjuvanted vaccine. A dose-response effect was seen in adjuvanted vaccine groups; with the highest response occurring in the full dose-7.5 and full dose-15 groups, with 44% and 52% seroconversion rates, respectively. HI antibody responses following administration of higher doses (7.5 µg and 15 µg) with a full dose of MF59 were higher than with the half dose formulation (Table 64 and Table 65). At Day 43, CHMP criteria for GMR and seroconversion were met in the 2 full dose groups. CBER criteria were not met except for seroconversion in the full dose-15 group.

Demographic and other baseline characteristics of the enrolled subjects were well balanced between the vaccine groups.

Overall, the results of the immunogenicity analyses performed using the MN assay were higher than the HI results, and showed higher immune responses in adjuvanted groups than nonadjuvanted group. A dose response effect was seen in adjuvanted groups, thus confirming the results observed using the HI assay. In MN assay the dose-response effect was also observed after the first vaccination, but was more pronounced after the second vaccination, with 78% seroconversion in the full dose-15 group at Day 43.

Although the antibody response to aH7N9c was lower than that observed for other pandemic or seasonal influenza vaccines, this study demonstrates that an immune response likely predictive of protection can be achieved, which may require multiple vaccinations with adjuvanted vaccines.

Table 64.

Study V131_01 - Percentages of Subjects Achieving Seroconversion (95% CI) and Percentages of Subjects Achieving an HI Titre ≥1:40 (95% CI) Against the Homologous Strain per Vaccine Group at Days 1, 22 and 43 – FAS, HI Assay

	18 to <65 Years			
	3.75 µg HA + 0.125 mL MF59	**7.5 µg HA + 0.25 mL MF59**	**15 µg HA + 0.25 mL MF59**	**15 µg HA**
	Seroconversion			
Day 22	N=96 0 (0, 4)	N=103 0 (0, 4)	N=97 0 (0, 4)	N=102 0 (0, 4)
Day 43	N=94 26 (17, 36)	N=103 ***44*** (34, 54)	N=97 ***52*** (**41**, 62)	N=102 3 (1, 8)
	HI Titre ≥1:40			
Day 1	N=98 0 (0, 4)	N=103 0 (0, 4)	N=98 0 (0, 4)	N=102 0 (0, 4)
Day 22	N=96 0 (0, 4)	N=103 0 (0, 4)	N=97 0 (0, 4)	N=102 1 (0.025, 5)
Day 43	N=94 26 (17, 36)	N=103 44 (34, 54)	N=97 52 (41, 62)	N=102 3 (1, 8)

Source: CSR V131_01: Table 14.2.1.1, Table 14.2.1.2.

Abbreviations: CBER = Center for Biologics Evaluation and Research; CHMP = Committee for Medicinal Products for Human Use; CI = confidence interval; FAS = full analysis set; HA = hemagglutinin; HI = hemagglutination inhibition; N = total number of subjects; HI titre ≥1:40 = percentages of subjects with HI titre ≥1:40; Seroconversion = subjects with a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40, or, subjects with a prevaccination HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titre.

Note: CBER and CHMP criteria were not part of the original study analyses, and were applied for the purpose of this document; **bold** = CBER criteria met; ***bold, italic*** = former CHMP criteria met.

Table 65.

Study V131_01 - GMTs and GMRs (95% CI) Against the Homologous Strain per Vaccine Group at Days 1, 22 and 43 – FAS, HI Assay

	18 to <65 Years			
	3.75 µg HA + 0.125 mL MF59	**7.5 µg HA + 0.25 mL MF59**	**15 µg HA + 0.25 mL MF59**	**15 µg HA**
GMT Day 1	N=98 5 (4.95-5.05)	N=103 5 (4.95-5.05)	N=98 5 (4.95-5.05)	N=102 5.05 (5-5.1)
GMT Day 22	N=96 5.07 (4.95-5.2)	N=103 5 (4.88-5.12)	N=97 5 (4.88-5.13)	N=102 5.1 (4.98-5.23)
GMR Day 22/ Day 1	N=96 1.01 (1-1.03)	N=103 1 (0.98-1.02)	N=97 1 (0.98-1.02)	N=102 1.01 (0.99-1.03)
GMT Day 43	N=94 12 (9.75-15)	N=103 19 (15-23)	N=97 26 (21-32)	N=102 5.75 (4.67-7.08)
GMR Day 43/ Day 1	N=94 2.42 (1.95-3)	N=103 ***3.7*** (3.02-4.55)	N=97 ***5.2*** (4.21-6.43)	N=102 1.14 (0.93-1.4)

Source: CSR V131_01: Table 14.2.1.3.

Abbreviations: CI = confidence interval; FAS = full analysis set; GMR = geometric mean ratio; GMT = geometric mean titre; HA = hemagglutinin; HI = hemagglutination inhibition; N = total number of subjects.

Note: CHMP criteria were not part of the original study analyses, and were applied for the purpose of this document; ***bold, italic*** = former CHMP criterion met.

2.6.6. Discussion on clinical efficacy

For Incellipan, the applicant submitted a marketing authorisation application for a 'pandemic preparedness vaccine', formerly known as 'mock-up' pandemic vaccine.

Pandemic influenza vaccines are intended for use only following the declaration of a pandemic at the level of the WHO. A dossier for such an application should include data obtained with a vaccine that is the same as the intended final pandemic vaccine in terms of construct (including amount of antigen, excipients and adjuvant, if any) and mode of manufacture. When a pandemic is declared by WHO, MAHs should submit a variation to include the declared pandemic strain in the pandemic vaccine.

The influenza subtype H5N1 investigated as antigen in this dossier is in line with the guideline requirements, since it represents a potential pandemic strain that is poorly immunogenic and to which the vast majority of humans are immunologically naïve.

It is not possible to conduct efficacy trials in the absence of an influenza pandemic. Therefore, an application based on immunogenicity and safety data is acceptable.

Immunogenicity data included in the application

The submitted dossier includes data from 5 clinical trials to determine the dosage, the immunogenicity and safety of aH5N1c, a monovalent, cell culture-derived, inactivated influenza vaccine that comprises surface antigens from a potential pandemic H5N1 virus strain candidate (A/turkey/Turkey/1/2005 [H5N1] NIBRG-23 strain) and the adjuvant MF59 (MF59C.1 proprietary adjuvant).

The applicant analysed haemagglutination inhibition (HI) titres based on GMTs/GMRs and on seroconversion rates, defined as the percentage of subjects achieving either: 1) a prevaccination (baseline) HI titre <1:10 and postvaccination HI titre ≥1:40 after vaccination; or 2) a prevaccination (baseline) HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI titre), which is considered appropriate.

The application further includes microneutralisation (MN) data from all studies, but MN assays have only been performed for samples of small subsets of participants. Microneutralisation was determined *post hoc*, presumably to fulfil the EMA requirements, and the data have been included in a CSR addendum (dated with 2022, phase 3 CSR: 2018).

The submission includes data on cross reactivity against other H5N1 strains for nearly all trials, except for the main phase 3 study V89_18. Additional data from three studies investigating immunogenicity and safety of the same vaccine construct but including different influenza strains (V110_04: H1N1, V129_01: H3N2v, V131_01: H7N9) are considered as supportive evidence. Of note, V110_04 is a paediatric study and V129_01 does also include paediatric (in addition to adult) data.

The recommendations within the Guideline on Influenza Vaccines (EMA/CHMP/VWP/457259/2014) to investigate cell-mediated immunity (CMI) and anti-neuraminidase (NA) antibodies have not been followed. Investigating CMI would have been particularly useful in the elderly where antibody responses are usually lower due to immune senescence.

The submitted clinical data package for nonelderly and elderly adults is overall acceptable. In contrast, the provided paediatric data are limited and not in line with the relevant guidance, especially regarding the clinical safety database in paediatric subgroups, which describes an expectation of "300 individuals for each of the infant, children and adolescent paediatric groups". The additional supportive paediatric data for the H1N1 and H3N2v strains (using the same vaccine construct) are however considered informative. In order to support the recommendation of the full dose for children independently of age, the applicant has presented immunogenicity and safety data stratified by three age cohorts (6-36 months, 3-9 years, 9-18 years). In consequence, a recommendation of the full dose for children of all

age groups can be agreed due to the high immunogenicity and the benign safety and tolerability profile (see safety section). According to the SmPC guideline information on the half dose results were also included in section 5.1 of the SmPC, with the higher incidence of fever in the full dose group mentioned.

The applicant will need to investigate clinical effectiveness and safety of the pandemic vaccine and submit this information to the CHMP for evaluation. The Annex II of the product Information therefore includes a specific obligation in this regard.

The former CHMP criteria (CPMP/BWP/214/96) and the more stringent CBER criteria (Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines, CBER 2007) were considered as thresholds for the evaluation of immunogenicity (as primary endpoints and secondary endpoints) in all clinical trials of aH5N1c.

The Guideline on Influenza Vaccines (EMA/CHMP/VWP/457259/2014) states: "The HI titre of 1:40 was previously suggested to represent a reasonable statistical correlate for an efficacy of 50–70% against clinical symptoms of influenza based on challenge studies in healthy adults. Since then, evidence has emerged to indicate that there remains a need to better define correlates of protection against influenza, which potentially may vary according to individual characteristics, populations, specific age group (e.g. paediatric population) and vaccine type." It should be noted that this guideline was published around the same time as the phase 3 trial was initiated (and came into effect around half a year later), while all phase 1, 2 and supportive trials were conducted before.

The former criteria recommended by CHMP to assess immunogenicity of influenza vaccines and the current criteria recommended by CBER (2007) are deemed informative, but not critical for the immunogenicity assessment of the vaccine. The totality of all provided immunogenicity data shows that the candidate vaccine is likely to be efficacious in the event of a pandemic.

Considering that no correlate of protection exists for Influenza, the interpretability of the HI (and MN) antibody titres achieved following vaccination with aH5N1c is limited. The protective potential of the elicited humoral immune response cannot be predicted. However, the use of haemagglutination inhibition (HI) and microneutralisation (MN) assays to assess vaccine-induced immunogenicity is in concordance with the relevant guideline (EMA/CHMP/VWP/457259/2014). While HI and MN titres are not a true surrogate marker, it has been widely shown that higher HI titres tend to correlate with better protection.

Regarding the uncertainty of the unknown protective potential, it further has to be considered that in case of a declared pandemic, the antigen (A/turkey/Turkey/1/2005 (H5N1) like strain (NIBRG 23)) included in the main clinical trials for the development of Incellipan will be replaced by the declared pandemic strain. It is unknown how such a strain change would affect the immune response.

Dose-response/dose-selection

Study P89P1 investigated immunogenicity and safety of two IM injections (3 weeks apart) of aH5N1c (A/Indonesia/5/2005 strain) at different dose levels of antigen (3.75 µg, 7.5 µg, 15 µg HA) and MF59 adjuvant (100% [= 0.25 mL MF59], 50%, 25%, 0%). All twelve possible antigen-adjuvant combinations were investigated. The data clearly support the decision to use adjuvanted formulations in adults. Of note, the H5N1 strain included in the vaccine construct of the phase I trial (A/Indonesia/5/2005 strain) differs from the strain used for the subsequent phase 2 trials (A/turkey/Turkey/1/2005). The latter strain was also used for the phase 3 trial (V89_18).

The phase I data show that a second dose (three weeks after the first, on Day 22) elicits a stronger response regarding HI, SRH and MN titres, since the GMTs/GMRs were much higher at D43 compared to D22.

Investigating a higher unadjuvanted dose would have been of interest, but the applicant's argumentation (in the Clinical Overview) regarding the advantage of an antigen sparing effect in a pandemic setting is understood.

One secondary endpoint of Study V89P1 was to investigate immunogenicity for heterovariant strains. According to the applicant, heterologous antibody response was only tested against one heterologous strain (Turkey, Clade 2.2) strain, which was used as the antigen for the subsequent studies. Not surprisingly, the GMRs at Day 43 were lower against the heterologous Turkey strain for all three assays (HI assay, MN assay, SRH assay), but with partly overlapping confidence intervals. Some cross-reactivity was expected since both strains belong to the same H5N1 Clade 2 (Clade 2.1.3 for A/Indonesia; Clade 2.2 for A/Turkey; both sharing some common epitopes).

Additional dose-response studies were conducted in three phase 2 trials (V89_04 in healthy adults 18 to <65 years of age, V89_13 in healthy adults ≥65 years of age, V89_11 in healthy children from 6 months to ≤17 years of age). Also based on the immunogenicity data from these additional trials, the chosen dose in adults and elderly seems appropriate. The paediatric phase 2 data (V89_11) are presented in more detail further below.

Design and conduct of clinical study V89_18

Study V89_18 was a Phase 3, stratified, randomised, observer-blind, multi-centre, placebo-controlled study to evaluate safety, immunogenicity and lot-to-lot consistency of aH5N1c in healthy adult subjects ≥18 years of age. Subjects were randomly assigned (1:1:1:1) to receive a series of 2 IM injections administered 3 weeks apart, of 1 of 3 lots aH5N1c containing 7.5 µg of the H5N1 (A/turkey/Turkey/1/2005 NIBRG-23 strain) influenza antigen with 0.25 mL MF59 (full dose) or placebo containing 0.5 mL sterile saline (0.9% NaCl). The randomisation was stratified by study centre and age (18 to <65 years of age and ≥65 years of age) with a goal to enrol 50% into each age cohort. This study was conducted at 26 centres in the USA. The overall study design is acceptable.

Only one regimen has been studied (two vaccinations 3 weeks apart). Due to experience with other influenza vaccines, there are no objections against this vaccination scheme. However, investigating different schemes would have been informative to estimate the immune response in case of delayed second vaccination due to sparse vaccine availability in a pandemic situation.

Study V89_18 managed to recruit 50% subjects ≥65 years of age, which is positively acknowledged. Pregnant women and individuals with known or suspected impairment of the immune system were excluded from the trials. Although approximately half of the participants were 65 years of age and above, there is lack of data in frail patients. While this is acceptable for an immunogenicity trial of a pandemic preparedness vaccine (investigating the immunogenicity in healthy adults is stated as a minimum in the GL), use of the vaccine in immunocompromised and frail individuals will be considered as an uncertainty in the benefit/risk evaluation.

Nearly all (99.8%) enrolled subjects were exposed to either vaccine or placebo, and 93.3% completed the protocol. The main reasons for discontinuation from the study were "lost to follow-up" and "withdrawal by subject". No significant imbalances between the groups are noted. The most common protocol deviations were "did not comply with blood draw schedule" (8.1%) and "did not comply with study vaccination schedule" (2.5%). These deviations were balanced between the groups.

Concerning analysis sets, the focus on the PP set for primary analysis was initially not supported. The applicant planned to perform the primary analysis in the FAS if PPS and FAS differed by more than 5%. However, the analysis was not conducted although FAS and PPS differed by more than 5% at day 43 ((3086-2856)/3086 ~ 7.5%), which is the time point of the primary endpoint. Following CHMP request, the applicant provided the analysis of the endpoints concerning seroconversion and GMT/GMRs at day

22, 43 and 183 with FAS, which were consistent with those in the PPS and all success criteria were reached at Day 43.

According to the EMA Guideline on missing data in confirmatory trials (EMA/CPMP/EWP/1776/99 Rev. 1), the FAS, which should be consistent with the intention to treat principle, should be used in confirmatory trials. The definition of the FAS in the study is not considered adequate since it included only subjects with complete measurement at the relevant time points. Therefore, the applicant was asked to conduct sensitivity analyses with an analysis set, where missing values at relevant time points after baseline and relevant values at/after protocol deviations were imputed, to comply with the ITT principle. Sensitivity analyses with a worst and a best-case scenario were carried out by the applicant for the primary and secondary immunogenicity endpoints at day 22, 43, 183, and led to the same conclusions as the analyses without imputation.

The statistical methods chosen for descriptive as well as inferential analyses were considered suitable.

Objectives/endpoints

The co-primary objectives were to demonstrate lot-to-lot consistency across 3 consecutively produced lots of aH5N1c vaccine, as assessed by the ratio of geometric mean titres of HI antibody responses, and to evaluate whether the immune response meets the immunogenicity criteria defined by Centre for Biologics Evaluation and Research (CBER) guidance. Both primary objectives were evaluated at Day 43, 3 weeks after the second vaccine administration.

The secondary objectives included determination of seroconversion, percentage of subjects achieving an HI antibody titre ≥1:40, and GMTs/GMRs at different time points (Day 22, Day 43, Day 187). Additionally, it was evaluated whether the immune response elicited by aH5N1c fulfils the former (no longer valid) immunogenicity criteria by CHMP.

An additional *post hoc* objective was included to evaluate MN antibody responses 3 weeks after the second vaccine administration (Day 43) in healthy adult subject 18 to <65 years of age.

Efficacy data and additional analyses of Study V89_18

The primary EP of lot-to-lot consistency was met. It is understood that this EP was primarily introduced due to FDA requirements.

At Day 43, 3 weeks after the second vaccination, there was no notable HI titre response in the placebo group, while the HI titres increased after each administration of aH5N1c. In the total study population, a Day 43 HI GMT of 130.6 (GMR in relation to Day 1: 7.96) was reached for the vaccine group, compared to 13.7 (GMR: 0.83) for the placebo group. Not surprisingly, a stronger immune response was noted in adults from 18 to 65 years of age (GMT 170.7, GMR 12.7), compared to participants ≥65 years of age (GMT 97.9, GMR 4.9). Seroconversion at Day 43 was achieved by 66.9% of the study population (18-64 years of age: 79.9%; ≥65 years of age: 54%), compared to 1% in the placebo group. At Day 43, 3 weeks after the second vaccination, all three former CHMP criteria and both CBER criteria were met for both age groups.

As shown from the results of earlier trials, the phase 3 data confirm the necessity of a second dose to achieve a more robust immune response.

The HI assay data at D183 (GMR overall population: 1.22, 18-64 years of age: 1.53, ≥65 years of age: 0.97) show that antibodies return to or close to baseline within half a year, suggesting that a booster dose may be necessary in case of an influenza pandemic. This is somewhat contradictory to the phase 2 results, where HI titres remained to some extent above baseline after one year (V89_04 = nonelderly adults: GMR=~2; V89_13 = adults ≥65 years of age, GMR = ~2; V89_11 = paediatric population: GMR = 12).

Interestingly, when only looking at the results for the placebo group, there was a clearly higher proportion of subjects with HI titres ≥1:40 at baseline (Day 1, 22.6% for all participants ≥ 18 years of age, GMT: 16.7) compared to Day 183 (5.2%, GMT: 5.2)). At baseline, the vaccine and the placebo groups were well balanced with nearly identical GMTs and proportions of subjects with HI titres ≥1:40. Considering that the results for the placebo group showed a >4-fold difference in proportion of subjects with HI titres ≥1:40 and >3-fold difference in GMTs when comparing Day 1 with Day 183, this raises the question whether this difference between two time points in the unvaccinated population was caused by assay variability. The samples from D183 were analysed at a different time point than the earlier samples, since the Day 43 results were part of an interim analysis. The applicant was asked to comment on potential causes for this finding. The applicant argued that the clearly higher HI titres at Day 1 (for both the vaccine and the placebo group) compared to the placebo group at Day 183 might have been caused by a potential exposure to a cross-reactive seasonal influenza strain. Further, it is pointed out that the percentages of subjects who received seasonal influenza vaccination within the last 12 months were similar between the groups and therefore did not impact the immune response at Day 1. It is not known whether the relatively higher titres at Day 1 compared to Day 183 were indeed caused by exposure to a cross-reactive seasonal influenza strain or by assay variability (D183 samples were analysed at a different time point, while the other samples were analysed earlier for an interim analysis). However, this observation is not expected to have a strong impact on the outcome of the study (i.e., the clear advantage of the vaccine vs. placebo regarding the ability to elicit an HI titre response at all investigated time points post-vaccination).

When comparing the HI titre response of vaccinated participants between trials in the adult population, it seems that the GMTs at Day 43 were significantly lower in the phase 3 study, although the baseline titres were (> 2-fold) higher. The MN titre responses were also weaker in the phase 3 trial vs the earlier trials (D43 GMTs in subjects from 18 to <65 years of age; V89_04: 410 vs. V89_18: 130.91; D43/D1 GMRs; V89_04: 61 vs. V89_18: 23.8). Unlike the HI titres, the baseline MN titres were comparable between the studies. The applicant was requested to discuss the differences in immunogenicity data for adult participants in the V89_04, V89_13 and V89_18 studies and the potential impact of using different laboratory sites for analysis. Although the assays used were validated, they were slightly different for example in the selection of reference sera (V89_18 used sera from ferrets, whilst V19_13 used control sera from sheep). The applicant acknowledged the differences in immunogenicity due to different laboratories but referred to assay validation and similar patterns of immunogenicity across the different studies. Data from the pivotal study (V89_18) is included in section 5.1 of the SmPC to provide data immunogenicity data for the age groups > 18 years. Data from the V89_13 and V89_04 studies are used in the SmPC to show cross-reactivity with other strains. While the noted differences in titres caused by different testing laboratories are not optimal for interpretation of the data, it is acceptable to keep the phase 2 results in the SmPC.

Upon request, the applicant provided a presentation of the proportion of participants who showed a relatively weak response to vaccination. In adult subjects (Study V89_18), the elicited immune response at Day 43 was below a 2-fold increase in 13.8% of the participants (compared to 90.6% in the placebo group). This indicates that a significant proportion of participants showed a weak immune response after 2 vaccinations. Based on other data presented with the responses, it seems that the weak responders were likely mainly elderly (see also the special populations section).

Microneutralisation data are only available for a small subpopulation (aH5N1c: n=76, placebo: n = 24) in participants between 18 and <65 years of age and only for the time points at baseline (Day 1) and 3 weeks after second vaccination (Day 43). Overall, these data confirm a robust response 3 weeks after the second vaccination (GMR 23.8). Of note, 89.5% showed seroconversion, 75% of participants had a MN titre ≥ 1:80 and 48.7% had a MN titre ≥ 1:160.

With the responses to the D120 LoQ, the applicant argued that heterologous responses were already investigated for all age groups during the phase 2 trials and that a similar pattern would be expected for Study V89_18. It was further pointed out that evaluating the response against these 5 historical strains would not have an added value. The applicant further argued that investigating cross-reactivity and cross-priming with the H5N6 strain (clade 2.3.4.4h), which is evaluated in Study V89_18E1, appears to be more relevant given the current outbreak of H5N1 clade 2.3.4.4b to which the H5N6 strain is more closely related, than determining cross-reactivity against the 5 older subclades of H5N1 strains (Anhui/1/2005 clade 2.3.4, Egypt/N03072/2010 clade 2.2.1, Hubei/1/2010 clade 2.3.2, Indonesia/5/2005 clade 2.1.3 and Vietnam/1203/2004 clade 1).

Design and conduct of Study V89_11 (paediatric data)

This phase 2, randomised, controlled, observer-blind multicentre study was designed to evaluate immunogenicity, tolerability and safety of 2 intramuscular (IM) doses of either low dose or high dose aH5N1c (A/turkey/Turkey/1/2005 NIBRG-23 strain) in healthy subjects 6 months through 17 years of age. A total of approximately 666 subjects were to be randomised at a 1:1 ratio to receive 2 vaccinations 3 weeks apart of either study vaccine (high or low dose aH5N1c). Randomisation was stratified by site and age cohort (6 through 35 months, 3 through 8 years and 9 through 17 years).

The exclusion criteria in the paediatric phase 2 trial were more extensive than in the adult phase 3 study. In addition to, for example, known or suspected impairment/alteration of the immune system, children with any serious chronic or progressive disease according to judgment of the investigator were also excluded. These include potential risk factors for severe influenza complications like severe asthma, autoimmune disease, or diabetes mellitus type I.

Although there were 10 sites in the United States, it has to be noted that the majority of participants were recruited at the 2 sites in Thailand (country of enrolment: 73% Thailand, 27% USA).

A substantial percentage (26%) of subjects had protocol deviations that led to exclusion of the subject or part of the subject's data from at least one analysis set. However, while not optimal, these exclusions did not concern all, but only certain visits (due to missing serology data).

The definition of most analyses sets was considered adequate except for FAS. Although FAS was defined as all subjects with at least one vaccination and measured titre values at baseline and the respective time point of the endpoint, it seemed like the actual conducted analysis was a complete case analysis, and missing immunogenicity values at baseline were ignored in the set for the analysis of HI titre ≥ 1;40 and GMTs. Similar to V89-18, the definition of FAS contradicted the EMA Guideline on missing data in confirmatory trials (EMA/CPMP/EWP/1776/99 Rev. 1) because MCAR was assumed for immunogenicity values, and an analogous sensitivity analysis was performed by the applicant resulting in similar conclusions as the original analysis. The described multiplicity correction for primary immunogenicity endpoints is deemed adequate. Although confidence intervals for secondary immunogenicity endpoints were also corrected, they were not part of the confirmatory strategy. These analyses are considered descriptive and reporting of 95% CIs would have been sufficient.

Efficacy data and additional analyses of Study V89_11

All pre-specified CBER and (former) CHMP criteria regarding HI titres ≥1:40, seroconversion and GMR at Day 43 were met in the paediatric population by both the full and the half dose. The higher dose performed significantly better for all three endpoints.

The GMTs and GMRs at D43 compared to Day 1 were high throughout all age subgroups, an increasing trend by decreasing age is noted (9 to <18 years of age: GMT=961, GMR=186; 3 to <9 years of age: GMT=1244, GMR=249; 6 to ≤ 36 months: GMT=1842, GMR=302). These titres are substantially higher compared to the values observed in the trials with adult participants.

A focussed discussion of the paediatric immunogenicity data by age cohorts and dose levels (full dose, half dose) was missing in the initial data submission. The safety data were also not adequately presented to allow assessment of paediatric age subgroups. Therefore, a combined safety and immunogenicity question was raised. The applicant was requested to present the safety and immunogenicity data for both dose levels stratified for more than 2 age groups (e.g., 6-36 months, 3-9 years, 9-18 years). In order to inform optimal paediatric dose levels and to justify the data to be included in section 5.1 of the SmPC, the applicant was requested to thoroughly discuss safety and immunogenicity data for each of the age subgroups and for both dose levels. In order to support the recommendation of the full dose for children independently of age, the applicant presented immunogenicity and safety data stratified by three age cohorts (6-36 months, 3-9 years, 9-18 years), as shown in the results section 3.3.4.4 above. In consequence, a recommendation of the full dose for children of all age groups in 4.2 can be agreed due to the high immunogenicity and the benign safety and tolerability profile (see safety section). According to the SmPC GL rev. 2, the half dose results were also included in section 5.1 of the SmPC, with the higher incidence of fever in the full dose group mentioned.

Of note, the majority of participants of the Paediatric Study V89_11 were recruited in Thailand (country of enrolment: 73% Thailand, 27% USA). There are substantial differences regarding the immunogenicity profile in various participant populations (particularly Thailand vs USA) at D22. The applicant was asked to discuss potential reasons for these differences in immune response. The applicant responded that immune responses may be influenced by a number of different factors, including genetic factors, age and study demographics. However, it is unlikely that such changes would affect only a single timepoint (D22) – after the first immunisation. Nevertheless, since two immunisations are required to achieve the prespecified immunogenicity criteria, this question was not pursued further.

Upon request, the applicant provided microneutralisation data by paediatric subgroups. MN testing was only performed for subjects between 6 months and 9 years of age, since adolescents are expected to have a comparable immune response as adults (for whom MN data are available from the other studies presented in the dossier). In line with the HI assay results, the youngest participants (6-36 months of age) had numerically higher MN titres at Day 43 (N=34, GMT [95% CI]: 1525.67 [1136.74, 2047.68]) compared to children from 3 to <9 years of age (N=35, GMT [95% CI]: 1085.86 [813.33, 1449.71]) but with overlapping confidence intervals.

Design and conduct of Study V89_13 (population ≥65 years of age)

Since the design of Study V89-13 was very similar to Study V89-11, partly the same questions have been raised. All questions have been resolved.

Immunogenicity data against heterologous H5N1 strains

HI analysis heterologous strains

Haemagglutination inhibition against different H5N1 strains was only performed for the phase 2 trials. The assays included five different strains (A/Anhui/1/2005, A/Egypt/N03072/2010, A/Hubei/1/2010, A/Indonesia/5/2005, A/Vietnam/1203/2004). The results show cross-reactivity at a varying degree, with weakest results against the Anhui and the Indonesia strains, and more robust immune responses against the Egypt, Hubei and Vietnam strains, as described below.

In adults, the seroconversion rates at Day 43 ranged between 17% and 59% and in children between 32% and 72%.

In adults below 65 years of age, the GMRs at Day 43 (vs. baseline) were ranging from ~2 (A/Anhui/1/2005) to 8.33 (A/Hubei/1/2010). In adults ≥65 years of age, the GMRs were weaker,

ranging from 1.5 (A/Anhui/1/2005) to 4.77 (A/Hubei/1/2010). In the paediatric population, the GMRs ranged from 8.39 (A/Anhui/1/2005) to 40 A/Egypt/N03072/2010).

MN analysis heterologous strains

Microneutralisation against the same heterologous H5N1 strains as described above was again only performed for the phase 2 studies. Similar to the HI assay, the results of the heterologous MN analyses show cross-reactivity at a varying degree. In adults below 65 years of age, the GMRs at Day 43 (vs. baseline) were ranging from ~2 (A/Anhui/1/2005) to 8.33 (A/Hubei/1/2010). In adults ≥65 years of age, the GMRs were weaker, ranging from 1.5 (A/Anhui/1/2005) to 4.77 (A/Hubei/1/2010). In the paediatric population, the GMRs ranged from 8.39 (A/Anhui/1/2005) to 40 A/Egypt/N03072/2010).

These data suggest that vaccination with aH5N1c elicits cross-reactive HI titres against heterologous H5N1 strains. Therefore, aH5N1c might also offer some protection against disease caused by different H5N1 strains. However, waning of antibodies has to be considered as well. The protective potential of these cross-reactive antibodies will depend on the circulating strain and can only be determined by effectiveness studies. There are no supportive nonclinical data regarding the immune response against heterologous H5N1 strains.

Immunogenicity in special populations

Immunogenicity data were presented by age, sex, race, ethnicity, country of enrolment, baseline titre, and by status of seasonal influenza vaccination. Overall, these data do not raise a concern, apart from the above raised question regarding differences in the immunogenicity profile between subjects from Thailand and the USA (Study V89_11). Interestingly, adult participants who did not receive seasonal influenza vaccination within the last 12 months had higher seroconversion rates and GMTs/GMRs, compared to participants who received seasonal influenza vaccination. This outcome was significant in the phase 3 trial.

The applicant was asked to present the HI results (seroconversion, GMT, GMR) by age groups (65-74, 75-84, 85+) for studies V89_18 and V89_13 for both the FAS and the PPS. As expected, the immune response (based on HI titres) decreased with increasing age. The interpretability of the data is hampered due to the low number of participants ≥85 years of age. The HI titres were higher in the phase 2 study compared to the phase 3 study, which was already observed before for adults below 65 years of age (when comparing results from Studies V89_18 vs V89_04). The consequence of these low titres in elderly for the protective potential is not known in the absence of efficacy data or a correlate of protection and without knowledge of the circulating strain.

Immunogenicity data from supportive studies

Overall, the results of the supportive studies with the same vaccine construct but containing different influenza subtypes (H1N1, H3N2v, H7N9) confirm the advantage of adjuvanted formulations, especially in adults. The extent of the immune response depends on the influenza subtype. Study V131_01 in adults confirms the necessity of a second dose, considering that there was no measurable immune response against H7N9 after only one vaccination. In the paediatric Study V110_04 (H1N1), the CHMP and CBER criteria were also met with the unadjuvanted formulations. One could argue that an unadjuvanted vaccine may elicit a sufficient immune response in children. However, since a potential pandemic strain cannot be predicted and bearing in mind that there was no response against H7N9 with the unadjuvanted vaccine (in adults, no data in children available for this subtype), the decision to include an adjuvant to aH5N1c is understood. This may increase the likelihood of a potent immune response against weakly immunogenic influenza subtypes.

Waning of antibodies and potential need of a booster dose

The duration of follow-up was 12 months with the exception of study V89_18 in individuals >18 years of age which was 6 months. The applicant was asked to provide information on the potential for booster immunisations since the duration of immunogenicity appeared to be less than one year. The applicant responded that results from the phase 2 trials provide evidence that immune responses persist up to 1 year following vaccination. While this may be true for the paediatric population, this is not agreed for the adult population in the phase 3 Study V89_18, where the HI titres at D183 returned to (or close to, depending on the age of the individuals) baseline.

Substantial waning of antibodies was noted for the adult population (especially in elderly) and the GMTs returned to (or very close to) baseline at the 6-month time point, as reflected in the SmPC. The duration of a potential protective effect cannot be predicted. In a pandemic situation the applicant has proposed safety and effectiveness studies, however, no immunogenicity studies are described. It is not likely that following zoonotic exposure or during a pandemic that national authorities will be monitoring immunogenicity of the vaccine over time.

There is currently no correlate of protection for influenza A(H5N1) vaccines (Guideline on Influenza Vaccines, EMA/CHMP/VWP/457259/2014). Only immunogenicity has been provided to support this application based on former CHMP criteria (1997) since several studies were carried out when these criteria were applicable. The immunogenicity assays HI and MN may show inter-laboratory variability. Although it is recognised that clinical efficacy is difficult to show against H5N1 which only rarely infects humans, the applicant was nevertheless asked to discuss the relationship between the immunogenicity results and the potential for vaccine efficacy. The applicant states that, although HI and MN assays may show interlaboratory variability, the pattern in antibody responses with both the HI and the MN assay observed following 2 doses of aH5N1c vaccine was similar across clinical trials. And though there is currently no correlate of protection for influenza vaccines, immunogenicity has been evaluated using the former CHMP-criteria as well as CBER criteria. It is acknowledged that the CBER criteria can be considered more stringent and that the CHMP-criteria were effective at the time of conduct for some of the clinical trials. Still, these criteria are deemed informative, not critical for the evaluation.

It is recognised that antibodies have been shown to be important in the prevention of infection and virus propagation, that haemagglutinin is one of the two main antigenic proteins and that HI and MN antibodies appear to play a major role in preventing infection. However, there are still uncertainties regarding the relationship between the immunogenicity results and vaccine efficacy for the vaccines in question. Especially, bearing in mind that for Incellipan, in the event of a declared pandemic, the antigen included in the main clinical trials will be replaced by the pandemic strain.

The applicant was asked whether there are plans to develop a multidose vial formulation (besides the pre-filled syringe) to enable different posologies. According to the response, the applicant leaves open the possibility of introducing a multidose vial formulation during an officially declared pandemic. The applicant further clarified that thiomersal would be used as preservative (containing 25 mcg mercury per 0.5 mL) in case such a multidose vial formulation were introduced.

In adult and paediatric individuals, two doses of aH5N1c administered three weeks apart elicit robust immune response as measured by HI titres against the H5N1 strain contained in the vaccine (A/turkey/Turkey/1/2005 [H5N1] NIBRG-23) three weeks after the second vaccination. The HI assay results are confirmed by microneutralisation data collected in rather small subpopulations of one phase 3 and three phase 2 trials.

Additional confirmatory evidence from three supportive studies investigating the immune response of vaccines using the same vaccine construct but containing different potential pandemic influenza subtypes (H1N1, H3N2v, H7N9) as antigens suggest that Incellipan may also be sufficiently immunogenic after a strain change.

There are no data regarding cell-mediated immunity or anti-neuraminidase (NA) antibodies.

The immunogenicity of aH5N1c has not been investigated in immunocompromised or frail individuals.

The totality of immunogenicity data suggests that aH5N1c is robustly immunogenic. Considering that higher HI titres tend to correlate with better protection, it is reasonably likely that the strain-adapted vaccine will be able to offer protection against disease caused by a pandemic influenza strain. The extent of protection cannot be estimated in the absence of an efficacy study and without knowledge of the potentially circulating influenza strain.

In adults, substantial waning of antibodies over time was noted and hardly any HI titres were detected already 6 months after vaccination, suggesting that there may be a need for a booster vaccination in this population in a scenario of an ongoing pandemic. In the paediatric population, the HI titres did also wane over time, but the seroconversion rates and GMRs were still considerably high one year after vaccination.

Overall, the presented immunogenicity data are considered favourable for Incellipan.

Additional efficacy data needed in the context of a conditional MA

Incellipan can only be marketed when there is an official WHO/EU declaration of an influenza pandemic, on the condition that the Marketing Authorisation Holder for Incellipan takes due account of the officially declared pandemic strain.

In order to confirm the efficacy of Incellipan, the MAH should conduct a non-interventional observational effectiveness study in children and adults against laboratory confirmed influenza during the next declared pandemic. The MAH should submit the final results of this study.

2.6.7. Conclusions on the clinical efficacy

In adult and paediatric individuals, two doses of aH5N1c administered three weeks apart elicit robust immune response as measured by HI titres against the H5N1 strain contained in the vaccine (A/turkey/Turkey/1/2005 [H5N1] NIBRG-23) three weeks after the second vaccination.

Additional confirmatory evidence from three supportive studies investigating the immune response of vaccines using the same vaccine construct but containing different potential pandemic influenza subtypes (H1N1, H3N2v, H7N9) as antigens suggest that Incellipan may also be sufficiently immunogenic after a strain change.

The totality of immunogenicity data suggest that aH5N1c is robustly immunogenic. Considering that higher HI titres tend to correlate with better protection, it is reasonably likely that the strain-adapted vaccine will be able to offer protection against disease caused by a pandemic influenza strain. The extent of protection cannot be estimated in the absence of an efficacy study and without knowledge of the potentially circulating influenza strain.

In adults, substantial waning of antibodies over time was noted suggesting that there may be a need for a booster vaccination in this population in a scenario of an on-going pandemic. In the paediatric population, the HI titres did also wane over time, but the seroconversion rates and GMRs were still considerably high one year after vaccination.

Incellipan is considered approvable from a clinical efficacy point of view.

The CHMP considers the following measures necessary to address the missing efficacy data in the context of a conditional MA.

Description	Due date
In order to confirm the efficacy of Incellipan, the MAH should conduct a non-interventional observational effectiveness study in children and adults against laboratory confirmed influenza during the next declared pandemic. The MAH should submit the final results of this study.	After declaration of a pandemic in the EU and after implementation of the pandemic vaccine

2.6.8. Clinical safety

The clinical safety database is presented in pooled form for the H5N1 studies in adults with a similar design: Phase 2 studies V89_04 (adults 18 to <65 years) and V89_13 (adults ≥65 years) as well as phase 3 study V89_18 (adults ≥18 years).

The safety data from the paediatric study V89_11 (6 months to ≤17 years of age) have not been combined with the adult aH5N1c safety data.

Furthermore, safety data from aH5N1c phase 1 dose finding study V89_P1 as well as the supportive studies undertaken with other influenza A strains, i.e. H1N1, H3N2 and H7N8 are presented separately.

The aH5N1c vaccine by Seqirus is adjuvanted with MF59, which is the adjuvant present in the pandemic egg-based vaccine Focetria that was licensed in EU in 2007 as mock-up H5N1 vaccine (EMEA/H/C/000710) and underwent a pandemic strain change to H1N1 in 2009. Millions of doses of Focetria were distributed during the influenza pandemic in 2009 with approximately a quarter of those doses being used. During the pandemic in 2009 the cell culture-derived vaccine Celtura was also licensed in Germany, Switzerland and Japan; some million doses were distributed and approximately 10% of those doses were used. In addition, MF59 is the adjuvant present in the pandemic preparedness egg-based vaccine Foclivia (EMEA/H/C/001208) and in the zoonotic egg-based vaccine Aflunov (EMEA/H/C/2094), licensed in EU since 2009 and 2010, respectively. In addition, MF59 is contained in seasonal influenza vaccines Fluad, Fluad Tetra, Flucelvax and Optaflu.

2.6.8.1. Patient exposure

Table 66. Overview of Subjects Exposed to Two Dose Levels

			Number of Subjects Exposed		
Vaccine	**Study**	**Population**	**Full Dose Level**	**Half Dose Level**	**Saline Placebo**
aH5N1c	V89_18	Phase 3, Adult	1198	-	398
		Phase 3, Elderly	1197	-	398
	V89_04	Phase 2, Adult	485	490	-
	V89_13	Phase 2, Elderly	699	689	-
	V89P1[a]	Phase 1/2, Adult	64	62	-
	V89_11	Phase 2, Paediatric	329	329	-
	Total	**Adult, Elderly, Paediatric**	**3972**	**1570**	**796**
aH1N1c	V110_04	Phase 3, Paediatric	298	290	-
aH3N2c	V129_01	Phase 1, Paediatric	105	107	-
		Phase 1, Adult, Elderly	105	104	
aH7N9c	V131_01	Phase 1, Adult	103	98	-
	Total	**Adult, Elderly, Paediatric**	**611**	**599**	**-**

[a] For Studies V89P1, V110_04, V129_01 and V131_01 the displayed numbers are the numbers for the full and half doses only.

Safety data from 3972 subjects, of whom 329 are in the paediatric age cohort, are available for the full dose vaccine. Supportive data from the vaccine construct using H1N1, H3N2 and H7N9 strains are available for a further 611 subjects, of whom 105 are in the paediatric age range. A further 1570 subjects (329 paediatric) received a half-dose of the H5N1 vaccine, and 599 (397 paediatric) received a half-dose of the other vaccine constructs. Furthermore, data from 796 adult and elderly subjects who were randomised to placebo are available in order to enable a meaningful comparison of the safety profile.

2.6.8.2. Adverse events

The following AEs were captured through the diary card, by interview of the subject, and by review of available medical records from all subjects:

- Solicited and unsolicited AEs, and medication/vaccinations given from day 1 to day 7 (inclusive); from day 22 to day 28 (inclusive);
- Unsolicited AEs, solicited AEs that continued beyond day 7 and 28 (respectively) and medications/vaccinations from day 8 to 21 and day 29 to 42 until the time of return to the clinic on day 22 and 43 (respectively);
- SAEs, all medications given to treat SAEs, NOCD, AEs leading to vaccine/study withdrawal, medically attended AEs, AESIs and all vaccinations from day 1 through day 387 (inclusive).

The period of observation for AEs extended from the time the subject signed informed consent until he or she completed the specified safety follow-up period, approximately 13 months after the first vaccination or terminated the study early (whichever came first).

Adult Trials

Solicited Local Adverse Events

Injection site induration, erythema, ecchymosis, pain.

Solicited Systemic Adverse Events

Nausea, generalised myalgia, generalised arthralgia, headache, fatigue, chills, loss of appetite, malaise, fever derived from measured body temperatures (defined as body temperature ≥38.0 ° C).

Other Solicited Adverse Event-Related Variables

Body temperature (summarised by route of measurement and in 0.5oC increments from 36.0oC) and the use of analgesics/ antipyretic medication for prophylaxis or treatment.

Paediatric Trials

Solicited local AEs:

For subjects < 6 years of age: injection site induration, erythema, ecchymosis and tenderness.

For subjects ≥ 6 years through ≤17 years of age: injection site induration, erythema, ecchymosis and pain.

Solicited systemic AEs:

For subjects < 6 years of age: change in eating habits, sleepiness, irritability and fever (defined as body temperature ≥38.0° C, preferably axillary).

For subjects ≥ 6 years through ≤17 years of age: nausea, myalgia, arthralgia, headache, fatigue, loss of appetite, malaise and fever (defined as body temperature ≥38.0° C, preferably axillary).

Other solicited AEs:

Body temperature, preferably axillary (summarised by route of measurement and in 0.5°C increments from 36.0°C) and the use of analgesics/antipyretic medication for prophylaxis or treatment.

Solicited Local and Systemic Adverse Events

Ages ≥18 Years, Full Dose and Placebo

Table 67 shows an overview of the frequency of solicited local and systemic AEs from Day 1 (excluding 30 minutes) through Day 7 after any vaccination and by vaccination for the pooled data of subjects ≥18 years of age and by major age group.

In total, 61.9% of the subjects in the aH5N1c full dose group and 38.0% of placebo subjects reported any solicited AE after any vaccination. The frequency of any solicited AE (local and systemic) was higher in the aH5N1c group than in the placebo group after each vaccination. The proportion of subjects for whom any solicited AE was reported was lower after the second vaccination than after the first vaccination both in the aH5N1c group and the placebo group (Table 67).

Table 67.

Ages ≥18 Years (Full Dose and Placebo) – Numbers (%) of Subjects with at Least One Solicited Local or Systemic Adverse Event from Day 1 (Excluding 30 Minutes) Through Day 7 After Any Vaccination and by Vaccination – Overall and by Major Age Group – Solicited Safety Set

	Overall		Age Group			
	≥18 Years		18 to <65 Years		≥65 Years	
	aH5N1c	Placebo	aH5N1c	Placebo	aH5N1c	Placebo
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Any Vaccination	N=3518	N=784	N=1636	N=387	N=1882	N=397
Any[a]	2177 (61.9)	298 (38.0)	1178 (72.0)	176 (45.5)	999 (53.1)	122 (30.7)
Local	1817 (51.6)	115 (14.7)	1071 (65.5)	77 (19.9)	746 (39.6)	38 (9.6)
Systemic[b]	1371 (39.0)	257 (32.8)	735 (44.9)	149 (38.5)	636 (33.8)	108 (27.2)
Vaccination 1	N=3506	N=782	N=1625	N=385	N=1881	N=397
Any[a]	1922 (54.8)	236 (30.2)	1086 (66.8)	146 (37.9)	836 (44.4)	90 (22.7)
Local	1573 (44.9)	80 (10.2)	957 (58.9)	57 (14.8)	616 (32.7)	23 (5.8)
Systemic[b]	1114 (31.8)	203 (26.0)	620 (38.2)	123 (31.9)	494 (26.3)	80 (20.2)
Vaccination 2	N=3430	N=770	N=1583	N=377	N=1847	N=393
Any[a]	1438 (41.9)	172 (22.3)	805 (50.9)	100 (26.5)	633 (34.3)	72 (18.3)
Local	1193 (34.8)	57 (7.4)	722 (45.6)	40 (10.6)	471 (25.5)	17 (4.3)
Systemic[b]	741 (21.6)	143 (18.6)	398 (25.1)	79 (21.0)	343 (18.6)	64 (16.3)

Source: ISS Table 1.4.1 and Table 1.4.1.1

Abbreviations: AE = adverse event; n = number of subjects with values in category; N = total number of subjects.

[a] Any AE refers to a subject reporting either local or systemic AEs but does not include subjects with other indicators of reactogenicity (body temperature and use of analgesics/antipyretics).

[b] Includes subjects with body temperature ≥38°C irrespective of route of measurement.

Threshold for erythema, ecchymosis and induration: Grade 0 (<25 mm), any (25-50 mm [Grade I], 51-100 mm [Grade II], >100 mm [Grade III]).

Overall, the solicited AEs were predominantly either mild or moderate in severity.

During the first 30 minutes after any vaccination, 4.8% of the subjects who received aH5N1c full dose and 3.4% of the subjects who received placebo reported any local and systemic AEs.

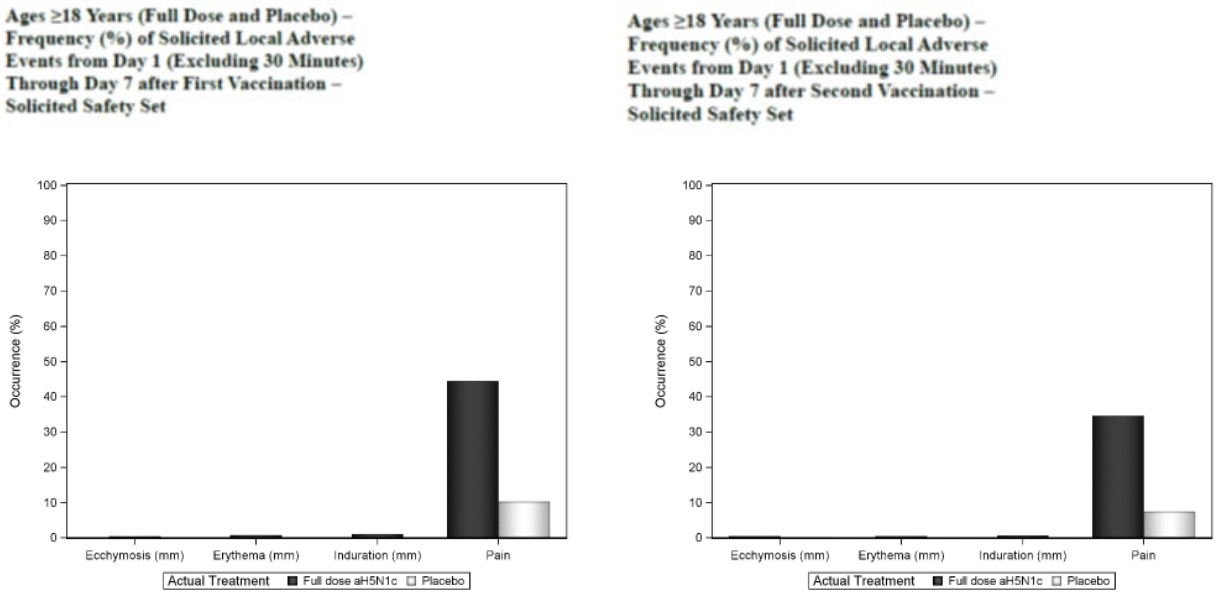
Solicited Local Adverse Events

Ages ≥18 Years, Full Dose and Placebo

As expected for an influenza vaccination, the most predominant local AE in the aH5N1c group was pain, with very low percentages of induration, erythema and ecchymosis reported. In the placebo group, the same pattern could be observed, but with a much lower incidence of pain.

Most local AEs were mild or moderate and resolved within a few days. After the first vaccination, the incidence of local AEs was higher than after the second.

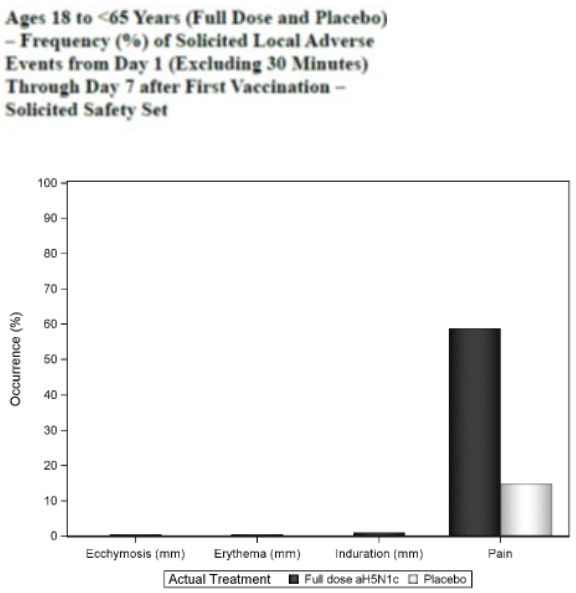
Figure 11.

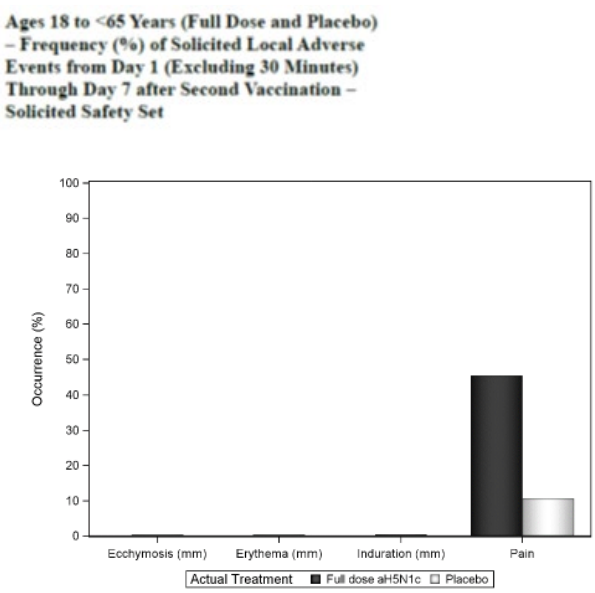


Ages 18 to <65 Years, Full Dose and Placebo

In the younger adult age cohort, the most predominant local AE in the aH5N1c group was pain, with very low percentages of induration, erythema and ecchymosis. The incidence of local AEs was higher than in the ≥ 18 age group, but as in the overall group, most local AEs were mild or moderate and resolved within a few days. Again, after the first vaccination, the incidence of local AEs was higher than after the second.

Figure 12.

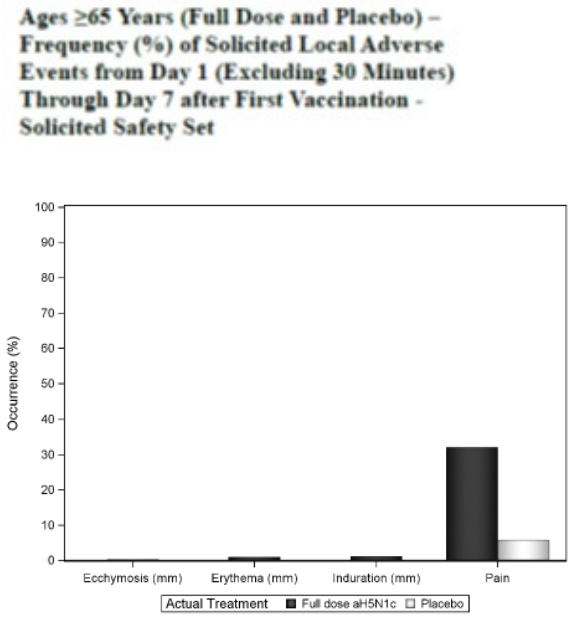


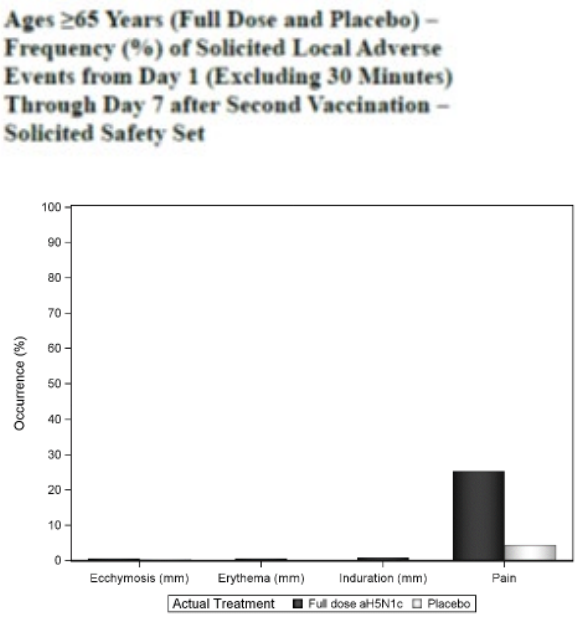


Ages ≥65 Years, Full Dose and Placebo

In the older adult age cohort, the most predominant local AE in the aH5N1c group was pain, with very low percentages of induration, erythema and ecchymosis. The incidence of local AEs, especially of pain, was notably lower than in the overall ≥ 18 age group and especially the <65 group. Most local AEs were of mild or moderate intensity and resolved within a few days. After the first vaccination, the incidence of local AEs was higher than after the second.

Figure 13.

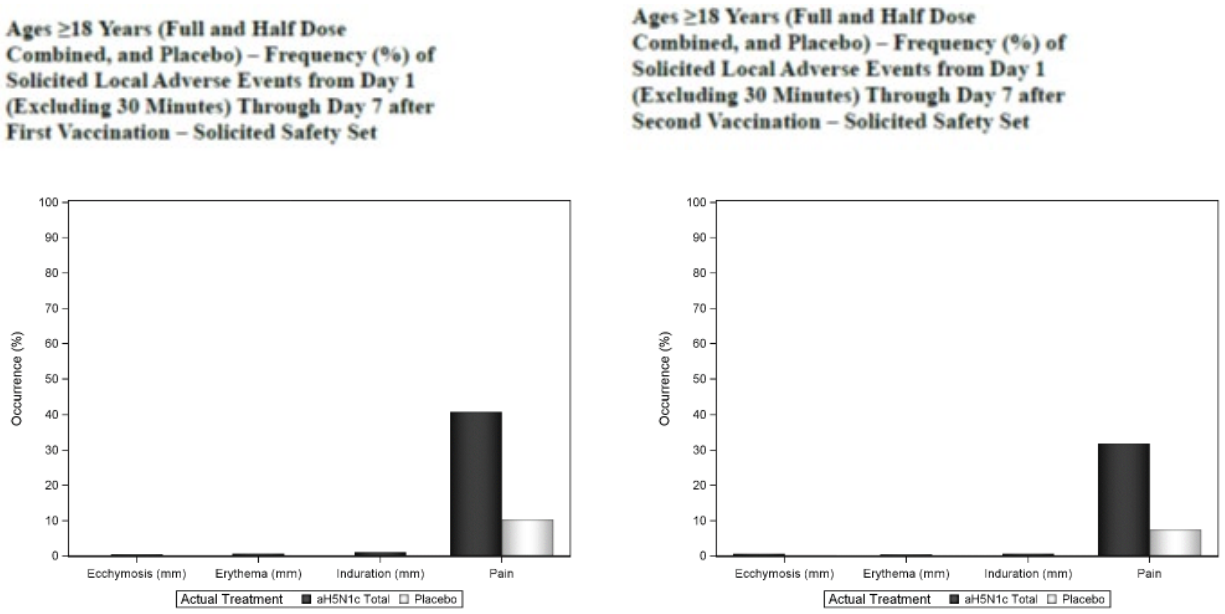




Ages ≥18 Years, Full and Half Dose Combined and Placebo

The addition of data from the half dose group shifts incidences of local solicited AEs towards slightly lower numbers.

Figure 141.



Paediatric Study V89_11 (6 months - ≤ 17 years)

Subjects aged <6 years

For subjects aged <6 years, tenderness was the mostly frequently (56% of subjects in both low dose and high dose group) observed solicited local AE between day 1 through day 7 after any vaccination (first vaccination: 49% in low dose group vs. 47% in high dose group; second vaccination: 38% vs. 43%, respectively).

Most of the solicited local AEs were mild to moderate in intensity in both the dose groups. Apart from severe tenderness after the first vaccination (1 subject in low dose group and 2 subjects in high dose group), none of the subjects in either of the dose groups experienced severe solicited local AEs.

Subjects aged 6 years through 17 years

In subjects aged 6 years through 17 years, pain was the most frequently reported solicited local AE; 72% of subjects in low dose group and 68% subjects in high dose group reported pain of mild to moderate intensity after any vaccination (first vaccination: 69% vs. 67%; second vaccination: 41% vs. 38%). Two subjects in high dose group reported erythema and none of the subjects in both dose groups reported ecchymosis after any vaccination. Induration was reported by 1% of subjects in the low dose group and 2% of subjects in high dose group. None of the subjects in either group reported severe local AEs, except for 1% of subjects who reported severe pain after each vaccination.

Overall, most local AEs were mild or moderate and resolved within a few days. After the first vaccination, the incidence of local AEs was higher than after the second. Incidences in the full and half dose group were similar in both age cohorts. However, all data concerning solicited local AEs are presented for two age cohorts for study V89_11, below 6 years and 6 years to 17 years, and should be

analysed and presented for at least 3 age cohorts (e.g., 6-36 months, 3-9 years, 9-18 years) analogous to data presented for these two cohorts.

Paediatric H1N1 Study V110_04 (6 months - ≤ 17 years)

Comparably to paediatric aH5N1c study VP98_11, the most predominant local AE was pain in the two older and tenderness in the two younger age cohorts, with very low percentages of erythema, induration and ecchymosis.

Most local AEs were of mild or moderate severity. After the first vaccination, the incidence of local AEs was higher than after the second. In contrast to the H5N1 study, incidences in the full dose group were higher across all age cohorts.

Phase 1 Study V89P1 (18 - ≤ 40)

The most commonly reported local reaction within 7 days of any vaccination in all vaccine groups was pain, which was reported by more subjects in the adjuvanted vaccine groups (54% to 74%) compared to the non-adjuvanted groups (17% to 43%). Other local reactions (ecchymosis, induration, erythema and swelling) were less frequently reported (≤3%) in all vaccine groups. The incidence of local reactions was lower after the second vaccination as compared to the first vaccination. Generally, the incidence of pain increased with increasing adjuvant content.

Supportive H3N2 Study V129_01 (paediatric 3 - ≤17 years; adult ≥18 years)

The local reactogenicity profile in this study was similar to the H5N1 studies.

Supportive H7N9 Study V131_01 (Adult 18 - ≤ 65 years)

The local reactogenicity profile in this study was similar to the H5N1 studies.

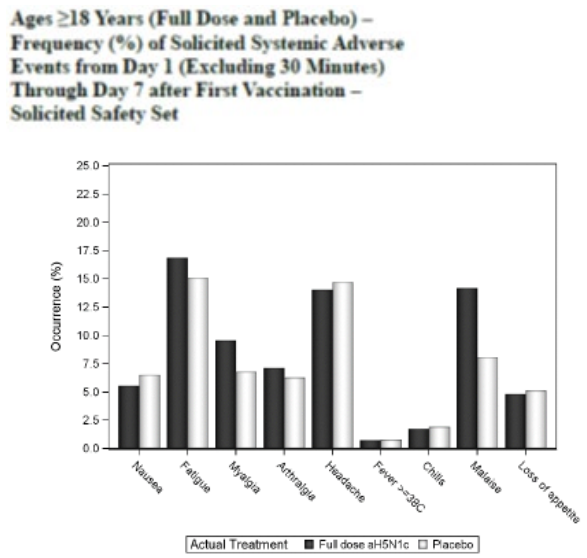
Solicited Systemic Adverse Events

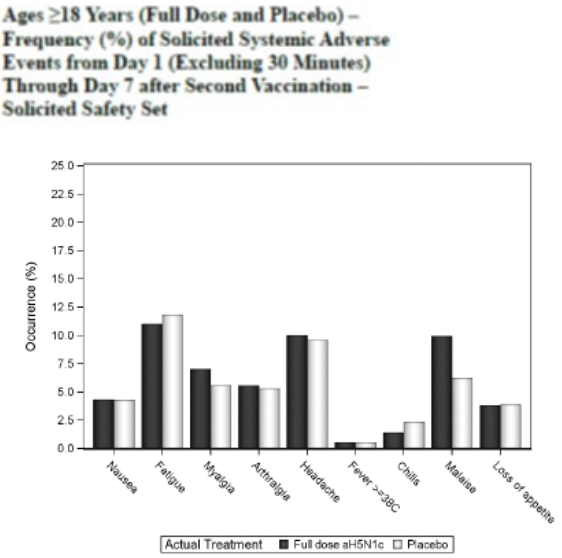
Ages ≥18 Years, Full Dose and Placebo

The most frequent solicited systemic AEs in the pooled adult population were fatigue, headache, myalgia, arthralgia and malaise. Incidences of systemic AEs were comparable in the aH5N1c and placebo group, apart from myalgia and malaise, which were less frequent in placebo recipients.

The majority of AEs were mild or moderate and resolved within several days. Systemic reactivity was lower after the second vaccination compared to the first.

Figure 15.



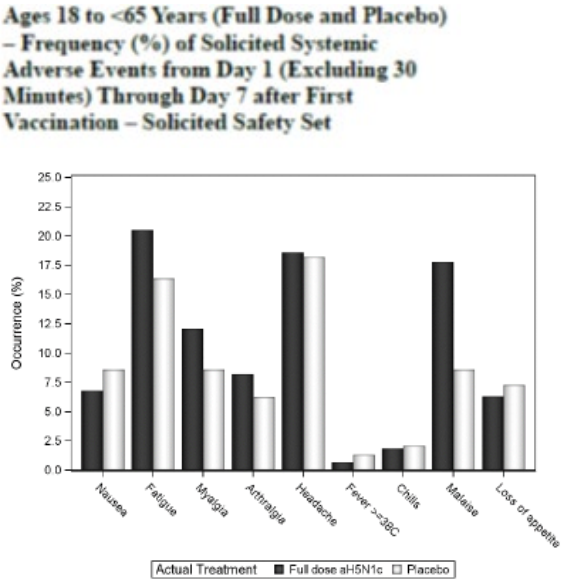


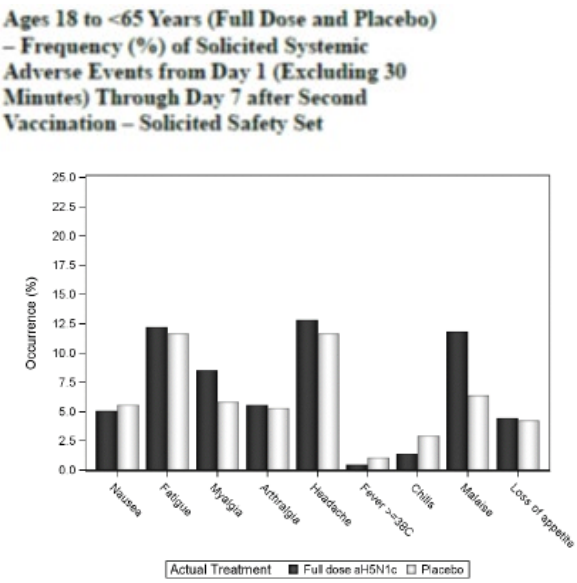
Ages 18 to <65 Years, Full Dose and Placebo

The most frequent solicited systemic AEs in the younger adult population <65 were fatigue, headache and malaise. Incidences of systemic AEs were comparable in the aH5N1c and placebo group, apart from fatigue, myalgia and malaise, which were less frequent in placebo recipients. Conversely, the incidence of fever was higher in the placebo group after both vaccinations.

As in the pooled group, the majority of AEs were mild or moderate and resolved within several days. Systemic reactivity was lower after the second vaccination compared to the first.

Figure 16.



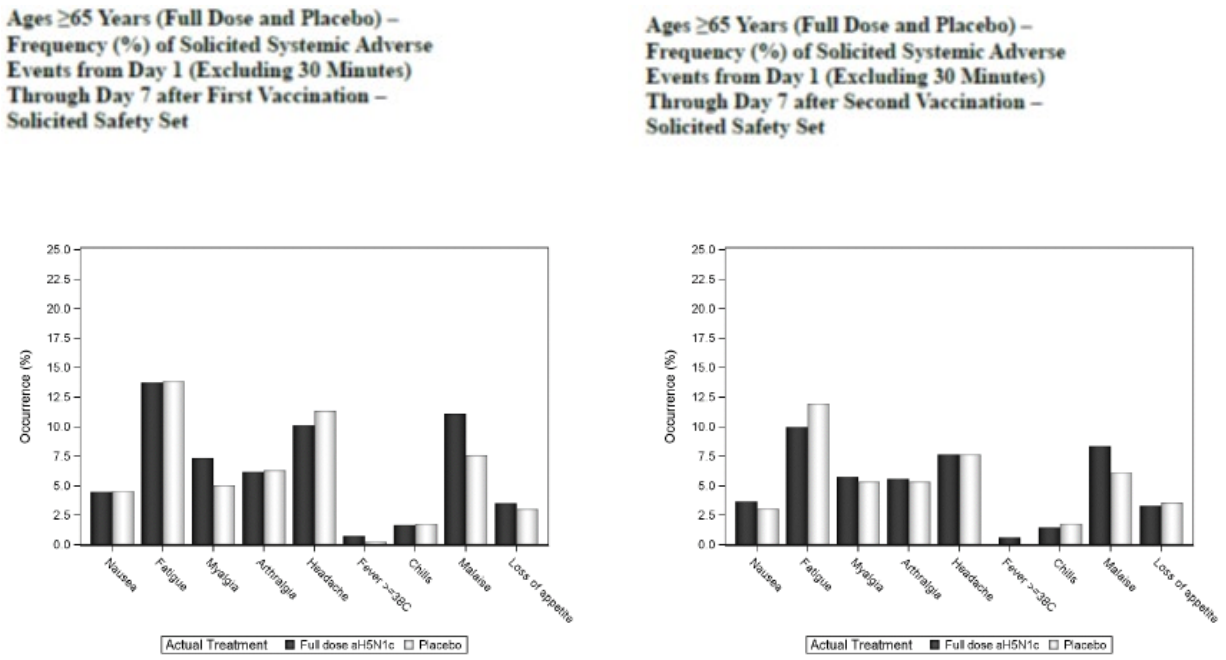


Ages ≥65 Years, Full Dose and Placebo

The most frequent solicited systemic AEs in the older adult population > 65 were fatigue, headache and malaise. Incidences of systemic AEs were comparable in the aH5N1c and placebo group, apart from malaise, which was less frequent in placebo recipients.

Comparable to the pooled population and the younger adult group, the majority of AEs were mild or moderate and resolved within several days. Systemic reactivity was lower after the second vaccination compared to the first.

Figure 17.

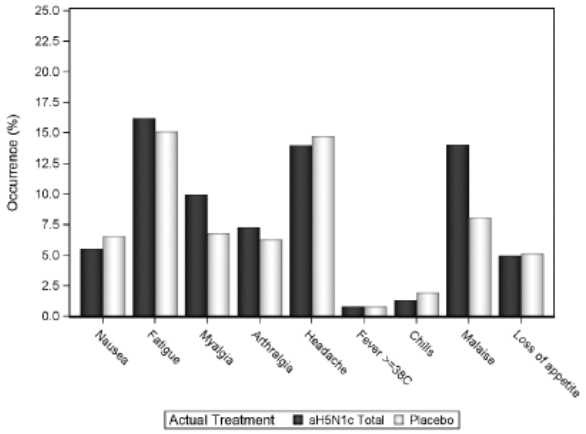


Ages ≥18 Years, Full and Half Dose Combined and Placebo

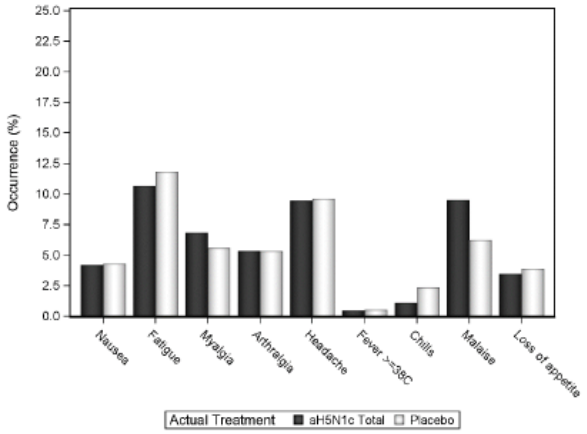
Adding solicited systemic reactogenicity data from the half-dose group did not change the systemic adverse event profile in a meaningful way.

Figure 18.

Ages ≥18 Years (Full and Half Dose Combined, and Placebo) – Frequency (%) of Solicited Systemic Adverse Events from Day 1 (Excluding 30 Minutes) Through Day 7 after First Vaccination – Solicited Safety Set



Ages ≥18 Years (Full and Half Dose Combined, and Placebo) – Frequency (%) of Solicited Systemic Adverse Events from Day 1 (Excluding 30 Minutes) Through Day 7 after Second Vaccination – Solicited Safety Set



Paediatric Study V89_11 (6 months - ≤17 years)

Subjects aged <6 years

The most frequently observed solicited systemic AE after any vaccination was irritability (28% in the low dose group vs. 30% in the high dose group) followed by sleepiness (25% in both dose groups) and change in eating habits (12% vs. 18%, respectively).

Across the dose groups, under severe solicited systemic AEs, ≤ 1% of subjects reported severe sleepiness and irritability after the first vaccination. Fever (body temperature ≥ 38.0° C) was experienced by 8% of subjects in the low dose group (first vaccination: 6%; second vaccination: 3%) and 16% of subjects in the high dose group (first vaccination: 7%; second vaccination 10%) after any vaccination. Only 1 subject in high dose group had body temperature ≥ 40.0° C, which occurred after the second vaccination.

For the prevention of pain and/or fever, 16% of subjects in the low dose group and 11% of subjects in the high dose group used prophylactic analgesic or antipyretic medication.

To treat pain and/or fever, 16% of subjects in low dose group and 23% of subjects in high dose group were prescribed with analgesic or antipyretic medication.

Subjects aged 6 years through 17 years

In subjects aged 6 years through 17 years, the most frequently reported solicited systemic AE after any vaccination was myalgia (28% in the low dose group vs. 30% in the high dose group) followed by fatigue (30% vs. 27%, respectively) and headache (29% vs. 22%, respectively). Fever (body temperature ≥38.0° C) was reported by 3% of subjects in the low dose group (first vaccination: 2%; second vaccination: 1%) and 4% of subjects (first vaccination: 3%; second vaccination: 1%) in the high dose group after any vaccination. None of the subjects developed a body temperature ≥ 40.0° C.

In both the dose groups, ≤1% of subjects reported severe solicited systemic AEs.

Prophylactic use of analgesic or antipyretic medication was reported by 6% of subjects in the low dose group and 9% of subjects in the high dose group. For the treatment of pain and/or fever after any vaccination, 14% vs. 15% of subjects in respective dose groups used analgesic or antipyretic medications.

Overall across the 2 age groups, apart from fever and use of analgesics and antipyretic medication in subjects aged <6 years, the percentage of subjects with solicited systemic AEs was similar between the 2 dose groups. In age group <6 years, a higher percentage of subjects in the high dose group reported fever and used analgesics/antipyretic medication than did subjects in the low dose group.

The majority of the solicited systemic AEs were of mild to moderate intensity. Most of the systemic AEs were resolved within day 7 after vaccination. As expected, compared to the first vaccination, the percentage of subjects with solicited systemic AEs was decreased after second vaccination.

However, all data concerning solicited systemic AEs for study V89_11 are presented for two age cohorts, below 6 years and 6 years to 17 years and should be analysed and presented for at least 3 age cohorts (e.g., 6-36 months, 3-9 years, 9-18 years) analogous to data presented for these two cohorts.

Phase 1 Study V89P1 (18 - ≤ 40)

Following the first vaccination, myalgia, fatigue, malaise, and headache were the most frequently reported systemic reactions. Similar percentages of subjects experienced fatigue and headache in the non-adjuvanted and adjuvanted vaccine groups, whereas myalgia was reported more frequently by subjects in the adjuvanted groups compared to the non-adjuvanted groups.

Similar to the reported solicited systemic reactions after the first vaccination, fatigue, headache and myalgia were also the most commonly reported systemic reactions experienced by subjects after the second vaccination. Similar percentages of subjects experienced fatigue and headache in the non-adjuvanted and adjuvanted vaccine groups (range across nonadjuvanted and adjuvanted groups: fatigue: 16% to 29% and 9% to 28%, respectively; headache: both 11% to 24%), whereas myalgia was more often experienced by subjects in the adjuvanted groups (13% to 36% compared to 12% to 13% in the non-adjuvanted groups).

Paediatric H1N1 Study V110_04 (6 months - ≤17 years)

The most common systemic reactions reported by the two oldest age cohorts were headache (first vaccination: 3% to 29%; second vaccination: 1% to 17%) and fatigue (first vaccination: 18% to 27%; second vaccination: 12% to 16%). The most common systemic reactions reported by the two youngest age cohorts were sleepiness (first vaccination: 16% to 32%; second vaccination: 14% to 19%), unusual crying (first vaccination: 21% to 30%; second vaccination:10% to 24%) and diarrhoea (first vaccination: 18% to 30%; second vaccination: 14% to 29%). The majority of the solicited systemic AEs were of mild or moderate severity.

Incidences were comparable between the dose groups, especially no trend towards increased fever could be observed in the younger age cohorts.

Supportive H3N2 Study V129_01 (paediatric 3 - ≤17 years; adult ≥18 years)

The systemic reactogenicity with the H3N2 strain was similar to that observed with the H5N1 strain.

Supportive H7N9 Study V131_01 (Adult 18 - ≤ 65 years)

The systemic reactogenicity with the H7N9 strain was similar to that observed with the H5N1 strain.

Unsolicited AEs

Ages ≥18 Years, Full Dose and Placebo

Day 1 Through Study TerminationTable ***68*** shows an overview of the numbers (%) of subjects with unsolicited AEs from Day 1 through study termination after any vaccination for subjects ≥18 years of age and by major age group.

Overall, the proportion of subjects for whom unsolicited (related) AEs were reported from Day 1 through Day 43 was comparable for subjects who received aH5N1c full dose and subjects who received placebo. The majority of the reported unsolicited AEs were of mild or moderate severity. Similar proportions of subjects in both treatment groups reported any unsolicited events, including SAEs, AESIs, AE(s) leading to NOCD, any AE leading to premature withdrawal and medically attended AE(s), from Day 1 through study termination.

Table 68.

Ages ≥18 Years (Full Dose and Placebo) – Numbers (%) of Subjects with Unsolicited Adverse Events from Day 1 Through Study Termination After Any Vaccination – Overall and by Major Age Group – Unsolicited Safety Set

	Overall		Age Group			
	≥18 Years		18 to <65 Years		≥65 Years	
	aH5N1c N=3579	Placebo N=796	aH5N1c N=1683	Placebo N=398	aH5N1c N=1896	Placebo N=398
Variable	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Subjects with any unsolicited AEs (Day 1-43)[a]	920 (25.7)	180 (22.6)	382 (22.7)	86 (21.6)	538 (28.4)	94 (23.6)
Subjects with any related unsolicited AEs (Day 1-43)[a]	294 (8.2)	49 (6.2)	121 (7.2)	22 (5.5)	173 (9.1)	27 (6.8)
Subjects with any SAE	225 (6.3)	74 (9.3)	55 (3.3)	13 (3.3)	170 (9.0)	61 (15.3)
Subjects with any related SAE	1 (0.0)	2 (0.3)	1 (0.1)	0	0	2 (0.5)
Subjects with any AE with outcome death	16 (0.4)	1 (0.1)	5 (0.3)	0	11 (0.6)	1 (0.3)
Subjects with any AESI	11 (0.3)	7 (0.9)	3 (0.2)	0	8 (0.4)	7 (1.8)
Subjects with any NOCD	348 (9.7)	73 (9.2)	105 (6.2)	20 (5.0)	243 (12.8)	53 (13.3)
Subjects with any AE leading to withdrawal[b]	18 (0.5)	3 (0.4)	7 (0.4)	1 (0.3)	11 (0.6)	2 (0.5)
Subjects with any medically attended AEs	1687 (47.1)	366 (46.0)	637 (37.8)	148 (37.2)	1050 (55.4)	218 (54.8)

Source: ISS

Abbreviations: AE = adverse event; AESI = adverse events of special interest; n = number of subjects with values in category; N = total number of subjects; NOCD = new onset of chronic disease; SAE = serious adverse event.

[a] Unsolicited AEs were collected during the treatment period Day 1-43; SAEs, AESIs, NOCDs, AEs leading to vaccine/study withdrawal, and medically attended AEs were collected from Day 1 through study termination.

[b] These numbers include deaths.

Percentages are based on the number of subjects in each treatment group.

Note: percentages of <0.1% are displayed as 0.0%.

The global incidences of AEs, solicited AEs, medically attended AEs, AESIs and SAEs appear comparable in the full dose group and the placebo group and are also similar when comparing adult and elderly subjects.

There is a difference in rates of NOCD observable between adult and elderly subjects, but the incidence in aH5N1c and placebo age cohorts is similar, most likely explained by the generally higher incidence of chronic conditions at a higher age.

Day 1 Through Day 43

An overview of the number (%) of subjects with "all" and "at least possibly related" unsolicited AEs reported from Day 1 through Day 43 after any vaccination is provided in Table 69 (by SOC) and in Table 70 (by PT).

Table 69.

Ages ≥18 Years (Full Dose and Placebo) – Number (%) of Subjects with All and at Least Possibly Related Unsolicited Adverse Events by System Organ Class Reported from Day 1 Through Day 43 After Any Vaccination[a] – Unsolicited Safety Set

	aH5N1c N=3579		Placebo N=796	
System Organ Class	All AEs n (%)	At Least Possibly Related AEs n (%)	All AEs n (%)	At Least Possibly Related AEs n (%)
Subjects with any AE	**920 (25.7)**	**294 (8.2)**	**180 (22.6)**	**49 (6.2)**
Infections And Infestations	247 (6.9)	17 (0.5)	52 (6.5)	3 (0.4)
General Disorders And Administration Site Conditions	210 (5.9)	158 (4.4)	40 (5.0)	27 (3.4)
Musculoskeletal And Connective Tissue Disorders	177 (4.9)	50 (1.4)	33 (4.1)	7 (0.9)
Nervous System Disorders	122 (3.4)	42 (1.2)	31 (3.9)	11 (1.4)
Gastrointestinal Disorders	113 (3.2)	27 (0.8)	27 (3.4)	9 (1.1)
Injury, Poisoning And Procedural Complications	103 (2.9)	6 (0.2)	16 (2.0)	0
Respiratory, Thoracic And Mediastinal Disorders	96 (2.7)	18 (0.5)	14 (1.8)	2 (0.3)
Skin And Subcutaneous Tissue Disorders	73 (2.0)	26 (0.7)	7 (0.9)	1 (0.1)
Metabolism And Nutrition Disorders	43 (1.2)	16 (0.4)	7 (0.9)	4 (0.5)
Eye Disorders	21 (0.6)	2 (0.1)	2 (0.3)	2 (0.3)
Ear And Labyrinth Disorders	20 (0.6)	5 (0.1)	1 (0.1)	0
Psychiatric Disorders	20 (0.6)	5 (0.1)	1 (0.1)	1 (0.1)
Cardiac Disorders	14 (0.4)	0	4 (0.5)	0
Vascular Disorders	14 (0.4)	1 (0.0)	3 (0.4)	0
Investigations	12 (0.3)	1 (0.0)	2 (0.3)	0
Reproductive System And Breast Disorders	12 (0.3)	0	1 (0.1)	0
Immune System Disorders	11 (0.3)	0	0	0
Neoplasms Benign, Malignant And Unspecified (Incl Cysts And Polyps)	11 (0.3)	2 (0.1)	3 (0.4)	0
Renal And Urinary Disorders	10 (0.3)	1 (0.0)	1 (0.1)	0
Blood And Lymphatic System Disorders	6 (0.2)	2 (0.1)	3 (0.4)	0
Surgical And Medical Procedures	6 (0.2)	0	0	0
Congenital, Familial And Genetic Disorders	2 (0.1)	0	1 (0.1)	0
Endocrine Disorders	2 (0.1)	0	0	0
Hepatobiliary Disorders	2 (0.1)	0	0	0
Product Issues[b]	1 (0.0)	0	0	0

Source: ISS Table 1.5.1 and Table 1.5.4

Abbreviations: AE = adverse event; MedDRA = Medical Dictionary for Regulatory Activities; n = number of subjects with values in category; N = total number of subjects.

[a] Throughout the document, this refers to unsolicited AEs with an onset from Day 1 through Day 43 of the study, regardless of whether 1 or 2 vaccinations were received.

[b] Product issues from Day 1 through Day 43 after vaccination was reported for Subject 50053 in Study V89_13, verbatim text: "Broken filling in tooth" (CSR V89_13: Listing 16.2.7.2).

Coded using MedDRA version 20.0.

Note: percentages of <0.1% are displayed as 0.0%.

Table 70.

Ages ≥18 Years (Full Dose and Placebo) – Number (%) of Subjects with All and at Least Possibly Related Unsolicited Adverse Events by Preferred Term (Occurring in ≥1% in Any Group) Reported from Day 1 Through Day 43 After Any Vaccination – Unsolicited Safety Set

	aH5N1c N=3579		Placebo N=796	
Preferred Term	All AEs n (%)	At Least Possibly Related AEs n (%)	All AEs n (%)	At Least Possibly Related AEs n (%)
Subjects with any AE	**920 (25.7)**	**294 (8.2)**	**180 (22.6)**	**49 (6.2)**
Headache	77 (2.2)	27 (0.8)	17 (2.1)	6 (0.8)
Injection Site Bruising	64 (1.8)	62 (1.7)	13 (1.6)	12 (1.5)
Fatigue	59 (1.6)	35 (1.0)	12 (1.5)	7 (0.9)
Arthralgia	56 (1.6)	23 (0.6)	10 (1.3)	3 (0.4)
Upper Respiratory Tract Infection	55 (1.5)	6 (0.2)	6 (0.8)	0
Viral Upper Respiratory Tract Infection	45 (1.3)	2 (0.1)	3 (0.4)	0
Myalgia	44 (1.2)	24 (0.7)	8 (1.0)	3 (0.4)
Back Pain	31 (0.9)	3 (0.1)	10 (1.3)	1 (0.1)
Urinary Tract Infection	27 (0.8)	1 (0.0)	12 (1.5)	0

Source: ISS Table 1.5.2 and Table 1.5.4

Abbreviations: AE = adverse event; MedDRA = Medical Dictionary for Regulatory Activities; n = number of subjects with values in category; N = total number of subjects.

Coded using MedDRA version 20.0.

Note: percentages of <0.1% are displayed as 0.0%.

The proportion of subjects for whom unsolicited AEs were reported during the treatment period from Day 1 through Day 43 after any vaccination was similar for the aH5N1c full dose and the placebo groups (25.7% vs. 22.6%). There was no clear increase in the frequency, or difference in nature of unsolicited AEs in the aH5N1c group compared with the placebo group. The proportion of unsolicited AEs considered to be at least possibly related to vaccination was similar in the aH5N1c full dose group and placebo group (8.2% vs. 6.2%).

The most frequently affected SOC in both treatment groups was 'Infections and infestations' (6.9% in the aH5N1c group vs. 6.5% in the placebo group) followed by 'General disorders and administration site conditions' (5.9% and 5.0%, respectively). The number of subjects with AEs categorised to the SOC 'Skin and subcutaneous disorders' (all AEs and possibly or probably related AEs) are shown per PT in ISS Table 71. The number of subjects with AEs categorised to the SOC 'Immune system disorders' (all AEs) are shown per PT in Table 72. There were no possibly or probably related AEs in the SOC 'Immune system disorders'.

Table 71.

Ages ≥18 Years (Full Dose and Placebo) – Numbers (%) of Subjects with All and at Least Possibly Related Unsolicited Adverse Events by Preferred Term Reported from Day 1 through Day 43 after Any Vaccination - Skin and Subcutaneous Tissue Disorders – Unsolicited Safety Set

	aH5N1c N=3579		Placebo N=796	
Preferred Term	**All AEs** n (%)	**At Least Possibly Related AEs** n (%)	**All AEs** n (%)	**At Least Possibly Related AEs** n (%)
Subjects with any AE	**73 (2.0)**	**26 (0.7)**	**7 (0.9)**	**1 (0.1)**
Rash	17 (0.5)	7 (0.2)	2 (0.3)	0
Dermatitis Contact	10 (0.3)	0	0	0
Ecchymosis	9 (0.3)	7 (0.2)	0	0
Pruritus Generalised	6 (0.2)	4 (0.1)	0	0
Pruritus	5 (0.1)	2 (0.1)	1 (0.1)	0
Hyperkeratosis	4 (0.1)	0	0	0
Actinic Keratosis	2 (0.1)	0	0	0
Erythema	2 (0.1)	1 (0.0)	0	0
Hyperhidrosis	2 (0.1)	0	0	0
Night Sweats	2 (0.1)	2 (0.1)	0	0
Acne	1 (0.0)	0	0	0
Dermal Cyst	1 (0.0)	1 (0.0)	0	0
Dyshidrotic Eczema	1 (0.0)	0	0	0
Eczema	1 (0.0)	0	0	0
Ingrowing Nail	1 (0.0)	0	0	0
Intertrigo	1 (0.0)	0	0	0
Miliaria	1 (0.0)	0	0	0
Papule	1 (0.0)	0	2 (0.3)	0
Precancerous Skin Lesion	1 (0.0)	0	0	0
Rash Macular	1 (0.0)	0	0	0
Rash Pruritic	1 (0.0)	1 (0.0)	0	0
Skin Hyperpigmentation	1 (0.0)	0	0	0
Skin Lesion	1 (0.0)	0	0	0
Solar Lentigo	1 (0.0)	0	0	0
Swelling Face	1 (0.0)	1 (0.0)	0	0
Urticaria	1 (0.0)	1 (0.0)	1 (0.1)	1 (0.1)
Keratosis Pilaris	0	0	1 (0.1)	0

Source: ISS Table 1.5.1 and Table 1.5.4
Abbreviations: AE = adverse event; n = number of subjects with values in category; N = total number of subjects.
Coded using MedDRA version 20.0.
Note: percentages of <0.1% are displayed as 0.0%.

Table 72.

Ages ≥18 Years (Full Dose and Placebo) – Numbers (%) of Subjects with All Adverse Events by Preferred Term Reported from Day 1 through Day 43 after Any Vaccination – Immune System Disorders – Unsolicited Safety Set

Preferred Term	aH5N1c N=3579 n (%)	Placebo N=796 n (%)
Subjects with any AE	**11 (0.3)**	**0**
Allergy to Arthropod Bite	2 (0.1)	0
Hypersensitivity	2 (0.1)	0
Seasonal Allergy	2 (0.1)	0
Allergy to Arthropod Sting	1 (0.0)	0
Drug Hypersensitivity	1 (0.0)	0
Food Allergy	1 (0.0)	0
Multiple Allergies	1 (0.0)	0
Type IV Hypersensitivity Reaction	1 (0.0)	0

Source: ISS Table 1.5.1
Abbreviations: AE = adverse event; n = number of subjects with values in category N = total number of subjects.
Coded using MedDRA version 20.0.
Note: percentages of <0.1% are displayed as 0.0%.

Both for subjects who received aH5N1c full dose or placebo, the most frequently reported unsolicited AEs (PT term) during the treatment period were headache (aH5N1c: 2.2%, placebo: 2.1%) and injection site bruising (aH5N1c:1.8%, placebo: 1.6%). At PT level, no notable differences were found between aH5N1c and placebo for unsolicited AEs that were considered related to study vaccination. The most frequently reported related unsolicited AEs were injection site bruising (aH5N1c: 1.7%, placebo: 1.5%) and fatigue (aH5N1c:1.0%, placebo: 0.9%). Across the 2 treatment groups, <1% of subjects reported the unsolicited AEs injection site bruising and fatigue which were solicited AEs continuing beyond Day 7 after the first and second vaccination.

Most of the unsolicited AEs reported from Day 1 through Day 43 after any vaccination were of mild (aH5N1c: 18.0%, placebo: 14.3%) or moderate severity (aH5N1c: 6.8%, placebo: 7.0%). Severe unsolicited AEs were experienced by 1.0% of subjects in the aH5N1c full dose group and 1.3% of subjects in the placebo group. The most frequent severe unsolicited AEs with aH5N1c full dose after any vaccination were fatigue (0.1%, n=5), headache (0.1%, n=5), and arthralgia (0.1%, n=4). Of these, 3 severe cases of fatigue, 2 severe cases of arthralgia and 1 severe case of headache were considered possibly or probably related to aH5N1c study vaccination.

The majority of the unsolicited AEs had resolved by the end of the studies.

The relative incidences of AEs up to 21 days after the second vaccination are generally comparable between active and placebo groups and do not give rise to concern.

Ages 18 to <65 Years, Full Dose and Placebo

Day 1 Through Day 43

The proportion of subjects in the age 18 to <65 years of age group for whom unsolicited (related) AEs were reported from Day 1 through Day 43 was comparable to the results in the ≥18 years of age population as a whole. Unsolicited AEs were reported by 22.7% of the subjects who received aH5N1c full dose and 21.6% of the subjects who received placebo.

The proportion of unsolicited AEs after any vaccination considered to be at least possibly related to vaccination was 7.2% in the aH5N1c full dose group and 5.5% in the placebo group.

The most frequently affected SOC in both treatment groups was 'Infections and infestations' (6.7% in the aH5N1c group vs. 5.3% in the placebo group) followed by 'General disorders and administration site conditions' (4.6% and 4.0%, respectively) and 'Musculoskeletal and connective tissue disorders' (4.6% and 5.0%, respectively).

The most frequently reported unsolicited AE during the treatment period was headache in both treatment groups (aH5N1c: 2.4%, placebo: 2.8%), followed by upper respiratory tract infection in the aH5N1c group (1.9%) and arthralgia in the placebo group (2.3%). All other unsolicited AEs occurred in ≤1.5% of the subjects in each treatment group.

Across the 2 treatment groups, <1% of subjects reported unsolicited AEs such as headache and arthralgia which were solicited AEs continuing beyond Day 7 after the first and second vaccination and the second.

Most of the unsolicited AEs reported from Day 1 through Day 43 after any vaccination in the 18 to <65 years of age group were of mild (aH5N1c: 16.2%, placebo: 13.8%) or moderate severity (aH5N1c: 5.8%, placebo: 6.3%). Severe unsolicited AEs were experienced by 0.8% of subjects in the aH5N1c full dose group and 1.5% of subjects in the placebo group.

The most frequent severe unsolicited AEs with aH5N1c full dose after any vaccination were headache (0.2%, n=4) and arthralgia (0.2%, n=4). Of these, 1 case of headache and 2 cases of arthralgia were considered possibly or probably related to aH5N1c study vaccination.

18 to <50 years vs. 50 to <65 years

During the treatment period, no notable differences were found in the proportion of subjects that reported unsolicited AEs, nor in the nature and severity of unsolicited AEs, between subjects 18 to <50 years of age (aH5N1c: 21.1%, placebo: 21.4%) and subjects 50 to <65 years of age (25.5%, 21.9%).

Ages ≥65 Years, Full Dose and Placebo

Day 1 Through Day 43

The overall nature and frequency of unsolicited AEs with the aH5N1c full dose in the≥65 years of age group were similar to those described for ≥18 years of age population as a whole. In the ≥65 years of age group, unsolicited AEs were reported by 28.4% of the subjects who received aH5N1c full dose and 23.6% of the subjects who received placebo.

The proportion of unsolicited AEs considered to be at least possibly related to vaccination was 9.1% in the aH5N1c full dose group and 6.8% in the placebo group.

The proportion of subjects with unsolicited AEs was higher in the ≥65 years of age group as compared to the 18 to <65 years of age group, for both aH5N1c full dose (28.4% vs. 22.7%) and placebo group (23.6% vs. 21.6%). There were no notable differences in the nature and severity of AEs between the ≥ 65 years of age group and 18 to <65 years of age group.

In subjects ≥65 years of age, the most frequently reported unsolicited AE during the treatment period was injection site bruising (aH5N1c: 2.4%, placebo: 1.8%), followed by headache (1.9%), fatigue (1.8%), and arthralgia (1.6%) in the aH5N1c group, and urinary tract infection in the placebo group (1.8%). All other unsolicited AEs occurred in ≤1.5% of the subjects in each treatment group. Across the treatment groups, ≤1% of subjects reported unsolicited AEs such as injection site bruising, headache, fatigue and arthralgia which were solicited AEs continuing beyond Day 7 after the first and second vaccination.

Most of the unsolicited AEs reported from Day 1 through Day 43 after any vaccination in the ≥65 years of age group were of mild (aH5N1c: 19.6%, placebo: 14.8%) or moderate severity (aH5N1c: 7.6%, placebo: 7.8%). Severe unsolicited AEs were experienced by 1.2% of subjects in the aH5N1c full dose group and 1.0% in the placebo group. The most frequent severe unsolicited AE with aH5N1c full dose after any vaccination in subjects ≥65 years of age was fatigue (0.2%, n=3). Two severe cases of fatigue were considered possibly or probably related to the aH5N1c vaccine.

65 to <75 years vs. ≥75 years

During the treatment period, no notable differences were found in the proportion of subjects that reported unsolicited AEs, nor in the nature and severity of the unsolicited AEs, between subjects 65 to <75 years of age (aH5N1c: 28.7%, placebo: 24.0%) and subjects ≥75 years of age (aH5N1c: 27.6%, placebo: 22.9%).

Ages ≥18 Years, Full and Half Dose Combined and Placebo

Day 1 Through Day 43

The proportion of subjects for whom unsolicited AEs were reported during the treatment period from Day 1 through Day 43 was 27.0% for the aH5N1c full and half dose combined group and 22.6% for the placebo group. There was no clear increase in the frequency, or difference in the nature of unsolicited AEs in the aH5N1c group compared with the placebo group. The proportion of subjects with AEs considered to be at least possibly related to vaccination was similar in the aH5N1c full and half dose combined group and placebo group (8.9% vs. 6.2%).

The most frequently reported unsolicited AEs during the treatment period were headache (aH5N1c: 2.2%, placebo: 2.1%) and injection site bruising (2.0%, 1.6%). At PT level, no notable differences were found between aH5N1c and placebo for unsolicited AEs that were considered related to study vaccination. The most frequently reported related unsolicited AEs were injection site bruising (aH5N1c: 1.9%, placebo: 1.5%) and fatigue (1.1%, 0.9%). Across the 2 treatment groups, <1% of subjects reported unsolicited AEs such as headache, injection site bruising, and fatigue which were solicited AEs continuing beyond Day 7 after the first or second vaccination.

Most of the unsolicited AEs reported from Day 1 through Day 43 after any vaccination were of mild (aH5N1c: 19.0%, placebo: 14.3%) or moderate severity (aH5N1c: 7.0%, placebo: 7.0%). Overall, the proportion of subjects experiencing severe unsolicited AEs was low, 1.0% in the aH5N1c full and half dose combined group and 1.3% in the placebo group. The most frequent severe unsolicited AEs in the aH5N1c full and half dose combined group after any vaccination were fatigue (0.2%, n=8), headache (0.1%, n=5) and arthralgia (0.1%, n=5). Six severe cases of fatigue, 3 severe cases of arthralgia and 1 severe case of headache were considered possibly or probably related to aH5N1c study vaccination.

The incidence of all AEs as well as related AEs when analysing the safety population including full and half-dose of the aH5N1c vaccine is consistent with the safety profile observed in the full dose population.

No safety concerns arise from the nature and frequency of the reported unsolicited adverse events.

Paediatric Study V89_11

Overall, the percentage of subjects reporting unsolicited AEs after any vaccination was similar between the half dose and full dose group (29% vs. 26%). In comparison to the first vaccination, the incidence of unsolicited AEs was lower after the second vaccination in both the half dose group (first vaccination:

18%; second vaccination: 14%) and full dose group (first vaccination: 20% vs. second vaccination: 11%).

Medically attended AEs were reported by 35% and 34% of subjects in the half and full dose group, respectively. Unsolicited AEs leading to NOCD were reported by 1% of subjects in the half dose group and in none of the subjects in the full dose group. The unsolicited AEs (bone cyst, ADHS, dyspepsia) leading to NOCD were assessed as not related to the study vaccine by the investigator.

Vaccination 1

After the first vaccination (Day 1 through Day 22), 18% of subjects in the half dose group and 20% of subjects in the full dose group reported unsolicited AEs. In total, only 3% to 4% of subjects had at least possibly or probably related unsolicited AEs as per the investigator. The most frequently affected SOC in both dose groups was 'Infections and infestations' (9% in half dose group vs. 10% in full dose group) followed by 'Gastrointestinal disorders' (5%) in the half dose group and 'General disorders and administration site conditions' (5%) in the full dose group. 'General disorders and administration site conditions' were considered to be possibly or probably related to the study vaccine as per the investigator in 2% of the subjects in each dose group. The most frequently reported unsolicited AE (by PT term) after first vaccination was upper respiratory tract infection (5% in both dose groups) followed by pyrexia (2% vs. 4%) and vomiting (3% vs. 2%). Across the 2 dose groups, ≤1% of subjects reported unsolicited AEs such as erythema, induration, and pain at the injection site, myalgia, arthralgia, headache and nausea which were solicited AEs continuing beyond Day 7 after the first vaccination.

Vaccination 2

After the second vaccination (Day 23 through Day 43), 14% of subjects in the half dose group and 11% of subjects in the full dose group reported unsolicited AEs. Among these, very few subjects (≤1%) reported unsolicited AEs assessed as possibly or probably related as per the investigator. The most frequently affected SOC after second vaccination was 'Infections and infestations' (9% vs. 7%, in respective dose groups. Upper respiratory tract infection (3% in half dose vs. 4% in full dose group) was the most frequently reported unsolicited AE (PT term) followed by pyrexia (2% vs. 1%) and nasopharyngitis (1% vs. 2%). Solicited AEs continuing beyond day 7 after the second vaccination (reported as unsolicited AEs) such as nausea, fatigue, injection site pain, myalgia and headache were reported by ≤1% of subjects.

However, all data concerning unsolicited AEs for study V98_11 are presented for the whole paediatric population and should be analysed and presented for the three age cohorts: 6 through 35 months, 3 through 8 years and 9 through 17 years analogous to data presented for the overall population. The requested analyses were submitted with the responses to the D180 LoOI. It is noticeable that the incidence of TEAEs decreases with increasing age. Only a minority of AEs were considered to be possibly or probably related, and no safety concern arises from the provided analyses.

Paediatric H1N1 Study V110_04 (6 months - ≤17 years)

Unsolicited AEs during the treatment period were reported by 33% to 35% of subjects in the 9 to 17 years cohort and 43% to 44% in the 3 to 8 years cohort. In the 12 to 35 months cohort (44% to 70%) and the 6 to 11 months cohort (68% in both dose groups) a higher percentage of subjects reported unsolicited AEs up to Day 43.

Of the AEs experienced, there was a slightly higher percentage of subjects in the 6 to 11 months cohort with AEs considered at least possibly related to the vaccine (27% to 30%) compared to the 9 to

17 years cohort (19% to 23%), the 3 to 8 years cohort (21% to 28%) and the 12 to 35 months cohort (6% to 21%). The rates were similar across groups within each age cohort.

In the older age cohorts (9 to 17 years and 3 to 8 years), the most common AEs (by PT) experienced were: rhinitis (1% to 10% across groups), oropharyngeal pain (3% to 8% across groups), diarrhoea (1% to 9% across groups) and abdominal pain (1% to 8% across groups).

In the younger age cohorts (12 to 35 months and 6 to 11 months), the most common AEs experienced were: nasopharyngitis (16% to 45% across groups), diarrhoea (4% to 23% across groups), and pyrexia (1% to 11% across groups).

The most common AEs in the 9 to 17 years cohort considered at least possibly related to vaccine were: injection site haemorrhage (3%), diarrhoea (1% to 5%), rhinitis (1% to 4%), abdominal pain (1%) and decreased appetite (0% to 3%). The most common AEs in the 3 to 8 years cohort considered at least possibly related to vaccine were: diarrhoea (3% to 6%), irritability (1% to 6%), abdominal pain (3% to 5%), and pyrexia (0% to 3%). The most common AEs in the 12 to 35 months cohort considered at least possibly related to vaccine were: nasopharyngitis (2% to 6%), cough (0% to 6%), diarrhoea (0% to 3%), and vomiting (2% to 3%). The most common AEs in the 6 to 11 months cohort considered at least possibly related to vaccine were: nasopharyngitis (9% to 15%), diarrhoea (5% to 11%), eating disorder (3% to 8%) and pyrexia (3% in both dose groups).

Supportive H3N2 Study V129_01 (paediatric 3 - ≤17 years; adult ≥18 years)

In the 3 to <9 years age cohort, 14% to 34% of the subjects across the vaccine groups reported unsolicited AEs, with 4% to 8% reporting AEs at least possibly related to the study vaccine. In the 9 to <18 age cohort, 9% to 28% of the subjects across the vaccine groups reported unsolicited AEs, with 4% to 9% reporting at least possibly related AEs.

In the 18 to <65 years age cohort, 13% to 25% of the subjects across the vaccine groups reported unsolicited AEs, with 6% to 8% reporting AEs at least possibly related to the study vaccine. In the ≥65 years age cohort, 24% to 38% of the subjects across the vaccine groups reported unsolicited AEs, with 8% to 10% reporting at least possibly related AEs.

The observed safety profile in this study is consistent with safety data generated with the H5N1 strain.

Supportive H7N9 Study V131_01 (Adult 18 - ≤ 65 years)

Across the groups, 24% to 30% of subjects reported unsolicited AEs from Day 1 through Day 43. Approximately 6% to 11% of these unsolicited AEs were assessed by the investigator as at least possibly or probably related to the study vaccine.

The observed safety profile in this study is consistent with safety data generated with the H5N1 strain.

2.6.8.3. Serious adverse event/deaths/other significant events

SAE

aH5N1c full dose

The frequency of subjects with SAEs from Day 1 through study termination was 6.3% in the aH5N1c full dose group and 9.3% in the placebo group.

Most of the SAEs were reported after Day 43, during the follow-up period (aH5N1c: 5.8% of subjects; placebo: 8.4% of subjects).

In total, 14 subjects in the ≥18 years of age, full dose population reported SAEs after the first vaccination comprising 9 subjects (0.3%) in the aH5N1c group and 5 subjects (0.6%) in the placebo group.

After the second vaccination, 14 subjects reported an SAE (aH5N1c: 11 subjects [0.3%]; placebo: 3 subjects [0.4%]).

Related SAEs

Only in 3 subjects (aH5N1c: 1 subject [<0.1%]; placebo: 2 subjects [0.3%]) the SAEs, all reported during the follow-up period, were considered possibly or probably related to study vaccination according to the investigator. These SAEs included spontaneous abortion (aH5N1c group) and immune thrombocytopenic purpura and polymyalgia rheumatica (both in the placebo group). Except for the spontaneous abortion (Study V89_04), these related SAEs occurred in subjects ≥65 years of age.

The SAE spontaneous abortion (aH5N1c full dose group) was considered related to study vaccination by the investigator. A subject of study V89_04 reported a previous spontaneous abortion in their medical history. During the reported pregnancy evidence of chromosomal XXX triploid aberration that is characteristically observed with partial (hydatidiform) molar pregnancy, therefore a plausible contribution from study vaccine or concomitant medication is not likely according to the sponsor, rather, the most probable causality for the molar pregnancy is the chromosomal abnormality from the time of the aberrant fertilisation. The causality assessment by the sponsor is supported.

No additional possibly or probably related unsolicited SAEs were reported in subjects in the half dose group.

Paediatric Study V89_11

In the paediatric Study V89_11, 24 SAEs were reported from Day 1 through study termination, of which, 14 SAEs were reported by 11 subjects in the half dose group, and 10 SAEs were reported by 8 subjects in the full dose group. None of these SAEs (mainly infections and bone fractures) were considered to be related to the study vaccine.

Paediatric H1N1 Study V110_04

In paediatric Study V110_04, from Day 1 to Day 366, a total of 37 subjects reported 49 SAEs (4 subjects in the 9 to 17 years cohort, 3 subjects in the 3 to 8 years cohort, 18 subjects in the 12 to 35 months cohort and 12 subjects in the 6 to 11 months cohort). The percentages of subjects who reported at least one SAE were balanced across the vaccine groups within each age cohort.

Two subjects reported SAEs at least possibly related to the vaccine:

☐ In the 9 to 17 years cohort 1 (full dose group), one subject experienced a convulsion (Day 98) and multiple episodes of epilepsy (Days 157 and 185), which were considered possibly related to vaccine. However, the 14-year-old Caucasian male subject who reported SAEs of convulsion and epilepsy had a medical history of meningitis, which confounds the causality assessment.

☐ In the 12 to 35 months cohort (half dose group), one subject who experienced a febrile convulsion (Day 10) considered as possibly related to vaccine.

Supportive H3N2 Study V129_01

From Day 1 through Day 366, 18 subjects experienced SAEs; 1 subject (full dose group) in the 3 to <9 years age cohort, 1 subject (15_None group) in the 9 to <18 years age group, 4 subjects (3 in the half dose and 1 in 15_None group) in the 18 to <65 years age cohort and 12 subjects (2 in the half dose, 5 in the full dose and 5 in 1the 15_None group) in the ≥65 years age cohort.

None of the SAEs were assessed as related to the study vaccination.

Supportive H7N9 Study V131_01

Across all vaccine groups a total of 8 subjects experienced 16 SAEs over the course of the study. None of the SAEs were judged by the investigator as related to the study vaccine.

Deaths

Collectively 21 deaths were reported from Day 1 through study termination in Studies V89P1, V89_04, V89_13, and V89_18. No deaths were reported in the paediatric study (V89_11).

Sixteen deaths were reported in the full dose group, 1 in the half dose group, 3 in non-adjuvanted vaccine groups, and 1 in the placebo group. All deaths occurred during the follow-up period (ie, >21 days after last vaccination received) with a mean of 234.4 days (range 38-512 days) for the aH5N1c group and 227 days for the placebo group. All deaths were reported in subjects with underlying diseases, taking multiple concomitant medications or were accidental (gunshot wounds). No patterns, trends or safety signals were identified during a review of the combined cases of death reported across the trials. None of the deaths were assessed as related to the study vaccine by either the investigator or by Seqirus. The submitted narratives of the deaths do not indicate a relationship of each event with IP.

AESI

Ages ≥18 Years, Full Dose and Placebo

In the pooled data, 18 subjects developed an AESI (as retrospectively categorised) during the course of the trial they were enrolled in, comprising 11 subjects (0.3%) with the full dose of aH5N1c and 7 subjects (0.9%) in the placebo group. For the subjects who were vaccinated with aH5N1c, all AESIs occurred beyond Day 43 during the follow-up period. In the placebo group, 1 subject (0.1%) experienced an AESI (colitis ulcerative) after Vaccination 2 prior to Day 43, and all other AESIs were reported during the follow-up period, after Day 43.

None of the AESIs in the aH5N1c full dose group were assessed as possibly related to study vaccination; however, 2 AESIs reported for subjects in the placebo group were assessed by the investigator as possibly related to study vaccination: one subject was diagnosed with immune thrombocytopenic purpura and the other subject was diagnosed with polymyalgia rheumatica.

Ages ≥18 Years, Full and Half Dose Combined and Placebo

No AESIs were reported in the half dose group, thus no additional AESIs were reported in the full and half dose combined group.

One case of facial paralysis occurred in, a 71 year old white female participant of phase 2 trial V89_13 in temporal relationship to the second vaccination. The investigator considered this AESI as related to a seasonal influnza vaccine (Fluvax), which was given concomitantly with the second vaccination of aH5N1c. As an influence of aH5N1c on the development of this adverse event cannot be legitimately excluded, this AE should be reflected in section 4.8 of the SmPC. With their response to the

outstanding questions, the applicant has clarified that the date for the seasonal infuenza vaccination given in the narrative of the SAE was a transcription error. The real date was 10 days before onset of the facial paralysis and, given the temporal relationship, is likely to be the underlying cause. A correction to the CSR was submitted.

The narratives provided for the other reported AESIs do not support a causal or temporal relationship with IP.

Paediatric Study V89_11

There were no AESIs reported throughout the study.

Paediatric H1N1 Study V110_04

At the time Study V110_04 was conducted, AESIs had not been prespecified yet.

Supportive H3N2 Study V129_01

There were no AESIs reported throughout the study.

Supportive H7N9 Study V131_01

There were no AESIs reported throughout the study.

2.6.8.4. Laboratory findings

No laboratory evaluations were assessed for the evaluation of safety in aH5N1c studies V89_04, V89_13, V89_11, and V89_18.

Only in Study V89P1 laboratory data regarding safety were evaluated. Blood samples were collected in the first 120 subjects (approximately 10 per vaccine group) enrolled for clinical laboratory testing prior to vaccination on Day 1, Day 8 and Day 29. Serum chemistry and haematology clinical laboratory tests included haemoglobin, red blood count (erythrocytes), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), white blood count (leukocytes), platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine. Urinalysis was not performed.

Mean changes from baseline in laboratory data were generally small and without clinical relevance. Mean values showed no antigen level or adjuvant content related differences.

No safety signals arise from the laboratory evaluations of the phase 1 study.

2.6.8.5. Safety in special populations

Table 73. Number (%) of Subjects Reporting Selected Unsolicited AEs by Age Group - Studies V89_04, V89_13, and V89_18 - Unsolicited Safety Set

	<65 years N=1674	**65-74 years N=1358**	**75-84 years N=482**	**85+ years N=52**
Total AEs	774 (46%)	829 (61%)	303 (63%)	35 (69%)
Serious AEs - Total	55 (3%)	105 (8%)	56 (12%)	9 (18%)
- Fatal	5 (<1%)	7 (<1%)	1 (<1%)	3 (6%)
- Hospitalization/prolong existing hospitalization	44 (3%)	90 (7%)	46 (10%)	8 (16%)
- Life-threatening	4 (<1%)	6 (<1%)	5 (1%)	3 (6%)
- Disability/incapacity	0	1 (<1%)	1 (<1%)	0
- Other (medically significant)	11 (<1%)	17 (1%)	11 (2%)	2 (4%)
AE leading to drop out	4 (<1%)	4 (<1%)	0	1 (2%)

Psychiatric disorders	48 (3%)	24 (2%)	10 (2%)	2 (4%)
Nervous system disorders	92 (5%)	99 (7%)	38 (8%)	5 (10%)
Injury, poisoning and procedural complications*	161 (10%)	169 (12%)	64 (13%)	9 (18%)
Cardiac disorders	24 (1%)	36 (3%)	19 (4%)	5 (10%)
Vascular disorders	34 (2%)	43 (3%)	17 (4%)	4 (8%)
Cerebrovascular disorders	0	0	0	0
Infections and infestations	353 (21%)	371 (27%)	120 (25%)	13 (25%)
Anticholinergic syndrome	0	0	0	0
Quality of life decreased	0	0	0	0
Sum of postural hypotension, black outs, syncope, dizziness, ataxia	16 (<1%)	18 (1%)	8 (2%)	0
Eye disorders	19 (1%)	48 (4%)	20 (4%)	3 (6%)
Metabolism and nutrition disorders	49 (3%)	76 (6%)	26 (5%)	1 (2%)
Musculoskeletal and connective tissue disorders	153 (9%)	235 (17%)	76 (16%)	6 (12%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	14 (<1%)	46 (3%)	32 (7%)	5 (10%)
Source: V89_04, V89_13, V89_18 Integrated: Table 14.3.1.1.99 *Falls and fractures are included in the category "Injury, poisoning and procedural complications"				

Pregnancy

Pregnancy was an exclusion criterion in all clinical trials with adjuvanted and non-adjuvanted H5N1c formulations. However, during Studies V89P1, V89_04, V89_11, and V89_18, a total of 55 pregnancies were reported after the administration of aH5N1c, 3 of the pregnancies with non-adjuvanted H5N1c and 5 with placebo.

Of the 50 subjects who did become pregnant following treatment with (non)adjuvanted H5N1c, 50% resulted in liveborn healthy babies, 18% ended with a therapeutic/elective abortion, 6% ended with spontaneous abortion and 2% in a missed abortion. For a total of 24% no outcome was available.

In the placebo group 5 subjects became pregnant following placebo administration, among them 40% resulted in liveborn healthy babies, 40% ended with a spontaneous abortion and for 20% no outcome was available. No reports were received of babies born with congenital malformations or other problems. Overall, a total of 8% spontaneous abortion/missed abortion were reported in the aH5N1c development programme and 40% in the placebo group, respectively.

The frequency of spontaneous abortions reported in the aH5N1c programme is aligned according to what is reported in the US general population (estimated background risk of miscarriage in clinically recognised pregnancies 15% to 20%).

The available sparse data in pregnancies do not indicate a safety signal for aH5N1c.

Adverse events by gender

Pooled Data, Ages ≥18 Years

Solicited Local Adverse Events

The frequency of solicited AEs after any vaccination was lower in male than in female subjects in both the aH5N1c group and placebo group. Solicited local AEs were reported for 44.5% male vs. 57.1% female subjects in the aH5N1c group and 11.7% vs. 17.1% in the placebo group, respectively.

Solicited Systemic Adverse Events

Solicited systemic AEs were reported for 34.3% (males) vs. 42.6% (females) in the aH5N1c group and 23.4% vs. 40.4% in the placebo group, respectively. This pattern was also seen for the individual AEs. A similar pattern, i.e., differences between male and female subjects, was found in the full and half dose combined.

There was no notable difference in the frequency of fever between male and female subjects from Day 1 (excluding 30 minutes) through Day 7 (aH5N1c: 1.0% vs. 1.4%, placebo: 0.6% vs. 1.8% for males vs. females). All 3 subjects who presented with fever ≥40°C in the aH5N1c group were male subjects. In the full and half dose combined group, there were no additional cases of fever ≥40°C.

Adverse events by country

Adult and elderly population (≥ 18 years)

The frequency of solicited AEs after any vaccination ranged from 54.9% in New Zealand to 78.2% in Australia with solicited AEs reported in 59.7% and 61.2% of subjects from Thailand and USA, respectively.

The proportion of unsolicited AEs in the half and full dose combined group was higher in subjects who were enrolled in Australia (41.4%) or New Zealand (44.4%) than in subjects who were enrolled in Thailand (21.7%) or the USA (25.5%).

The proportions of subjects with unsolicited AEs that were considered related to aH5N1c by the investigator in the full dose group were 17.8% in Australia, 18.1% in New Zealand, 8.0% in the USA and 6.2% in Thailand.

Paediatric population

The proportion of subjects <6 years of age reporting AEs after any vaccination was higher in subjects from the USA than in subjects from Thailand both for any solicited AEs (half dose: 80% vs. 69%, full dose: 86% vs. 64%), solicited local AEs (half dose: 63% vs. 55%, full dose: 69% vs. 51%) and solicited systemic AEs (half dose: 56% vs. 35%, full dose group: 57% vs. 37%).

Similarly, the proportion of subjects ≥6 years of age reporting AEs after any vaccination was higher in subjects from the USA than in subjects from Thailand both for any solicited AEs (half dose: 91% vs. 70%, full dose: 89% vs. 70%), solicited local AEs (half dose: 86% vs. 66%, full dose: 77% vs. 65%) and systemic AEs (half dose: 64% vs. 46%, full dose: 59% vs. 45%).

In the entire study cohort, the proportion of subjects with unsolicited AEs was higher in subjects from the USA than in subjects from Thailand in both dose groups after any vaccination (62% vs. 39% respectively in the full dose group and 66% vs. 40%, respectively in the half dose group)."

2.6.8.6. Immunological events

For a vaccine, antibody formation is the desired outcome and is evaluated in the efficacy part of this dossier. No related AEs were reported in the SOC Immune disorders.

2.6.8.7. Safety related to drug-drug interactions and other interactions

Interactions with seasonal influenza vaccine were only evaluated for immunogenicity outcomes.

2.6.8.8. Discontinuation due to adverse events

Ages ≥18 Years, Full Dose and Placebo

In total, 21 of the 4375 subjects in pooled data for the ≥18 years full dose population experienced AEs that led to premature study withdrawal: 18 subjects (0.5%) in the aH5N1c full dose group and 3 subjects (0.4%) in the placebo group. Most subjects discontinued after Day 43, during the follow-up period.

Two subjects (0.1%) in the aH5N1c group and 1 subject (0.1%) in the placebo group experienced AEs (assessed as possibly or probably related) that led to study withdrawal during the treatment period, all after the first vaccination (Day 1 through Day 22). In the aH5N1c group, these AEs were rash (1 subject) and constipation (1 subject).

Ages ≥18 Years, Full and Half Dose Combined and Placebo

Pooled data for the ≥18 years of age full and half dose combined population showed similar results as for the full dose population. In total, 19 (0.4%) of the subjects in the ≥18 years aH5N1c full and half dose combined group experienced AEs that led to premature study withdrawal, similar to the proportion of subjects (0.4%, n=3) in the placebo group. Most subjects discontinued after Day 43, during the follow-up period. Two subjects (<0.1%) in the aH5N1c group and 1 subject (0.1%) in the placebo group experienced AEs that led to study withdrawal during the treatment period, all after the first vaccination (Day 1 through Day 22). In the aH5N1c group, these AEs were rash (1 subject) and constipation (1 subject).

In addition to the 3 prematurely withdrawn subjects (2 subjects from the aH5N1c full dose group and 1 subject from the placebo group), no additional subjects who received aH5N1c half dose had unsolicited AEs possibly related to study treatment.

Paediatric Study V89_11

Only one subject (<1%) in the full dose group was prematurely withdrawn from the study on Day 22 due to unsolicited AEs, gastroenteritis rotavirus and rash, which were considered to be not related to study vaccine by the investigator.

Paediatric H1N1 Study V110_04

From Day 1 to Day 366, overall, there were 8 subjects (2 subjects in each age cohort) who had at least 1 AE that led to withdrawal. Three subjects reported AEs leading to withdrawal that were considered at least possibly related to study vaccine (all received half dose; 9-17 yoa: skin irritation; 12-35 months: crying and irritability; 6-11 months: crying and eating disorder).

Overall, a very small proportion of subjects experienced AEs that led to discontinuation of the study and only a minority of these AEs were considered as related to IP by the investigator. No safety concern arises from these events.

2.6.8.9. Post marketing experience

No post-marketing data are available for aH5N1c.

2.6.9. Discussion on clinical safety

Exposure

Safety data from 3972 subjects, of whom 329 are in the paediatric age cohort, are available for the full dose vaccine. Supportive data from the vaccine construct using H1N1, H3N2 and H7N9 strains are available for a further 611 subjects, of whom 105 are in the paediatric age range. A further 1570 subjects (329 paediatric) received a half-dose of the H5N1 vaccine, and 599 (397 paediatric) received a half-dose of the other vaccine constructs. Furthermore, data from 796 adult and elderly subjects who were randomised to placebo are available in order to enable a meaningful comparison of the safety profile.

The majority of subjects in the main safety data set for adult and elderly were White subjects from the USA (76.9% in the vaccine group and 83.3% in the placebo group) with Black or African American being the second biggest subgroup (11.8% in the vaccine group and 14.1% in the placebo group).

In contrast to the adult and elderly population, the majority of subjects in the main paediatric study V89_11 were Asians from Thailand (71%) with white subjects from the USA (21%) being the second biggest subgroup. Thus, the paediatric study population does not fully reflect the demographic characteristics of the intended treatment population for Incellipan in Europe.

A substantial number of elderly subjects > 65 (n=1896) and > 75 (n=533) years was exposed to the full dose of the aH5N1c vaccine. The in- and exclusion criteria selected for primarily healthy subjects, therefore, even though the medical history specified a variety of past and concomitant diseases, there are no specific safety data for chronically ill subjects or subjects who are immune compromised.

The available safety database in adults, i.e. safety data from 3643 subjects ≥ 18 years, fulfils the requirements of the Guideline on Influenza Vaccines EMA/CHMP/VWP/457259/2014. For paediatric subjects, however, the GL specifies that data from at least 900 subjects across three age strata (infants, children, adolescents) should be available, which is not met by the 329 subjects exposed to the full dose in study V89_11. The low number of paediatric subjects is noted, but in the event of a H5N1 pandemic declared by WHO, the observed benign safety profile as well as supportive data from children vaccinated with the H1N1 and H3N2 construct are considered able to overcome this deficiency.

Safety Evaluation

The safety evaluation in the clinical study programme was conducted in accordance with the Guideline on clinical evaluation of vaccines (EMEA/CHMP/VWP/164653/05 Rev. 1), defining solicited adverse events during the first week after each vaccination and capturing unsolicited AEs up to D 43, medically attended AEs, SAEs, NOCD and AESIs for approximately one year after the second vaccination.

In study V89_11, solicited AEs assessed for the youngest age group (6 months-6 years) did not include well-known and frequent AEs of influenza vaccines such as for instance Swelling, Diarrhoea, Vomiting, Shivering, and unusual crying. Consequently, these AEs are not listed as ADRs in the SmPC. In contrast, these AEs are listed as ADRs with the frequency common or very common in the SmPC for other approved unadjuvanted and MF59 adjuvanted influenza vaccines (source: SmPC for Foclivia, Aflunov, Flucelvax Tetra and Fluad Tetra). The applicant was asked to justify the exclusion of the AEs Swelling, Diarrhoea, Vomiting, Shivering and unusual crying from the list of solicited AEs assessed in study V89_11. No justification or rationale for the exclusion has been provided by the applicant, however no unsolicited AEs of swelling, shivering and persistent/unusual crying have been reported in study V89_11 and diarrhoea and vomiting have been included as ADRs in children and adolescents with the frequency common in the SmPC.

The age categories used in the analysis of adverse events in the paediatric population (6 months to <6 years and 6 to ≤ 17 years) were considered inadequate and too wide. A reanalysis for at least three age cohorts (e.g., 6-36 months, 3-9 years, 9-18 years) was requested for all unsolicited adverse events and was submitted by the applicant.

Adverse Events

Solicited Adverse Events

In total, 51.6% of adult and elderly subjects (≥18 years) in the full dose group and 14.7% of subjects in the placebo group reported a solicited local AE after any vaccination. As expected for an influenza vaccination, the most predominant local AE in the aH5N1c treatment group ≥18 years was pain (51.2%), with very low percentages of induration, erythema and ecchymosis reported. In the placebo group, the same pattern could be observed, but with a much lower incidence of pain (14.7%). Most local AEs were mild or moderate and resolved within a few days. After the first vaccination, the incidence of local AEs was higher than after the second. In the older adult age cohort ≥ 65, the incidence of local AEs, especially of pain (39.1%), was notably lower than in the overall ≥ 18 age group and especially the <65 group (65%). Most local AEs were of mild or moderate intensity and resolved within a few days. After the first vaccination, the incidence of local AEs was higher than after the second.

The most frequent solicited systemic AEs in the pooled adult population who received the full dose were fatigue (21.7%), headache (19.5%), malaise (19.4%), myalgia (13.6%) and arthralgia (10.6%). Incidences of systemic AEs were comparable in the cH5N1 and placebo group, apart from myalgia and malaise, which were less frequent in placebo recipients. The majority of AEs were mild or moderate and resolved within several days. Systemic reactivity was lower after the second vaccination compared to the first.

Overall, the reactogenicity in adults and elderly after vaccination with the full dose aH5N1c vaccine is comparable to other, already licenced, MF59 adjuvanted influenza vaccines e.g. Aflunov and no safety concerns are identified.

In general, the frequencies reported overall for solicited adverse events were higher in the paediatric population (70% in the 6 months to 6 years group and 75% in the 6 to ≤18 years group) than in the adult and elderly population (61.9%). This is mainly due to the lower frequency of solicited AEs in elderly subjects (53.1%) compared to adult subjects (72%).

As observed for adult subjects, in paediatric subjects the most predominant local AE was pain (72% half dose, 68% full dose) in the older (>6) and tenderness (56% in both full and half dose) in the younger age cohort (<6), with very low percentages of erythema, induration and ecchymosis. As in adults, most local AEs were mild or moderate and resolved within a few days. After the first vaccination, the incidence of local AEs was higher than after the second. Incidences in the full and half dose group were similar in both age cohorts for most AEs apart from fever in the younger age cohort.

The most predominant systemic AE in children was myalgia (28% half dose, 30% full dose) and fatigue (30% half dose, 27% full dose) in the older and irritability (28% half dose, 30% full dose) and sleepiness (25% in both full and half dose) in the younger age cohort. In the younger age cohort, fever was observed twice as frequent in the full dose group (8% vs.16% after any vaccination, half vs full dose), however, temperatures beyond 38.9° were rare. Fever was much less frequently reported in the older age cohort (3% vs 4% after any vaccination half vs. full dose).

Most systemic AEs were mild or moderate and resolved within a few days. After the first vaccination, the systemic reactogenicity was higher than after the second. Incidences in the full and half dose group were similar in both age cohorts for most AEs apart from fever.

Unsolicited Adverse Events

The proportion of subjects ≥18 years for whom unsolicited AEs were reported during the treatment period from Day 1 through Day 43 after any vaccination was similar for the aH5N1c full dose and the placebo groups (25.7% vs. 22.6%). There was no clear increase in the frequency, or difference in

nature of unsolicited AEs in the aH5N1c group compared with the placebo group. The proportion of unsolicited AEs considered to be at least possibly related to vaccination was similar in the aH5N1c full dose group and placebo group (8.2% vs. 6.2%). The most frequently affected SOC in both treatment groups was 'Infections and infestations' (6.9% in the aH5N1c group vs. 6.5% in the placebo group) followed by 'General disorders and administration site conditions' (5.9% and 5.0%, respectively). The incidence of all AEs as well as related AEs when analysing the safety population including full and half-dose of the cH5N1 vaccine is consistent with the safety profile observed in the full dose population. The most frequently reported unsolicited AEs (day 1 through 43) were headache (full dose:2.2% vs. placebo: 2.1%), injection site bruising (1.8% vs. 1.6%) and fatigue (1.6% vs. 1.5%) and the most common related AEs were injection site bruising (full dose: 1.7%, placebo: 1.5%) and fatigue (full dose: 1.0%, placebo: 0.9%). Severe unsolicited AEs were experienced by 1.0% of subjects in the full dose group and 1.3% of subjects in the placebo group. No specific safety signals arise from the nature and frequency of the reported unsolicited adverse events.

The incidence of all AEs (29%) in the paediatric aH5N1c trial was slightly higher than that reported for the adult trials, but the frequency of related AEs (in 4%) was lower than observed in the adult subjects. This reflects most likely the higher occurrence of infections and accidents in paediatric age cohorts. The most common unsolicited AEs for both the half and full dose after the first and after the second vaccination were reported in the SOC 'Infections and infestations' followed by 'Gastrointestinal disorders' and 'General disorders and administration site conditions'. The most frequently reported unsolicited AE by PT after the first vaccination was upper respiratory tract infection (5% in both dose groups) followed by pyrexia (2% vs. 4%) and vomiting (3% vs. 2%), and after the second vaccination upper respiratory tract infection (3% vs. 4%) followed by pyrexia (2% vs. 1%) and nasopharyngitis (1% vs. 2%). The most common related AEs were vomiting (2 and 4 cases in the half and full dose group, respectively), pyrexia (2 and 4 cases) and injection site pain (2 and 3 cases). Overall, unsolicited AEs including related AEs were comparable in the half and full dose group. All data are presented for the overall paediatric population and should be analysed and presented for at least three age cohorts (e.g., 6-36 months, 3-9 years, 9-18 years) analogous to data presented for the overall adult population. The applicant submitted the requested analyses. It is noticeable that the incidence of TEAEs decreases with increasing age. Only a minority of AEs were considered to be possibly or probably related, and no safety concern arises from the provided analyses.

SAEs

The frequency of subjects with SAEs from Day 1 through study termination was 6.3% in the aH5N1c full dose group and 9.3% in the placebo group in subjects ≥18. Most of the SAEs were reported after Day 43, during the follow-up period (aH5N1c: 5.8% of subjects; placebo: 8.4% of subjects.

Only in 3 subjects (aH5N1c: 1 subject [<0.1%]; placebo: 2 subjects [0.3%]) the SAEs, all reported during the follow-up period, were considered possibly or probably related to study vaccination according to the investigator. These SAEs included spontaneous abortion (aH5N1c group) and immune thrombocytopenic purpura and polymyalgia rheumatica (both in the placebo group). However, the main contributing factor to the spontaneous abortion reported in a subject of study V89_04 is considered to be the chromosomal aberration of the foetus and not an effect of aH5n1c vaccination.

In the paediatric Study V89_11, 24 SAEs were reported from Day 1 through study termination, of which, 14 SAEs were reported by 11 subjects in the half dose group, and 10 SAEs were reported by 8 subjects in the full dose group. None of these SAEs (mainly infections and bone fractures) were considered to be related to the study vaccine. In study V110_04, one subject experienced serious febrile convulsions and one subject serious convulsions and epilepsy, which all were considered to be related to the study vaccine. In study V89_11, 1 serious case of convulsion and 1 serious case of febrile convulsion were reported (day 1 through day 387), although none of these cases were

considered to be related to the study vaccination. In addition, one non-serious case of febrile convulsion occurred in the half dose group. Warnings regarding convulsions in general and febrile convulsions in particular in paediatric subjects with history of epilepsy were included in 4.4 of the SmPC, in line with those of other MF59 adjuvanted influenza vaccines e.g. Foclivia and Focetria.

Other Significant Adverse Events

AESIs

The incidence of AESIs was higher in the placebo group (0.9%) than in the full dose vaccine group (0.3%) in adult subjects. The onset of AESIs was in nearly all cases in the observation period after D 43, there were only two cases of AESIs reported with an onset less than 60 days: one case of Basedow's Disease (54 days) and one case of Colitis Ulcerative (42 days). All AESIs assessed by the investigator as possibly related occurred in the placebo group (i.e. immune thrombocytopenic purpura and of polymyalgia rheumatica). One case of facial paralysis occurred in, a 71 year old White female participant of phase 2 trial V89_13 in temporal relationship to the second vaccination. The investigator considered this AESI as related to a seasonal influenza vaccine (Fluvax), which was given concomitantly with the second vaccination of cH5N1. As an influence of cH5N1 on the development cannot be legitimately excluded, this AE should be reflected in section 4.8 of the SmPC. With their response to the outstanding questions, the applicant clarified that the date for the seasonal infuenza vaccination given in the narrative of the SAE was a transcription error. The real date was 10 days before onset of the facial paralysis and, given the temporal relationship, is likely to be the underlying cause. A correction to the CSR was submitted. The narratives provided for the other reported AESIs do not support a causal or temporal relationship with IP.

No AESIs were reported in paediatric subjects.

New onset of chronic disease (NOCD)

Overall, the proportion of subjects ≥18 years of age with unsolicited AEs leading to NOCD was similar for subjects who received aH5N1c full dose (9.7%) and subjects who received placebo (9.2%). The most frequently reported PTs for NOCDs were hypertension (0.9%), osteoarthritis (0.5%), and atrial fibrillation (0.4%) in the aH5N1c full dose group, and coronary artery disease (0.6%), hypertension and atrial fibrillation (both 0.5%) in the placebo group. In total 3 subjects (1%) in the paediatric population, all in the half dose group, had NOCDs: bone cyst, attention deficit/hyperactivity disorder, and dyspepsia. None were considered related to the study vaccine by the investigator.

AEs leading to premature withdrawal

In the adult and elderly population (≥18 years), 19 subjects in the half and full dose group and 3 subjects (0.4%) in the placebo group prematurely withdrew from the study due to experiencing AEs. These AEs were fatal in 17 subjects in the aH5N1c group and in 1 subject in the placebo group. In the paediatric population only 1 subject prematurely withdrew from the study due to experiencing AEs.

Discontinuation due to AEs

In total, 3 adult and elderly subjects in the half and full dose group and 2 subjects in the placebo group discontinued from the study due to experiencing an AE. In addition, discontinuations in 17 subjects in the aH5N1c group (16 cases in the full dose and 1 case in the half dose group) and 1 subject in the placebo group were due to fatal AEs. One subject in the paediatric study population withdrew due to experiencing an AE.

Medically attended adverse events

Overall, 47.1% subjects ≥18 years of age in the full dose group and 46.0% in the placebo group reported a medically attended AEs (day 1 through study termination). The most frequent medically attended AEs were upper respiratory tract infection (full dose: 3.7% vs. placebo: 5.0%), urinary tract infection (3.2% vs. 4.0%). All other events occurred in less than 3% (full dose) and 4% (placebo) of the subjects. In the paediatric population, unsolicited medically attended AEs were reported in 34% of the subjects in the full dose. The most frequently reported medically attended AEs by PT was upper respiratory tract infection (13%), nasopharyngitis (3%) diarrhoea (2%) and Otitis media (2%).

Deaths

Collectively 21 deaths were reported from Day 1 through study termination in Studies V89P1, V89_04, V89_13, and V89_18. No deaths were reported in the paediatric study (V89_11). Sixteen deaths were reported in the full dose group, 1 in the half dose group, 3 in non-adjuvanted vaccine groups, and 1 in the placebo group. All deaths occurred during the follow-up period (i.e., >21 days after last vaccination received) with a mean of 234.4 days (range 38-512 days) for the H5N1c group and 227 days for the placebo group. None of the deaths were assessed as related to the study vaccine by either the investigator or by the applicant. The submitted narratives of the deaths do not indicate a relationship of each event with IP.

No deaths were reported in the paediatric study population (study V89_11, V110_04 and V129_01).

Special Populations

Adult and elderly population

Overall, there were more female (56.5%) than male subjects (43.5%) included in the adult/elderly study population. This was consistent throughout the different vaccination and age groups. Both solicited local and systemic AEs were reported more frequently by female than male subjects but was less pronounced for SAEs, AESIs and NOCDs. No notable differences in solicited local and systemic AEs and AEs leading to deaths are apparent between different race groups in the full dose group. Both any and related unsolicited AEs and SAEs occurred more frequently in White than Black or African American or Asian subjects. Similar results were observed in the half and full dose combined group. Overall, the frequency of solicited, unsolicited, SAEs and AEs leading to death and other significant AEs were comparable in subjects of Hispanic or Latino origin and non-Hispanic or Latino subjects. In general, adult and elderly subjects from Australia or New Zealand reported higher frequencies of any or related unsolicited AEs compared to subjects from Thailand or USA. Of note, NOCDs occurred more frequently in New Zealand (22%) than in the other countries (5.1% to 9.7%), which is likely explained by the higher age of the study population in New Zealand (all subjects were ≥65 years).

Paediatric population

The majority of subjects in the paediatric population was Asian (73%) followed by White (21%) and Black or African American subjects (5%). There were substantial differences in solicited as well as unsolicited AEs between Asian and White subjects in the paediatric population. Both solicited local and systemic AEs and unsolicited AEs were reported more frequently in White subjects than in Asian subjects consistently throughout different age and vaccination groups. Corresponding differences were seen for subjects from Thailand and USA, since 76.7% of subjects from USA were of White origin and all subjects from Thailand were of Asian origin. There is an imbalance in vaccination history between subjects enrolled in study centres in the USA and Thailand; the majority of subjects (61.7%) from the USA had received influenza vaccines previous to study V89-11 compared to only 2.9% of from Thailand. Although the limited size of the subgroups with different vaccination history makes a detailed analysis difficult, no clear trend was identified regarding the safety profile in previously vaccinated vs.

unvaccinated subjects. The applicant's explanation that the observed differences in the incidence of adverse events between USA and Thailand is likely due to differences in reporting between these countries, for instance, attributable to differences in the health care system, and not an impact of previous vaccination is accepted.

Both suspected impairment/alteration of immune function as well as pregnancy or breastfeeding was an exclusion criterion in all clinical trials with adjuvanted and non-adjuvanted H5N1c formulations. The available sparse data in pregnancies do not indicate a safety signal for aH5N1c.

Post-marketing experience

No post-marketing data are available for Incellipan. However, in the SmPC for Aflunov, a zoonotic MF59C.1 adjuvanted inactivated H5N1 vaccine, adverse events reported in the whole population from post-marketing surveillance with Focetria as well as a seasonal trivalent MF59C.1-adjuvanted H1N1 subunit influenza vaccine are listed in section 4.8 under the subsection "description of selected adverse reactions". The applicant has therefore included the subheading "description of selected adverse reactions" in section 4.8 of the SmPC presenting up-to-date data on adverse events from post marketing experience with other similar vaccines in line with the guideline on summary of product characteristics (2009, rev.2).

2.6.10. Conclusions on the clinical safety

The reported safety profile indicates that the aH5N1c vaccine is well tolerated after both vaccinations and did not reveal unexpected safety signals or lead to significant safety concerns. Data from studies undertaken with other vaccine constructs, i.e. H1N1, H3N2 and H7N9, further support this conclusion.

Incellipan is considered approvable from a clinical safety point of view.

2.7. Risk Management Plan

2.7.1. Safety concerns

Below is summary of safety concerns in the RMP version 0.4:

Summary of safety concerns	
Important identified risks	None
Important potential risks	Neuritis Convulsions Encephalomyelitis Vasculitis Guillain-Barre syndrome Demyelination Bell´s palsy Immune thrombocytopenia
Missing information	Use in pregnancy

2.7.2. Pharmacovigilance plan

Ongoing and planned additional pharmacovigilance activities

Study (Status)	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
Not applicable				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are specific obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
Not applicable				
Category 3 - Required additional pharmacovigilance activities				
V89_20OB is a postmarketing observational cohort safety study of pandemic influenza A/H5N1c* vaccine in pregnant women (Planned)	To evaluate the safety of pandemic influenza vaccine in pregnant women	Use in pregnancy	Protocol to be provided once pandemic is declared. Milestones to be confirmed	To be confirmed

**The strain is subject to change to be matched with the next pandemic strain*

Planned post-authorisation efficacy studies

Study (Status)	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Efficacy studies which are conditions of the marketing authorisation				
Not applicable				
Efficacy studies which are specific obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
A non-interventional observational effectiveness study in children and adults* against laboratory confirmed influenza (Planned)	To perform an analysis of pandemic vaccine effectiveness against laboratory confirmed influenza for aH5N1c** versus no vaccination	Not applicable	Protocol to be provided when pandemic is declared. Milestones to be confirmed	To be confirmed

**The age population may be subject to change based on Health Authority recommendations once pandemic is declared*

*** The strain is subject to change to be matched with the next pandemic strain*

2.7.3. Risk minimisation measures

Safety concern	Risk minimisation measure	Pharmacovigilance Activity
Important Identified Risk		
None		
Important Potential Risk		
Neuritis	Routine risk minimisation measures: Neuritis is described in: *Incellipan: SmPC Section 4.8*	Routine pharmacovigilance activities beyond adverse

	Incellipan: PL Section 4 Additional risk minimisation measures: *No additional measures*	reaction reporting and signal detection: *S-PSUR (in situation of pandemic)* *EPSS (in situation of pandemic)* Additional pharmacovigilance activities: *No additional Pharmacovigilance (PV) activities*
Convulsions	Routine risk minimisation measures: Convulsions are described in: *Incellipan: SmPC Sections 4.4 and 4.8* *Incellipan: PL Sections 2 and 4* Additional risk minimisation measures: *No additional measures*	Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection: *S-PSUR (in situation of pandemic)* *EPSS (in situation of pandemic)* Additional pharmacovigilance activities: *No additional PV activities*
Encephalomyelitis	Routine risk minimisation measures: Encephalomyelitis is described in: *Incellipan: SmPC Section 4.8* *Incellipan: PL Section 4* Additional risk minimisation measures: *No additional measures*	Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection: *S-PSUR (in situation of pandemic)* *EPSS (in situation of pandemic)* Additional pharmacovigilance activities: *No additional PV activities*
Vasculitis	Routine risk minimisation measures: Vasculitis is described in: *Incellipan: SmPC Section 4.8* *Incellipan: PL Section 4* Additional risk minimisation measures: *No additional measures*	Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection: *S-PSUR (in situation of pandemic)* *EPSS (in situation of pandemic)* Additional pharmacovigilance activities: *No additional PV activities*
Guillain-Barré syndrome	Routine risk minimisation measures: Guillain-Barre syndrome is described in: *Incellipan: SmPC Section 4.8* *Incellipan: PL Section 4* Additional risk minimisation measures: *No additional measures*	Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection: *S-PSUR (in situation of pandemic)* *EPSS (in situation of pandemic)* Additional pharmacovigilance activities: *No additional PV activities*
Demyelination	Routine risk minimisation measures: *None; included as a potential safety concern based on pharmacological class effects* Additional risk minimisation measures: *No additional measures*	Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection: *S-PSUR (in situation of pandemic)* *EPSS (in situation of pandemic)*

		Additional pharmacovigilance activities: *No additional PV activities*
Bell's palsy	Routine risk minimisation measures: *None; included as a potential safety concern based on pharmacological class effects* Additional risk minimisation measures: *No additional measures*	Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection: *S-PSUR (in situation of pandemic)* *EPSS (in situation of pandemic)* Additional pharmacovigilance activities: *No additional PV activities*
Immune thrombocytopenia	Routine risk minimisation measures: *None; included as a potential safety concern based on pharmacological class effects* Additional risk minimisation measures: *No additional measures*	Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection: *S-PSUR (in situation of pandemic)* *EPSS (in situation of pandemic)* Additional pharmacovigilance activities: *No additional PV activities*
Missing information		
Use in pregnancy	Routine risk minimisation measures: Pregnancy is described in: *Incellipan: SmPC Section 4.6* *Incellipan: PL Section 2* Additional risk minimisation measures: *No additional measures*	Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection: *S-PSUR (in situation of pandemic)* Additional pharmacovigilance activities: *V89_20OB (in situation of pandemic)*

2.7.4. Conclusion

The CHMP considers that the risk management plan version 0.4 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request the alignment of the new PSUR cycle with the international birth date (IBD). The IBD is 30.01.2020. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Incellipan (Pandemic influenza vaccine (H5N1) (surface antigen, inactivated, adjuvanted, prepared in cell cultures)) is included in the additional monitoring list as it is a biological product authorised after 1 January 2011 and it is to be approved under a conditional marketing authorisation.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Pandemic influenza outbreaks occur when a new highly infectious virus strain enters a population with low immunity from previous exposure. A pandemic outbreak is expected to spread quickly and cause substantial global morbidity and mortality. Infection with influenza virus usually occurs by droplet spread from infected people to uninfected people through inhalation. The virus can also be spread by hands contaminated with influenza viruses.

Morbidity and mortality from A/H5N1 influenza virus infections are also age-specific, with the highest mortality rates reported for young people between 10 and 40 years of age. In a global patient registry of 193 children across 13 countries, the case fatality rate (CFR) associated with H5N1 infections declined with decreasing age among children who were younger than 5 years of age. The slightly improved CFR in the youngest affected may be related to earlier identification, and related treatment.

Clinical signs and symptoms of A/H5N1 pandemic influenza may be characterised by the abrupt onset of fever (>38ºC), headache, myalgia, severe malaise, prostration, non-productive cough, sore throat and rhinitis. More severe illness can result when influenza virus moves from the tracheal epithelium to invade the lungs (primary viral pneumonia), or when secondary bacterial pneumonia occurs.

In some reports of infection with an A/H5N1 virus, diarrhoea and shortness of breath were prominent features. In severe cases the clinical picture can present with dyspnoea leading to acute respiratory distress syndrome (ARDS) which may lead to respiratory failure and death. Typical findings of chest

radiographs in individuals infected with an H5N1 virus are interstitial infiltration, patchy lobar infiltrates in a variety of patterns (single lobe, multiple lobes, unilateral or bilateral distributions) progressing in some cases to a diffuse bilateral ground-glass appearance characteristic of ARDS. The median time from onset of fever to ARDS was reported as 6 days (range 4-13 days).

Transmission of H5N1 to humans leading to onward transmission between humans is uncommon. Recently, substantial outbreaks in both birds and mammals with influenza H5N1 clade 2.3.4.4b have heightened awareness of this strain. From 2020 to December 2022, six human cases of influenza A(H5N1) belonging to the 2.3.4.4b clade were reported to WHO of which two were severe.

3.1.2. Available therapies and unmet medical need

Vaccination is the primary method for prevention of influenza and its severe complications. Immunisation with surface antigens, especially HA, reduces the likelihood of infection and severity of disease, and currently represents the most important measure for reducing the impact of influenza. Vaccination with seasonal influenza vaccine is associated with a reduction in influenza-related respiratory illness and physician visits at all ages, in hospitalisations and deaths among high-risk persons, otitis media among children, and work absenteeism among adults.

Currently two zoonotic vaccine, Aflunov and Zoonotic Influenza Vaccine Seqirus and three pandemic preparedness vaccines (Foclivia, Adjupanrix and Pandemic Influenza Vaccine H5N1 AstraZeneca) are approved within the EU.

A number of antiviral therapies are also currently approved for the treatment of influenza virus infection in the EU. These target viral proteins such as neuraminidase (Oseltamivir contained in Ebilfumin and Tamiflu) and zanamivir contained in Dectova. Relenza (zanamivir) is administered via inhalation in contrast to Dectova which is by intravenous infusion. Xofluza was recently approved in the EU and acts on the influenza virus cap-dependent endonuclease (Baloxivir marboxil). These antiviral therapies are also approved for use in children. Except for Dectova, these antiviral therapies can also be used in the context of post-exposure prophylaxis.

3.1.3. Main clinical studies

The main evidence for immunogenicity in adult individuals is a single phase III randomised, stratified, observer-blind, multi-centre, placebo-controlled study to evaluate safety, immunogenicity and lot-to-lot consistency of aH5N1c in healthy adult subjects ≥18 years of age (V89_18). Participants were randomly assigned (1:1:1:1) to receive a series of 2 IM injections administered 3 weeks apart, of 1 of 3 lots aH5N1c containing 7.5 µg of the H5N1 (A/turkey/Turkey/1/2005 NIBRG-23 strain) influenza antigen with 0.25 mL MF59 (full dose) or placebo. The randomisation was stratified by study centre and age (18 to <65 years of age and ≥65 years of age) with a goal to enrol 50% into each age cohort. In total, 3191 individuals were exposed, 2394 participants received the vaccine, and 797 participants received placebo.

In children, a submitted phase II study is considered as main evidence. Study V89_11 was a randomised, controlled, observer-blind multicentre study to evaluate immunogenicity, tolerability and safety of 2 intramuscular (IM) doses of either low dose or high dose aH5N1c (A/turkey/Turkey/1/2005 NIBRG-23 strain) in healthy participants 6 months through 17 years of age. Participants were randomised at a 1:1 ratio to receive 2 vaccinations 3 weeks apart of either study vaccine (high or low dose aH5N1c). Randomisation was stratified by site and age cohort (6 through 35 months, 3 through 8 years and 9 through 17 years). In total, 658 were exposed to the vaccine and 329 children received the relevant dose (=high dose or full dose).

3.2. Favourable effects

In clinical trials, at Day 43, 3 weeks after the second vaccination, the pre-defined criterion of seroconversion based on HI titres against the H5N1 strain contained in the vaccine was achieved by 66.9% [95% CI: 64.9 - 68.9] of the study population (18-64 years of age: 79.9% [95% CI: 77.4 - 82.3]; ≥65 years of age: 54% [95% CI: 51.0 - 57.0]).

Compared to baseline, the geometric mean HI titres at Day 43 increased ~8-fold (GMR: 7.96 [95% CI: 7.61 - 8.33]) in the vaccine group, while no increase was observed in the placebo group (GMR: 0.83, [95% CI: 0.77 - 0.9]). In adults from 18 to 65 years of age (GMR 12.7, 95% CI: 11.9, 13.5), compared to participants ≥65 years of age (GMR 4.9, 95% CI: 4.6, 5.2).

In the overall paediatric population, the HI titre response at Day 43 was substantially higher (seroconversion: 96% [97.5% CI 40 - 54%], GMR 262 [97.5% CI 190 - 361]) with a consistent trend for higher responses with decreasing age. At Day 22, the geometric mean HI titres in children already increased 13-fold (97.5% CI 9 – 18), compared to baseline.

HI titres against 5 different heterologous H5N1 strains at Day 43 reveal GMRs between 2.09 and 7.3 for adults 18 to <65 years of age, between 1.53 and 4.77 for adults ≥65 years of age, and between 8.39 and 40 for children, depending on the strain.

Microneutralisation (MN) data at Day 43 in small subpopulations of the studies confirm that two doses of aH5N1c elicit an immune response (GMR in adults 18 to 64 years of age: 23.8 [95% CI: 18.7 – 30.3], GMR in children: 257 (97.5% CI: 209 – 315]) against the homologous H5N1 strain.

MN data against 5 different heterologous H5N1 strains at Day 43 show GMRs between 4.76 and 34 for adults 18 to <65 years of age, between 3.68 and 12 for adults ≥65 years of age, and between 13 and 160 for children, depending on the strain.

3.3. Uncertainties and limitations about favourable effects

No efficacy or effectiveness data are available.

There is no immune correlate of protection for pandemic influenza strains. Therefore, there is uncertainty regarding the relationship between the immunogenicity and the potential for vaccine efficacy.

Haemagglutination inhibition (HI) and microneutralisation (MN) assays for the phase 3 trial were performed in a different laboratory compared to the phase 2 trials and variability of the results is noted. For example, the HI GMRs of the phase 3 trial are roughly 3-fold lower compared to phase 2 trials. While the HI seroconversion rates are comparable for adults 18 to <65 years of age, there is a significant difference for adults ≥65 years of age between the phase 3 (54% [CI: 51 – 57]) and the phase 2 trials (74% [CI: 70 – 77]). The MN titre responses were also weaker in the phase 3 trial vs the earlier trials (D43 GMTs in subjects from 18 to <65 years of age; phase 2: 410 vs. phase 3: 130.91; D43/D1 GMRs; phase 2: 61 vs. phase 3: 23.8).

HI and MN assay data against heterologous H5N1 strains are only available for the phase 2 trials and the statement above regarding lower titres in phase 3 should be considered. There are no supportive nonclinical data in this regard.

Substantial waning of antibodies was noted for the adult population and the GMTs returned to (or very close to) baseline at the 6-month time point in the phase 3 trial. The duration of a potential protective effect cannot be predicted.

No booster data are available (neither homologous, nor heterologous).

No data regarding cell-mediated immunity or anti-neuraminidase antibodies are available.

There is lack of data in immunocompromised individuals (exclusion criterion).

A detailed analysis of the immunogenicity data of the phase 3 trial in elderly by age subgroups revealed that the immune response (as measured by HI titres) three weeks after the second vaccination was weak in individuals ≥85 years of age (N=37, Day 43/1 GMR: 2.22 [95% CI: 1.43 - 3.44], seroconversion rate at Day 43: 18.9% [95% CI: 8.0 – 35.2%]).

3.4. Unfavourable effects

The tolerability of the aH5N1c vaccine was good in adult and paediatric subjects, with pain at the injections site (51.2%) in adults and pain at the injection site (72% half dose, 68% full dose) in children ≥ 6 and tenderness (56% in both full and half dose) in children < 6 the most frequently reported local solicited adverse event. The most frequent systemic solicited AEs were fatigue (21.7%), and headache (19.5%) in adults. Myalgia (28% half dose, 30% full dose) and fatigue (30% half dose, 27% full dose) in the older and irritability (28% half dose, 30% full dose) and sleepiness (25% in both full and half dose) in the younger paediatric age group were the systemic AE most often experienced in children.

Most solicited AEs were of mild or moderate severity and resolved within a few days.

The proportion of subjects ≥18 years for whom unsolicited AEs were reported during the treatment period from Day 1 through Day 43 after any vaccination was similar for the aH5N1c full dose and the placebo groups (25.7% vs. 22.6%). There was no clear increase in the frequency, or difference in nature of unsolicited AEs in the aH5N1c group compared with the placebo group. The proportion of unsolicited AEs considered to be at least possibly related to vaccination was similar in the aH5N1c full dose group and placebo group (8.2% vs. 6.2%). The most frequently affected SOC in both treatment groups was 'Infections and infestations' (6.9% in the aH5N1c group vs. 6.5% in the placebo group) followed by 'General disorders and administration site conditions' (5.9% and 5.0%, respectively). The incidence of all AEs as well as related AEs when analysing the safety population including full and half-dose of the cH5N1 vaccine is consistent with the safety profile observed in the full dose population.

The incidence of all AEs in the paediatric aH5N1c trial was slightly higher than that reported for the adult trials, but the frequency of related AEs (4%) was lower than observed in the adult subjects. This reflects most likely the higher occurrence of infections and accidents in paediatric age cohorts. However, all data are presented for the whole paediatric population and should be analysed and presented for the three age cohorts: 6 through 35 months, 3 through 8 years and 9 through 17 years analogous to data presented for the overall population. The requested safety analyses were submitted withing the procedure. It is noticeable that the incidence of TEAEs decreases with increasing age. Only a minority of AEs were considered to be possibly or probably related, and no safety concern arises from the provided analyses.

No significant safety concern has been identified based on the safety data presented in the dossier for Incellipan.

3.5. Uncertainties and limitations about unfavourable effects

Some unsolicited AEs, for instance hypothyroidism, showed imbalances in overall or related cases between the aH5N1c vaccine and placebo group. According to the applicant, case narratives for non-serious events are not available, however, short narratives for cases of hypothyroidism including two related in the full dose group have been provided. The information provided is insufficient to conduct a

causality assessment. The applicant has committed to follow up hypothyroidism as safety topic in PSURs for Incellipan.

Based on the more frequent reporting of osteoarthritis (de novo and particularly flare up) and one related case in the full dose group and half dose group further follow-up post-licensure is warranted to gain more insight in the potential relation between Incellipan and osteoarthritis (de novo and flare up). The applicant has committed to follow-up of osteoarthritis (de novo and flare up) in PSURs. There are limited data on use in pregnant women, and no data on use in breast-feeding women or in immunocompromised subjects. There were few participants with autoimmune disease included in the study.

3.6. Effects Table

Table 74. Effects Table for Incellipan

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Phase III Study (adults)			**aH5N1c**	**Placebo**		
SCR	Percentage of subjects with HI seroconversion at Day 43 (95% CI)	%	66.9 (64.9 – 68.9)	1.0 (0.4 – 2.0)	SoE: HI titre increase confirmed by MN assay Uncertainty: No correlate of protection, the clinical relevance of increase in both HI and MN antibodies is unknown.	V89_18
HI GMR	HI GMR Day 43/Day 1 (95% CI)	Ratio	7.96 (7.61 – 8.33)	0.83 (0.77 – 0.90)		V89_18
MN GMR	MN GMR Day 43/Day 1 (95% CI)	Ratio	23.8 (18.7 – 30.3)	0.97 (0.6 – 1.5)		V89_18 Addendum (*post hoc*)
Phase II Study (children)			**aH5N1c (full dose)**	**aH5N1c (half dose)**		
SCR	Percentage of subjects with HI seroconversion at Day 43 (97.5% CI)	%	96% (93% – 98%)	86% (81% – 90%)	SoE: HI titre increase confirmed by MN assay Uncertainty: No correlate of protection, the clinical relevance of increase in both HI and MN antibodies is unknown.	V89_11
HI GMR	HI GMR Day 43/Day 1 (97.5% CI)	Ratio	262 (190 – 361)	84 (61 – 116)		V89_11
MN GMR	MN GMR Day 43/Day 1 (97.5% CI)	Ratio	257 (209 – 315)	Not determined		V89_11
Unfavourable Effects						
Adults ≥ 18 (Pooled across all H5N1 studies)						
			Full dose	Placebo		
Local Solicited AE	Injection site pain	%	51.2	14.7	SoE: injection site pain is the most frequently reported local solicited AE	Clin Summary of Safety

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
					across studies	
Systemic solicited AE	Fatigue	%	21.7	20.4	SoE: most frequently reported systemic solicited AEs across studies	
	Headache	%	19.5	19.3		
	Myalgia	%	13.6	9.8		
Unsolicited AEs	Any AE	%	12.6	10.8		
	Related AE	%	3.6	3.1		
Children > 6 and <18 (Study VP89_11)						
			Full dose	Half dose		
Local Solicited AE	Injection site pain	%	68.0	72.0	*SoE*: injection site pain is most frequently reported local solicited AE in paediatric studies.	CSR V89_11
Systemic solicited AE	Myalgia		30.0	28.0	*SoE*: Myalgia, fatigue is most frequently reported systemic solicited AE in paediatric studies.	
	Fatigue		27.0	30.0		
	Fever		4.0	3.0		
Children 6 months to < 6 years (Study V89_11)						
			Full dose	Half dose		
Local Solicited AE	Injection site tenderness	%	56.0	56.0	*SoE*: injection site pain is most frequently reported local solicited AE in paediatric studies	
Systemic solicited AE	Irritability	%	30.0	28.0	*SoE*: Irritability and sleepiness most commonly reported systemic solicited AE	
	Sleepiness	%	25.0	25.0		
	Fever	%	16.0	8.0		
	Fever ≥ 39°	%	2.0	2.0		
Children 6 months to < 18 years (Study V89_11)					Uncertainty: Small size of safety database	
Unsolicited AEs	Any AE		26.0	29.0		
	Related AE		4.0	4.0		

Abbreviations: SoE = Strength of evidence, HI = haemagglutination inhibition, MN = microneutralisation, SCR = seroconversion rate (defined as subjects with a prevaccination HI titre <1:10 and postvaccination HI titre ≥1:40, or, subjects with a prevaccination HI titre ≥1:10 and a ≥4-fold increase in postvaccination HI antibody titre), GMR = geometric mean ratio, CI = confidence interval

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

In all investigated age groups, two doses of aH5N1c administered three weeks apart elicit robust HI titres against the H5N1 strain contained in the vaccine (A/turkey/Turkey/1/2005 [H5N1] NIBRG-23). These findings are confirmed by microneutralisation data collected in relatively small subpopulations of one phase 3 and three phase 2 trials.

It is acknowledged that once a pandemic has been declared, Incellipan will undergo a strain change to include the strain causing the pandemic. This will also rely on the generation of new clinical data for

the pandemic strain although the details of these studies in adults and the elderly remain to be described.

Additional confirmatory evidence from three supportive studies investigating the immune response of vaccines using the same vaccine construct but containing different potential pandemic influenza subtypes (H1N1, H3N2v, H7N9) as antigens suggest that two doses of the vaccine are also immunogenic after a strain change.

The totality of immunogenicity data suggest that aH5N1c is robustly immunogenic. Considering that higher HI titres tend to correlate with better protection, it is reasonably likely that the vaccine will be able to offer protection against (severe) disease and death caused by pandemic influenza. The extent of protection cannot be estimated in the absence of an efficacy study and without knowledge of the potentially circulating influenza strain.

There are no data in immunocompromised individuals and the above statement that the protective potential of aH5N1c cannot be estimated in advance is even more relevant for this vulnerable population. It should also be noted that the immune response against the H5N1 strain contained in the vaccine was weak in individuals ≥85 years of age.

In adults, substantial waning of antibodies over time was noted and hardly any HI titres were detected already 6 months after vaccination, suggesting that there may be a need for a booster vaccination in this population to maintain protection. In the paediatric population, the HI titres did also wane over time, but antibody titres were still considerably high one year after vaccination.

The reported safety profile indicates that the aH5N1c vaccine is well tolerated after both vaccinations and did not reveal unexpected safety signals or lead to significant safety concerns. Data from studies undertaken with other vaccine constructs, i.e. H1N1, H3N2 and H7N9, further support this conclusion.

3.7.2. Balance of benefits and risks

While there is no immunological correlate of protection, two injections of aH5N1c elicited an immune response in all investigated age cohorts, which could be replicated in several phase 2 and one phase 3 trial and is supported by similar immunological outcomes from other vaccine constructs, i.e. H1N1, H3N2 and H7N9. Considering that higher HI titres tend to correlate with better protection, the observed immunogenicity is reasonably likely assumed to translate into protective efficacy and to prevent (severe) morbidity and mortality in all investigated age groups. The effectiveness of the vaccine will be investigated in case of an officially declared pandemic.

The safety profile of Incellipan in the adult and elderly population is comparable with the safety profile of other MF59 adjuvanted influenza vaccines.

3.7.3. Additional considerations on the benefit-risk balance

Conditional marketing authorisation

As comprehensive data on the product are not available, a conditional marketing authorisation was requested by the applicant in the initial submission.

The product falls within the scope of Article 14-a of Regulation (EC) No 726/2004 concerning conditional marketing authorisations, as it aims at the prevention of a seriously debilitating, life-threatening disease. In addition, the product is to be used in emergency situations in response to public health threats duly recognised by the World Health Organisation.

Clinical signs and symptoms of A/H5N1 pandemic influenza may be characterised by the abrupt onset of fever (>38ºC), headache, myalgia, severe malaise, prostration, non-productive cough, sore throat and rhinitis. More severe illness can result when influenza virus moves from the tracheal epithelium to invade the lungs (primary viral pneumonia), or when secondary bacterial pneumonia occurs.

In some reports of infection with an A/H5N1 virus, diarrhoea and shortness of breath were prominent features. In severe cases the clinical picture can present with dyspnoea leading to acute respiratory distress syndrome (ARDS) which may lead to respiratory failure and death.

Furthermore, the CHMP considers that the product fulfils the requirements for a conditional marketing authorisation:

- The benefit-risk balance is positive, as discussed.
- It is likely that the applicant will be able to provide comprehensive data. Once a pandemic has been declared and following a type II variation to include the pandemic strain A post-marketing observational cohort safety study of pandemic influenza A/H5N1c vaccine in pregnant women and a non-interventional study of vaccine effectiveness for aH5N1c are foreseen in case of a pandemic.
- Unmet medical needs will be addressed, at the time of strain variation once the pandemic situation has been declared by the WHO. The pandemic influenza strain will represent an influenza variant or re-assorted virus that is new to mankind and where no or very limited immunogenicity exists in the human population.

  Three other pandemic preparedness vaccines are currently authorised in the EU for use against a future pandemic influenza strain. It can therefore be expected that variations will also be submitted for these vaccines to address the pandemic, thereby providing a number of vaccine options within the EU once the pandemic has been declared. The timing of their availability may differ according to the technology used and method for manufacture. It is considered needed that although there are a number of other approved influenza vaccine candidates, that the availability of additional vaccines that can contribute to controlling the pandemic will be beneficial.

  In addition, antiviral therapies for the treatment of influenza infection are available in the EU that target viral proteins such as neuraminidase (Oseltamivir contained in Ebilfumin and Tamiflu), zanamivir contained in Dectova and the cap-dependent endonuclease (Baloxivir marboxil) contained in Xofluza. Relenza (zanamivir) was approved nationally in all member states and is administered via inhalation in contrast to Dectova which is administered by intravenous infusion. These antiviral therapies are also approved for use in children. With the exception of Dectova, these antiviral therapies can also be used in the context of post-exposure prophylaxis.
- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required.

  Incellipan represents a pandemic preparedness vaccine, intended for use once a pandemic situation has been declared by the WHO or within the European Union, and the identity of the pandemic strain is known.

3.8. Conclusions

The overall benefit/risk balance of Incellipan is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Incellipan is favourable in the following indication(s):

Incellipan is indicated for active immunisation against influenza in an officially declared pandemic.

Incellipan should be used in accordance with official recommendations.

The CHMP therefore recommends the granting of the conditional marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Official batch release

In accordance with Article 114 Directive 2001/83/EC, the official batch release will be undertaken by a state laboratory or a laboratory designated for that purpose.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of PSURs for this medicinal product are set out in Article 9 of Regulation (EC) No 507/2006 and, accordingly, the marketing authorisation holder (MAH) shall submit PSURs every 6 months.

The requirements for submission of PSURs for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14-a of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
In order to confirm the efficacy of Incellipan, the MAH should conduct a non-interventional observational effectiveness study in children and adults against laboratory confirmed influenza during the next declared pandemic. The MAH should submit the final results of this study.	After declaration of a pandemic in the EU and after implementation of the pandemic vaccine

Paediatric data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0141/2022 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.