01 April 2016
EMA/CHMP/323530/2016
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Pandemic influenza vaccine H5N1 MedImmune

Common name: pandemic influenza vaccine (H5N1) (live attenuated, nasal)

Procedure No. EMEA/H/C/003963/0000

Note

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

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List of abbreviations

Abbreviation or Specialized Term	Definition
AE	adverse event
AFI	any febrile illness
AGM	African Green Monkeys
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AOM	acute otitis media
ASC	antibody secreting cell
AST	aspartate aminotransferase
att	attenuated
ca	cold-adapted
CAIV-T	influenza virus vaccine, trivalent, types A and B, live cold-adapted
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CMI	cell-mediated immunity
CSR	clinical study report
CRADA	cooperative research and development agreement
CTL	cytotoxic T-lymphocyte
DOD	Department of Defense
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot assay
EU	European Union
FDA	Food and Drug Administration
FFA	fluorescent focus assay
FFU	fluorescent focus units
FURI	febrile upper respiratory illness
GMFR	geometric mean fold-rise
GMR	geometric mean ratio
GMT	geometric mean titre
HA	hemagglutinin
HAI	hemagglutination inhibition
ICH	International Conference on Harmonization
IFN-γ	interferon gamma
IgA	immunoglobulin A
IgG	immunoglobulin G
IIV	inactivated influenza vaccine
ILI	influenza-like illness
ISIV	Inactivated subvirion influenza vaccine
LAIV	live attenuated influenza vaccine
LRI	lower respiratory illness
MAA	Marketing Authorisation Application
MAARI	medically attended acute respiratory illness
MDCK	Madin-Darby canine kidney

Abbreviation or Specialized Term	Definition
MDV	master donor virus
MMR	measles, mumps, rubella trivalent vaccine
MN	microneutralization
NA	neuraminidase
NAI	neuraminidase inhibiting
NIH	National Institutes of Health
NOCD	new onset of chronic diseases
OD	optical density
OPV	oral polio virus vaccine
OTC	over-the-counter
PBMC	peripheral blood mononuclear cell
PDCO	Paediatric Committee
P/IIV	pandemic inactivated influenza vaccine
PIP	Paediatric investigation plan
P/LAIV	pandemic live attenuated influenza vaccine
Q/LAIV	quadrivalent live attenuated influenza vaccine
RE/AE	Reactogenicity event/adverse event
RTI	respiratory tract infection
RT-PCR	Reverse transcriptase polymerase chain reaction
rRT-PCR	real-time reverse transcriptase polymerase chain reaction
SAE	serious adverse event
SAWP	Scientific Advice Working Party
SE	solicited event
SFI	severe febrile illness
SmPC	Summary of Product Characteristics
SNMC	significant new medical conditions
SPF	specific pathogen free
SWC	Scott & White clinic
TCID50	median tissue culture infectious dose
TIV	trivalent inactivated influenza virus vaccine
ts	temperature-sensitive
URI	upper respiratory illness
USA	United States of America
VAR or VARIVAX	varicella vaccine
WHO	World Health Organization
wt	wildtype

1. Background information on the procedure

1.1. Submission of the dossier

The Applicant MedImmune LLC submitted on 5 March 2015 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Pandemic influenza vaccine H5N1 MedImmune, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 20 February 2014.

The Applicant applied for the following indication: "Prophylaxis of influenza in an officially declared pandemic situation in children and adolescents from 12 months to less than 18 years of age. The use of MedImmune pandemic influenza vaccine H5N1 should be based on official recommendations."

The legal basis for this application refers to:

Article 8(3) of Directive 2001/83/EC - complete and independent application. The Applicant indicated that pandemic influenza vaccine (H5N1) (live attenuated, nasal) was considered to be a new active substance.

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).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0313/2014 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0313/2014 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

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.

Applicant's request for consideration

Conditional Marketing Authorisation

The Applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14(7) of Regulation (EC) No 726/2004 as it is claimed that this is a medicinal product to be used in emergency situations, in response to public health threats duly recognised either by the World Health Organisation or by the EU (Article 2(2) of Commission Regulation (EC) No 507/2006) –.

The Applicant claimed that:

- The risk-benefit balance of the medicinal product is positive;
- he will be in a position to provide comprehensive data when a pandemic occurs;

- there is a an unmet medical need fulfilled by providing a live attenuated pandemic influenza preparedness vaccine in children and adolescents;
- the benefit to public health of authorising this pandemic preparedness vaccine outweighs the risk inherent in the fact that additional data are still required on the actual pandemic strain.

New active Substance status

The Applicant requested the active substance contained in the above medicinal product to be considered as a new active substance in itself, as the Applicant claims that it is not a constituent of a product previously authorised within the Union. The active substance is:

Reassortant influenza virus* (live attenuated) of the following strain**:

A/Vietnam/1203/2004 (H5N1) strain

(A/Vietnam/1203/2004, MEDI 0141000136)

* propagated in fertilised hens' eggs from healthy chicken flocks.

** produced in VERO cells by reverse genetic technology. This product contains a genetically modified organism (GMO).

Scientific Advice

The Applicant received Scientific Advice from the CHMP on 2 August 2013, 11 September 2013 and 15 January 2014. The Scientific Advice pertained to quality, non-clinical and clinical aspects of the dossier.

Licensing status

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

1.2. Steps taken for the assessment of the product

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

Rapporteur: Jan Mueller-Berghaus Co-Rapporteur: Karsten Bruins Slot

- The application was received by the EMA on 5 March 2015.
- The procedure started on 25 March 2015.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 15 June 2015. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 12 June 2015.
- The PRAC Rapporteur Risk Management Plan (RMP) Assessment Report was adopted by PRAC on 09 July 2015.
- During the meeting on 23 July 2015, the CHMP agreed on the consolidated List of Questions to be sent to the Applicant. The final consolidated List of Questions was sent to the Applicant on 24 July 2015.
- The Applicant submitted the responses to the CHMP consolidated List of Questions on 23 November 2015.
- The Rapporteurs circulated the Joint Assessment Report on the Applicant's responses to the List of Questions to all CHMP members on 05 January 2016.
- The PRAC Rapporteur Risk Management Plan (RMP) Assessment Report was adopted by PRAC on

14 January 2016.

- During the CHMP meeting on 28 January 2016, the CHMP agreed on a list of outstanding issues to be addressed in writing by the Applicant.
- The Applicant submitted the responses to the CHMP List of Outstanding Issues on 26 February 2016.
- The Rapporteurs circulated the Joint Assessment Report on the Applicant's responses to the list of outstanding issues to all CHMP members on 11 March 2016.
- The PRAC Rapporteur Risk Management Plan (RMP) Assessment Report was adopted by PRAC on 17 March 2016.
- During the meeting on 29 March to 1st April 2016, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Conditional Marketing Authorisation to Pandemic influenza vaccine H5N1 MedImmune.

2. Scientific discussion

2.1. Introduction

Problem statement

An influenza pandemic occurs when a novel influenza virus appears to which no baseline immunity prevails in the human population, thus causing substantial numbers of deaths and severe disease worldwide. A pandemic may target age groups that are not traditionally affected by seasonal epidemics, e.g. young adults. Since the 20th century, several influenza pandemics occurred, including the “Spanish Flu” caused by H1N1 virus in 1918, the “Asian Flu” caused by H2N2 in 1957, the “Hong Kong Flu” caused by H3N2 in 1968, and the “Russian Flu” in 1977 and the “Swine Flu” in 2009 both caused by H1N1 viruses. The worst pandemic ever was the one in 1918, which killed an estimate of 50 million people worldwide, whereas the others brought about a lesser death toll.

It is hardly predictable which novel influenza virus strain will trigger the next pandemic and when a new pandemic will occur. At present, H5N1 strain poses a great concern, despite the limited human-to-human transmission so far, mainly due to its history of causing severe disease with a high case fatality rate. The consecutive isolations of several distinguishable clades of the H5N1 subtype in the world suggest that the virus underwent continuous minor antigenic changes.

During a pandemic, mass vaccination of the community represents an important measure to protect people individually from contracting disease and also from the spread of the infection within the population. The establishment of the “core dossier” procedure with the purpose of evaluating and authorising such vaccines during inter-pandemic periods could favour the timely supply of such vaccines to large parts of the population upon pandemic declaration. The dossier of this type of vaccine, the pandemic preparedness vaccine, will be developed on the basis of a potential pandemic strain. Such strain should be characterised mainly by the potential to cause a pandemic in humans, low immunogenicity and absence of specific baseline immunity in the population. Clinical testing of a pandemic preparedness vaccine will be undertaken during the interpandemic period, using clinical trial material that is produced by the same manufacture process and that is tested against the same specifications as will be the actual pandemic vaccine. The antigen content and dose formulation, as well as the route of vaccine administration in clinical trials have to be identical to that of the future pandemic vaccine. Once a pandemic is duly recognised in the EU, inclusion of the actual pandemic strain into the pandemic preparedness vaccine and subsequent authorisation of the pandemic vaccine will be processed via a variation procedure after submission of a dossier specific to the pandemic strain. Evaluation of this dossier will therefore be faster, in light of the previous knowledge on the vaccine construct acquired through the core dossier before the pandemic, and will focus mainly on quality data that are new and relevant to the actual pandemic virus strain to be included into the vaccine.

For this Marketing Authorisation Application (MAA), H5N1 was selected as the pandemic preparedness subtype due to the particular virulence of this subtype, the threat that it poses to human health and the fact that shows low immunogenicity and low baseline immunity. Since 2003, a total of 668 cases of human H5N1 infection have been reported including 393 deaths. While the virulence of the virus may decrease as it becomes adapted for sustained human-to-human transmission, the case fatality rate of approximately 60% is extremely alarming and a pandemic caused by H5N1 could equal or exceed that seen in 1918 in terms of overall severity.

Considerations on clinical evaluation of pandemic live attenuated influenza vaccine (P/LAIV)

The conduct of protective efficacy trials for a pandemic preparedness vaccine is neither practical nor ethical (if human challenge study is intended). Thus, a core dossier necessitates that immunogenicity and safety studies are instead conducted during interpandemic period. It is expected that the pandemic vaccine effectiveness will be evaluated in the post-authorisation phase during the next pandemic.

Due to the potential for shedding and transmission of a P/LAIV candidate virus strain and the resulting potential concern for reassortment between the vaccine virus and the wild type circulating viruses, conduct of clinical safety and immunogenicity trials generally requires isolation units and the recruitment of healthy adults in early phase of clinical development. Studies recruiting children, especially less than 2 years of age, would not be approvable by regulatory authorities without adequate clinical data from an adult population, especially when taking into account specific safety issue encountered for seasonal T/LAIV (Fluenz). For the latter vaccine a statistically significant increase in wheezing in children aged < 24 months and an increased rate of all-cause hospitalisation in children below the age of 12 months was observed.

Evaluation of P/LAIV immunogenicity is considered very challenging. For the most part difficulties are due to lack of knowledge regarding immunological correlates of protection for influenza vaccines in general but in particular for LAIV. Specific antibody responses, which are commonly examined for determining immunogenicity of inactivated influenza vaccines, are hardly detectable by existing immunoassays after vaccination with LAIV. Currently, no clear recommendation exists on how the immunogenicity of a P/LAIV vaccine should be measured. Nevertheless, relevant principles are described in the draft EMA Guideline on Influenza Vaccines (EMA/CHMP/VWP/457259/2014).

About the product

MedImmune pandemic preparedness vaccine H5N1 (P/LAIV) for intranasal administration is a monovalent live attenuated vaccine that contains a single attenuated (*att*), cold-adapted (*ca*), and temperature-sensitive (*ts*) reassortant influenza virus of the highly pathogenic avian influenza virus strain A/Vietnam/1203/04 (H5N1). The surface glycoproteins HA and NA of the H5N1 strain have been integrated by reverse genetics into the backbone of the long established A/AnnArbor/6/60 (H2N2) Master donor virus (MDV) that contributes the *ts*, *ca*, and *att* phenotype, creating a 6+2 chimeric virus. Hence, the vaccine contains a genetically modified organism (GMO). Each 0.2 mL dose will contain $10^{7.0 \pm 0.5}$ fluorescent focus units (FFU) of the genetically modified A/Vietnam/1203/2004 H5N1 strain.

P/LAIV is formulated in a refrigerated liquid formulation. For the list of excipients please see section 6.1 of the SmPC.

The vaccine is supplied in a carton of 10 single-use nasal sprayers (0.2 ml each, in trays). It will be administered using the Becton Dickinson Accuspray™ device, which delivers a 0.1 mL volume intranasal vaccine dose into each nostril.

The vaccination schedule proposed for children and adolescents consists of 2 doses of the vaccine separated by an interval of at least 4 weeks.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as nasal spray suspension containing $10^{7.0 \pm 0.5}$ fluorescent focus units (FFUs) of H5N1 A/Vietnam/1203/2004 in each 0.2 mL dose as active substance. Other ingredients are sucrose, dibasic potassium phosphate, monobasic potassium phosphate, gelatin (porcine, Type A), arginine hydrochloride, monosodium glutamate monohydrate and water for injection.

The product is supplied as a 0.2 ml suspension in a single use nasal sprayer (Type 1 glass), with nozzle (polypropylene with polyethylene transfer valve), nozzle tip protector cap (synthetic rubber), plunger rod, plunger stopper (butyl rubber), and a dose divider clip in a pack size of 10.

P/LAIV contains the same type of components as currently included in the approved vaccine, Fluenz Tetra, and differs only in the fact that it is a monovalent formulation containing a single Type A strain. The two vaccines are produced by the same manufacturing process using the same attenuated master donor virus and excipients and are blended with the same potency specification: $10^{7.0 \pm 0.5}$ fluorescent focus units (FFUs) per strain. Both vaccines are administered intranasally using the Becton Dickinson (BD) Accuspray™ device.

2.2.2. Active Substance

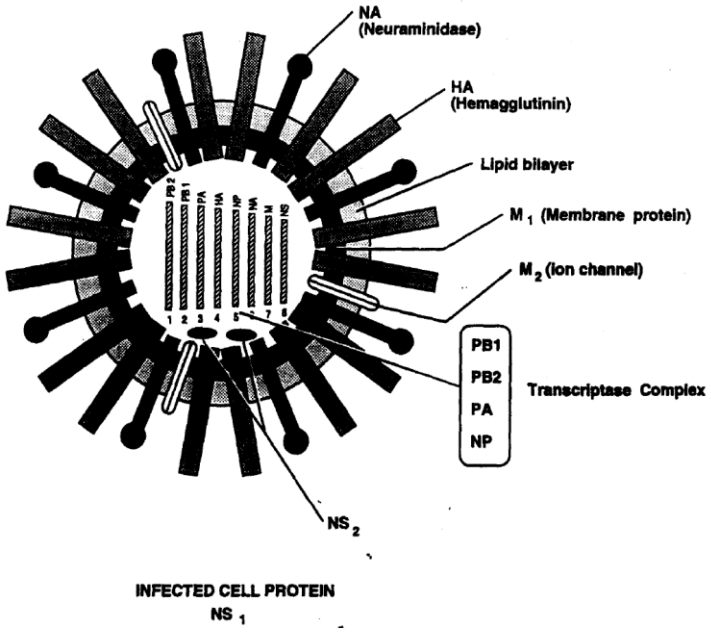
General information

The pandemic influenza A/Vietnam/1203 (H5N1) vaccine is a monovalent version of the already approved seasonal live attenuated vaccine “Fluenz Tetra” (tetravalent formulation).

Influenza virus Type A belongs to the family of Orthomyxoviruses. Influenza viruses are enveloped and do not have a rigid capsule structure (see Figure 1). The internal core of influenza virus particles consists of a segmented RNA genome, which is associated with the nucleoprotein <NP> and polymerase proteins. The viral envelope surrounds the viral nucleocapsid. The internal layer of the viral envelope contains viral matrix protein <M>, and the external layer of the envelope consists of a lipid bilayer that is derived from the host cell membrane during release of newly formed virus particles from infected cells. The external surface of the lipid bilayer of influenza viruses is decorated with two major viral transmembrane protein spikes of which approximately 80% are rod-shaped haemagglutinin (HA) protein trimers, and 20% are mushroom-shaped neuraminidase (NA) tetramers.

The epidemiology of influenza viruses dictates incorporation of contemporary protective antigens (the haemagglutinin (HA) and neuraminidase (NA) antigens) into the vaccine on an annual basis. The HA protein is responsible for several of the biological properties of influenza viruses and the NA protein contributes to the antigenic characteristics and functional properties of influenza virus. Both the HA and NA protein epitopes contribute to the induction of a protective response in humans. Alterations in the primary structure of HA and NA proteins are directly related to antigenic variation of influenza viruses, which serves as the basis for antigenic and immunogenic characterization of influenza viruses using strain-specific antiserum.

Figure 1. Structure of the influenza A virus particle (Lamb, 1996)



The cold-adapted reassortant vaccine strains for P/LAIV are produced by genetic reassortment between wild-type influenza virus and a master donor virus (MDV). Such reassortant viruses contain gene segments encoding HA and NA antigens contributed by the wild-type circulating virus, and gene segments encoding the remaining proteins derived from the cold-adapted MDV (polymerase basic protein 1 <PB1>, polymerase basic protein 2 <PB2>, polymerase acidic protein <PA>, nucleoprotein <NP>, matrix protein <M>, and non-structural protein <NS>). These vaccine strains are thus called 6:2 reassortants. Hence, cold-adapted reassortant vaccine strains derive their antigenic phenotypes from the circulating strains HA and NA antigens and their cold-adapted (*ca*: efficient growth at 33°C and 25°C), temperature-sensitive (*ts*: highly restricted growth at 39°C), and attenuated (*att*) phenotypes from the MDV. The MDV used to generate the P/LAIV vaccine reassortant is A/Ann Arbor/6/60 that was developed by H.F. Massaab in 1982.

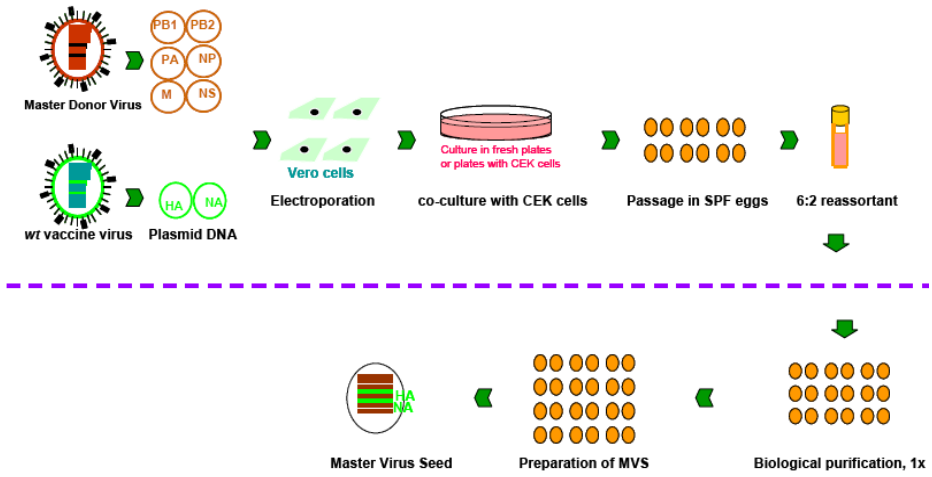
Manufacture, process controls and characterisation

The monovalent bulk active substance is produced at MedImmune UK Limited, Speke, Liverpool, United Kingdom.

Manufacture of the Master Virus Seed (MVS)

The 6:2 reassortant Master Virus Seed (MVS) containing six viral gene segments from the attenuated Master Donor Virus (MDV) and two gene segments from wild-type (wt) influenza vaccine virus encoding haemagglutinin (HA) and neuraminidase (NA) antigens. MVS are prepared by a plasmid rescue process / reverse genetics in Vero cells. The process is depicted in Figure 2.

Figure 2. MVS Manufacturing Process



CEK: chicken embryo kidney cells

The plasmid rescue process is initiated by extracting viral RNA from the MDV and from the wt strain, and converting six viral gene segments (<PB1>, <PB2>, <PA>, <NP>, <M>, <NS>) from the MDV, and the HA and NA gene segments from the wt strain, into cDNA by Reverse Transcription Polymerase Chain Reaction (RT-PCR). These amplified cDNAs are inserted into plasmids and transformed into E. coli cells. The transformed E.coli cells are grown and plasmid DNA is purified for testing and further processing.

The cDNA containing plasmids corresponding to the MDV gene segment, as well as the cDNA containing plasmids corresponding to the wt HA and NA gene segments are combined by electroporation into serum-free Vero (African green monkey kidney) cells that are derived from an extensively tested and characterized cell bank. The electroporated Vero cells are then co-cultured with CEK cells. The 6:2 reassortants are then passaged in SPF embryonated chicken eggs to produce material, referred to as accession seed. The accession seed is biologically purified and amplified in SPF eggs to produce the MVS batch.

Each MVS is tested for sterility (bacterial/fungal contamination), mycoplasma and viral adventitious agents before release for further manufacturing.

Manufacture of the monovalent bulk

Manufacturing of the monovalent bulk active substance is performed at the MedImmune UK facility in Speke, Liverpool. Monovalent bulks are prepared by inoculation and growth of the master virus seed (MVS) in embryonated Specific Pathogen Free (SPF) eggs. The batch scale for LAIV manufacture is defined by the number of SPF chicken eggs used. Downstream of the allantoic fluid harvesting stage, the process is defined by the volume of clarified harvest fluid loaded on the ultracentrifuge rather than the number of eggs.

Upon shipment SPF eggs are compliance checked, washed, dried and transferred into the Primary Incubation suite where they are held.

Following the primary incubation period, eggs are candled for any cracks etc. The trays of acceptable eggs are held under controlled temperature conditions until inoculation. The eggs are inoculated with inoculum (based on the infectivity titre ($\log_{10}$ FFU/ml) of diluted Master Virus Seed (MVS)) and incubated. After incubation, allantoic fluid is removed. Clear allantoic fluid is dispensed into a sterile polycarbonate bottle and transferred to the Harvest Cold Room until released for further processing.

Harvest bottles are screened for lack of bioburden and pooled. The pooled material is filtered to obtain Clarified Harvest Fluid (CHF). The CHF is mixed and samples are removed for further bioburden in-process testing. Pooled virus harvest is concentrated using continuous flow ultracentrifugation in a sucrose gradient to increase the density of virus particles and to reduce egg-derived proteins, nucleic acids and other components. Concentrated virus harvest is pooled, diluted and sterile filtered to obtain the monovalent bulk.

The viral eluate is then filled into polycarbonate bottles that are stored for a maximum period of 24 months.

The container closure system for the final active substance is a polycarbonate (PC) bottle, sealed with polypropylene screw-on cap containing a silicon rubber liner.

Following freeze-down and placement of the active substance containers in the MedImmune-UK storage freezers bottles are subsequently shipped to the MedImmune Pennsylvania Facility for further processing (blending). Monovalent bulk active substance shipping containers have been validated to maintain frozen temperatures during transit activities and utilize dry ice as the refrigerant.

Critical Process Parameters (CPP) and Key Operating Parameters (KOP) were defined for the manufacturing of the monovalent bulk AS, based on their ability to impact on Critical Quality Attributes (CQAs) and process performance, respectively.

In-process control (IPC) limits have been established for key process parameters that are used to monitor ongoing production of the monovalent bulk AS.

Process validation

Validation of the LAIV monovalent bulk AS manufacturing process at MedImmune UK was originally performed in 2004. The process has been subject to further validation studies to support subsequent process improvements and equipment changes. These changes were designed to improve the manufacturing process and microbiological control and have been well documented. A review of the monovalent bulk validation approaches, acceptance criteria and validation results has been provided. Taken together these data and attached reports confirm the validity and applicability of process steps/improvements implemented after the initial validation. The formulation of the monovalent bulk AS has not changed and is the same as that used in the pivotal clinical trials. The study results demonstrated that the manufacturing process is capable of consistently yielding monovalent bulk AS that is compliant with the specifications.

Manufacturing process development

As previously stated, MedImmune is applying the same manufacturing process to P/LAIV that has been used for more than 90 full-scale lots of a monovalent A/California/2009 (H1N1) vaccine produced during the “swineflu” pandemic in 2009/2010. Consequently, for the seasonal (Fluenz tetra) and the H1N1-pandemic counterparts of the H5N1-pandemic vaccine substantial experience exists regarding the manufacturing process and the control regimen applied (i.e. millions of doses).

The clinical H5N1 batches were manufactured in 2005-2006 at pilot scale. The present application dossier does not contain full scale A/Vietnam/1203-specific information for active substance manufacturing and testing. Instead available and supportive data is provided for seasonal and H1N1-pandemic vaccines. This is due to the situation that no large scale production of A/Vietnam/1203-specific active substance batches has been initiated so far and only small scale lots of clinical trial material have been produced in 2005/2006. The CHMP considered that given the extensive experience with MedImmunes manufacturing process for LAIVs, it would not be necessary to generate additional H5N1 commercial scale material, which may never be used as it can be expected that the strain will

need to be varied prior to commercial use of the vaccine. Thus data provided from the seasonal influenza vaccine and the pandemic A/H1N1 strain is considered supportive and sufficient.

Specification

The active substance is intended to be tested according to the programme that is implemented and approved for the seasonal LAIVs. This is considered acceptable. The monovalent bulk active substance is tested for appearance, sterility, endotoxin, identity HAI, genotype, phenotype, attenuation, and potency. A panel of additional tests, mostly to confirm the absence of microbial contaminations, is conducted on the pooled harvest.

Analytical methods

The proposed analytical procedures are the same as applied for the testing of the approved seasonal versions of the vaccine and are adequately validated. During the procedure the Applicant provided the requested relevant information on specific steps and characteristics related to the A/Vietnam/1203 descendent reassortant seed strain generated and used for vaccine manufacture.

During process development the potency assay and the specifications have been changed from the tissue culture infectious dose 50 (TCID50) to the fluorescent focus assay (FFA). The FFA utilizes immunofluorescent staining of virus-infected Madin-Darby Canine Kidney (MDCK) cells with anti-influenza hemagglutinin (HA) antibodies specific for each individual vaccine strain.

According to the release certificate provided, the A/Vietnam/1203 monovalent bulk (used for production of clinical trial vaccine lots in 2005/2006) was tested for potency by the TCID50 method. Comparative data on vaccine potency determined by either the TCID50 or the FFA method was requested during the procedure and has been provided by the Applicant. These data were generated for monovalent bulks as well as for trivalent seasonal finished product and demonstate the compatibility of potency determination by both methods.

Batch analysis

Batch analysis data for monovalent bulks from three consecutive process validation lots used to manufacture pandemic H1N1 consistency batches in 2009 (at pilot scale) have been provided instead of commercial scale data for a contemporary pandemic strain (A/Vietnam/1203/2004). Further batch analysis data for three lots of LAIV Monovalent Bulk (B/Massachusetts/2/2012) manufactured during January to February 2014 for influenza strain B/Massachusetts/2/2012 have been provided as supportive.

Taken together the available data and extensive manufacturing experience (i.e. seasonal (Fluenz tetra) and the H1N1-pandemic counterparts of the H5N1-pandemic vaccine) have been considered acceptable to confirm the consistency of the manufacturing process. The specifications for the monovalent bulk active substance have been established based on manufacturing history, clinical data and commercial experience, i.e. for seasonal LAIVs with respect to bioburden, endotoxin, appearance and potency.

Reference standard

There is no reference standard according to ICH Q6B used in the release tests performed on monovalent bulk AS for the LAIV. This is acceptable given the specificity of the active substance.

During the procedure the Applicant was requested to present a strategy for the production of anti-sera and reference reagents in the pandemic situation in light of time constraints in order to ensure the correct virus titre per dose. The proposed strategy to produce anti-sera and reference reagents in a pandemic situation was considered acceptable in order to determine the virus titre per dose using the FFA is a direct measure of the infectivity.

Stability

A stability period of 24 months at ≤ 60°C is proposed for the monovalent bulk active substance.

The proposed container closure system, the polycarbonate (PC) bottle was selected for the primary packaging of the monovalent bulk active substance due to its resistance to storage at extreme conditions. Stability data provided demonstrate that the container is suitable for long-term storage of the monovalent bulk active substance, and that there are no components of the container closure system that affect the quality or the consistency of the product during such storage for up to 24 months.

No stability data are available for the clinical A/Vietnam/1203 monovalent bulk produced in 2005-2006. However, MedImmune has provided stability data from three monovalent LAIV bulks produced at the Speke UK facility generated in 2009 (pilot scale to obtain pivotal clinical trial material) in support of the H1N1 A/California/07/2009 pandemic stored in the intended container for 28 months and over. In addition, active substance stability data have been included from the 2012/2013 and 2014/2015 influenza season which supports the current manufacturing process using the same container closure system.

In conclusion the available experience and data on stability indicate that the active substance is sufficiently stable and justify the proposed shelf life in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

The Finished product (FP) contains 7.0 ±0.5 $\log_{10}$FFU/0.2 mL dose of strain A/H5N1 (A/Vietnam/1203/2004).

Excipients in the finished product are sucrose, dibasic potassium phosphate, monobasic potassium phosphate, gelatin hydrolysate (gelatin <porcine, Type A>), arginine hydrochloride, and monosodium glutamate monohydrate. The volume of a single dose is 0.2 ml. The vaccine contains no preservative.

The P/LAIV finished product is formulated from the pandemic monovalent bulk active substance. Because the P/LAIV formulation was developed based on the seasonal vaccine formulation, compatibility and the excipient concentration has been satisfactorily justified and further confirmed by clinical and process validation studies with Fluenz tetra.

The excipients used are well controlled using Ph.Eur. and MedImmune methods and sufficient detail on the specifications has been provided.

The container/closure system for the monovalent, attenuated influenza vaccine (P/LAIV) finished product is the Becton Dickinson (BD) Accuspray™ Nasal Spray System. The nasal spray system consists of a nasal sprayer barrel, nasal sprayer nozzle, plunger stopper and plunger rod. The same device is in use for the seasonal version of the vaccine (i.e. Fluenz tetra).

Formulation development

The pandemic live attenuated influenza vaccine (P/LAIV) vaccine formulation was established based on the quadrivalent (Fluenz tetra) formulation and was considered suitable for intranasal administration. Extensive experience has been gained with the quadrivalent (Fluenz tetra) formulation with respect to compatibility of the influenza vaccine monovalent bulk active substance and the excipients used in the finished product. The excipients and their concentrations are identical between P/LAIV and Fluenz tetra.

Further studies to optimize the grade of gelatin and pH studies for trivalent LAIV resulted in the current formulation for all LAIV formulations including the pandemic vaccine formulation.

Manufacture of the product and process controls

The finished product is produced by MedImmune LLC, Philadelphia, PA, USA, and is released in the EU by MedImmune UK Limited, Speke, Liverpool, UK.

The manufacturing process consists of thawing of the monovalent bulk, followed by a blending of the AS with concentrated Gelatin-Arginine-Glutamate (cGAG) buffer and dilution to final volume with Sucrose Phosphate (1X SP) buffer.

The blended monovalent formulated bulk is aseptically filled as a 0.2 ml deliverable dose into 0.5 ml Accuspray nasal sprayer barrels, without any additional sterilisation step. The product is frozen at ≤ -20°C prior to or after final packaging (with secondary labelling). The product is then transported to a warehouse prior to shipment. In the EU, the product is batch released by MedImmune UK Ltd, Speke, UK.

Commercial batch size may vary and are based on forecasted demand.

Critical steps in the manufacture of the finished product are controlled at several stages of the manufacturing process at both the blending and filling operations to ensure that the process performs as intended. Manufacturing controls are performed throughout the process. The process parameters are established based upon that of the Fluenz tetra process parameters. No in-process tests are performed during the manufacture of the finished product, which is accepted because the manufacturing controls and batch release tests/ specifications for the limited operations for finished product manufacture adequately assure product of consistent quality.

Product specification

Batch release tests are performed on samples collected at the bulk blend and filled blend stage for the pandemic live attenuated influenza vaccine (P/LAIV) to confirm conformance with the finished product specifications. Sterility is performed on the bulk P/LAIV blend. Lot-specific tests performed on filled/packaged P/LAIV blend are pH, potency (by Fluorescent Focus Assay), identity (Fluorescent Focus Assay), endotoxin, ovalbumin, total protein, colour, opalescence and appearance, sterility, and thermal stability.

The analytical methods have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines. Potency is determined with the fluorescent focus assay (see under active substance).

The potential impurities in the P/LAIV finished product are endotoxin, ovalbumin and other egg-derived proteins, and residual process and excipient components (Gentamicin Sulfate), which are also present for the seasonal influenza vaccine Fluenz tetra.

The Endotoxin limits of ≤ 30 EU/mL (equivalent to 6 EU/human dose) and the ovalbumin limit of ≤ 1.2 µg/mL (equivalent to 0.24 µg/human dose) are well below the Ph. Eur. requirements for inactivated influenza vaccines (NMT of <100 EU/human endotoxin per dose and NMT of 1 µg/human ovalbumin per dose). Gentamicin Sulfate is a component of the media used in the MVS manufacturing process. The theoretical concentration of Gentamicin Sulfate in the final product, based on dilutions used in the manufacture of the final product, is approximately 1x10-10 µg/ml. Such low levels of gentamicin sulfate are not detectable in the process using current assay methods and thus a corresponding specification has not been included. The control of potential impurities has been extensively discussed during MAA of Fluenz tetra and is considered acceptable.

Batch analysis data (three validation batches from H1N1 pandemic finished product at commercial scale and three manufacturing batches of Fluenz tetra at commercial scale produced during 2014/2015) were provided and are within the pre-set acceptance criteria with no apparent trends. However, as already discussed above, no commercial scale batch data are presented for H5N1 P/LAIV, which has been considered justified on the basis of the experience generated with the seasonal and the H1N1 Pandemic vaccine.

There are no reference standards used in the release tests for P/LAIV, as defined by ICH Q6B (please also see active substance section).

Stability of the product

The proposed shelf life of the P/LAIV finished product is defined as up to 20 weeks at -25°C ± 5°C prior to distribution and subsequent storage at 2°C to 8°C not to exceed 18 weeks.

According to the special precautions for storage the vaccine should be stored in a refrigerator and not be frozen. Before use, the vaccine may be taken out of the refrigerator once for a maximum period of 12 hours at a temperature not above 25°C. If the vaccine has not been used after this 12 hour period, it should be discarded. These instruction are in line with the SmPC for Fluenz tetra.

The claimed shelf life is not supported by H5N1 P/LAIV finished product data. Instead, data from the US-licensed H1N1 pandemic vaccine are presented, which were generated using the same container closure system as for the proposed commercial vaccine.

It was considered that data provided from the monovalent H1N1 pandemic (2009) formulation did not support the claimed shelf life as all three lots were below specification prior to the end of the proposed shelf life. However, the Applicant has in the meantime developed and implemented a thermostability assay that allows detection of “thermolabile” HA molecules. This assay will be applied to all future new virus strains with the goal of preventing stability failures during the shelf life period. On the basis of this assay, the proposed shelf life for the H5N1 pandemic preparedness vaccine of 20 weeks at -25°C ± 5°C (prior to distribution) and subsequent storage at 2°C - 8°C for not more than 18 weeks is considered acceptable in accordance with the seasonal vaccine counterparts. As requested by the CHMP, the Applicant will generate strain-specific stability data for the actual pandemic vaccine strain in order to define the shelf life on an evidence-based, strain-specific basis. This obligation will be reflected in the Annex II of the marketing authorisation.

Adventitious agents

Viral safety

The master virus seed (MVS) is prepared by a plasmid rescue process and contains a specific constellation of viral gene segments from an attenuated Master Donor Virus (MDV) and a wild-type (wt) influenza virus.

The MVS are used to inoculate Specific Pathogen Free (SPF) embryonated eggs to produce individual monovalent bulks.

The viral safety is satisfactorily assured by i) quality/virological controls of raw materials of animal origin used during the process and ii) virological controls performed on cell substrates (Vero and CEK cells), SPF eggs and during the production process at MVS level and pooled harvest fluid level.

TSE

Raw materials of animal origin used in the production of P/LAIV are fetal bovine serum, new born calf serum and porcine trypsin, which were used for establishment of Vero cell banks and for culture of CEK. Gelatin of porcine origin is added as an excipient.

In accordance with the Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01), Certificates of suitability for TSE safety have been provided for all raw materials derived from TSE relevant animal species used in the manufacturing process.

Overall, sufficient data is provided to exclude a risk of TSE transmission through P/LAIV. The risk of transmitting TSE is thus considered very remote.

GMO

Like Fluenz Tetra, P/LAIV is a live attenuated influenza vaccine containing virus strains generated through reverse genetic technology. For this reason, the vaccine is classified a genetically modified organism (GMO) as defined in Directive 2001/18/EC.

Please see section 2.3.5. Ecotoxicity/environmental risk assessment for the assessment and conclusions on the environmental risk assessment.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The manufacturing process to be used for P/LAIV production of monovalent bulk active substance has been described in detail. It is the same as applied for the seasonal LAIV versions and no H5N1-specific process optimisation is proposed. The same holds true for the control testing scheme.

The development of the manufacturing process and formulation of the finished product has been described in detail and the different process changes were highlighted. The batch formula has been adequately described and information on the required potency is provided. Furthermore, information on the volume range and the resulting doses is provided. Validation of the finished product manufacturing process was appropriately performed. Descriptions of the analytical methods as well as the validation studies were provided. The proposed specifications for the P/LAIV finished product are cosidered to be acceptable. However, no batch data have been provided for the H5N1 strain (A/Vietnam/1203) and the assessment is based on data generated from the seasonal tetravalent formulation and the monovalent H1N1 pandemic (2009) formulation, which has been discussed and considered to be acceptable.

The proposed container closure system for the final product is acceptable and sufficient data supporting the suitability of the Accuspray™ Nasal Spray System were provided.

The claimed finished product shelf life (up to 20 weeks at -25°C ± 5°C prior to distribution and storage at 2°C to 8°C for not more than 18 weeks) has not been supported with H5N1 P/LAIV finished product data. It was considered that data provided from the monovalent H1N1 pandemic (2009) formulation did not support the claimed shelf life as all three lots were below specification prior to the end of the proposed shelf life. This was not considered acceptable at day 120 of the procedure. However, the Applicant has in the meantime developed and implemented a thermostability assay that allows to detect “thermolabile” HA molecules and has confirmed that he will generate strain-specific stability data for the actual pandemic vaccine strain. On the basis of this commitment from the Applicant that will be reflected as an obligation in Annex II of the MA the CHMP agreed to the proposed finished product shelf life.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The CHMP has identified the following measure necessary to address the identified quality developments issues that may have a potential impact on the safe and effective use of the medicinal product:

- to generate strain-specific stability data for any new pandemic vaccine strain.

2.2.6. 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 did not recommend any point for investigation at the time of opinion.

2.3. Non-clinical aspects

2.3.1. Introduction

The P/LAIV H5N1 vaccine is intended to be delivered intranasally (IN) twice, separately by at least 4 weeks, using a AccusprayTM device that delivers 0.2 mL/dose divided into each nostril (0.1 mL/nostril). The vaccine is intended for prophylaxis of influenza in an officially declared pandemic situation in children and adolescents from 12 months to less than 18 years of age.

Non-clinical evaluation of *ca* A/Vietnam/1203/2004 (H5N1) P/LAIV includes the immunogenicity and protective efficacy evaluation of this candidate vaccine in mice, ferrets and African green monkeys (AGMs), a repeat-dose toxicity study in ferrets, attenuated phenotype in chickens and ferrets and the neurotropism in mice.

In addition, extensive supportive data from other P/LAIV candidates and from seasonal LAIVs are included in this application, to support general toxicity, reproductive toxicity aspect, as well as eye irritation and environmental safety of the candidate vaccine.

A repeat-dose toxicity study with *ca* A/Vietnam/1203/2004 (H5N1) P/LAIV was conducted in ferrets in compliance with GLP. In addition, a great majority of supportive toxicity studies were also GLP-compliant.

2.3.2. Pharmacology

Non-clinical pharmacologic testing for *ca* A/Vietnam/1203/2004 (H5N1) P/LAIV was the following:

- Testing for attenuated phenotypes in ferrets and mice by measuring replication of the vaccine viruses in the upper (nasal turbinates) and lower (lung) respiratory tracts.
- Testing for pathogenicity in chickens, and replication in brains of mice and ferrets using P/LAIV strains derived from H5N1 (safety pharmacology).
- Evaluation of immunogenicity and protective efficacy of the candidate vaccine against homologous and heterologous wt virus challenges in seronegative animals, including ferrets, mice, and African Green Monkeys (AGMs).
- Similar nonclinical studies performed on other P/LAIV candidates as supporting data [A/California/7/2009 (H1N1pdm09), A/chicken/Hong Kong/G9/97 (H9N2), A/Ann Arbor/6/60 (H2N2), A/swine/Missouri/4296424/2006 (H2N3), A/teal/Hong Hong/W312/97 (H6N1), A/chicken/British Columbia/CN-6/2004 (H7N3), A/Netherlands/219/2003 (H7N7), A/Anhui/1/2013 (H7N9)].

A list of non-clinical pharmacology studies is provided in the table below.

Table 1. List of non-clinical pharmacologic studies supporting *ca* A/Vietnam/1203/2004 (H5N1) P/LAIV

Strain/Reference	Study	Species/ROA	GLP
H5N1 (A/Vietnam/1203/2004) (A/Hong Kong/213/2003) Suguitan et al, 2006	Pathogenicity and replication in chickens; Replication in the respiratory tract and brain of mice and ferrets; Immunogenicity and protection against homologous and heterologous *wt* H5N1 challenges in mice and ferrets.	Chicken/ IV, IN Ferret, Mouse /IN	No
H1N1pdm09 (A/California/7/2009) Chen Z et al, 2010 Ferret study 081-09-033 Ferret study 081-11-035	Immunogenicity, replication kinetics and protection against *wt* A/California/7/2009 challenge in ferrets	Ferret/IN	No
H9N2 (A/chicken/Hong Kong/G9/97) Chen H et al, 2003	Replication in mice; Immunogenicity in ferrets; Immunogenicity and protection against homologous and heterologous *wt* H9N2 in mice	Ferret, Mouse /IN	No
H2N2 (A/Ann Arbor/6/60) Chen G et al, 2010	Replication in mice and ferrets; Immunogenicity and protection against homologous and heterologous *wt* H2 challenges in mice and ferrets	Ferret, Mouse /IN	No
H2N3 (A/swine/Missouri/4296424/2006) Chen G et al, 2014 Chen Z et al, 2012	Replication and lung histopathology in mice and ferrets; Immunogenicity and protection against homologous and heterologous *wt* H2 challenges in mice and ferrets	Ferret, Mouse /IN	No
H6N1 (A/teal/Hong Kong/W312/97) Chen Z et al, 2009 Chen Z et al, 2012	Replication in ferrets; Immunogenicity and protection against homologous and heterologous *wt* H6 challenges in mice and ferrets	Ferret, Mouse /IN	No

H7N3 (A/chicken/British Columbia/CN-6/2004) Joseph et al. 2008	Replication kinetics, immunogenicity, and protection against homologous and heterologous *wt* H7 challenges in mice and ferrets	Ferret, Mouse /IN	No
H7N7 (A/Netherlands/219/2003) Min et al. 2010	Pathogenicity and replication in chickens; Replication kinetics in the respiratory tract of mice, ferrets and AGMs, in the brain of mice and ferrets; Immunogenicity and protection against homologous and heterologous *wt* H7 challenges in mice, ferrets and AGMs.	Chicken/ IV+IN Ferret, Mouse /IN AGM /IN+IT	No
H7N9 (A/Anhui/1/2013) Chen Z et al. 2014	Replication in ferrets; Immunogenicity and protective efficacy against homologous and heterologous *wt* H7 challenges in ferrets	Ferret/IN	No
H5N1 (A/Vietnam/1203/05) H7N3 (A/chicken/British Columbia/CN-6/2004) H6N1 (A/teal/Hong Kong/W312/97) H9N2 (A/chicken/Hong Kong/G9/97) Matsuoka et al. 2014	Replication, immunogenicity and protective efficacy against homologous *wt* virus challenges in AGMs	AGM/IN+IT	No
wt A/NWS-33 (Control) and seasonal *ca* H1N1, H3N2 and B viruses ACF-07-001	Neurovirulence Testing of Influenza Strains in Mice	Mouse/IN	No

ROA = route of administration; GLP = Good Laboratory Practice; IN=intranasal; IT = intratracheal; IV = intravenous; wt = wild-type; AGM = African green monkey

Primary pharmacodynamic studies

Protective efficacy of the P/LAIV H5N1 VN04 candidate has been demonstrated in mouse, ferret, and non-human primate models.

In pre-pubertal ferrets, the proposed 2-dose regime was able to completely protect challenged animals from wild-type virus replication in the lungs, either homologous (wt A/Vietnam/1203/2004) or heterologous (A/Indonesia/05/2005) H5N1 strains. Challenge virus titres in nasal turbinates were also significantly lower in the P/LAIV H5N1 VN04-vaccinated animals than those of mock-immunised ferrets following challenge with homologous or heterologous viruses. Similar was true for virus titres in brains.

In the ferret model, low HAI and neutralisation titres were induced by *ca* H5N1 vaccines against respective homologous wt virus. Even lower functional antibody titres against heterologous wt H5N1 virus were observed. Despite that, there was complete protection against viral replication in the lungs following homologous or heterologous wt H5N1 virus challenge. In this experiment, the control group vaccinated with *ca* H1N1 virus did not show detectable serum antibody titres against H5N1 viruses, and did not confer protection in wt H5N1 virus-challenged animals. The control was included to determine protection contributed by internal protein genes of the *ca* A/Ann Arbor/6/60 virus (which shared 6 internal protein genes with the *ca* H5N1 viruses). It is agreed that other immune responses

such as cellular immune responses and mucosal IgA may also contribute to the protective efficacy of P/LAIV in this model.

In adult African Green Monkeys, 2-dose regime at 1/5 the proposed dosage of P/LAIV H5N1 VN04, administered intranasally and intratracheally (each $1x10^6$ TCID50) provided complete protection against homologous wild-type virus replication both in the respiratory tissues (lung, trachea, nasal turbinate) and in the secretion samples (tracheal lavage fluid, nasal pharyngeal swab). In this model a single dose of vaccine was ineffective. Serum hemagglutination-inhibiting (HAI) antibody, neutralising (MN) antibody and nasal wash IgA antibody were detected in the vaccinated animals. Notably, a correlation between serum antibody levels, especially MN, and the level of protection against virology endpoints was observed. Such correlation was not seen for nasal wash IgA response. The underlying mechanism(s) involved in protection has not been dissected.

In compliance with previous scientific discussions with the Applicant, an array of ferret and monkey challenge studies conducted with P/LAIVs containing other subtypes, including H9, H2, H6, H7, as well as H1N1pdm09, were included in the H5N1 dossier as supportive evidence. Each vaccine candidate demonstrated a consistent pattern of efficacy in preventing homologous and heterologous wild-type virus challenge, although levels of serum antibodies varied by vaccine. In the ferret studies, a low level of serological responses does not necessarily indicate lack of protective efficacy, a finding consistent with the clinical observation made for Fluenz in clinical studies.

In the Day 120 responses, the Applicant has provided references to published studies, which show that seasonal LAIV and P/LAIV H7N7 induce cellular immune responses in animals. Based on the similarities between these vaccines and P/LAIV H5N1, the Applicant expects that P/LAIV H5N1 is able to induce both humoral and cellular immune responses in animals.

Secondary pharmacodynamic studies

Secondary pharmacodynamic studies are generally not performed with vaccines and were not performed with P/LAIV. As the vaccine did not show any effects apart from the expected immune response, this was considered acceptable.

Safety pharmacology programme

Safety pharmacology studies were performed with the LAIV construct to investigate its neurotropism in mice and ferrets, viral replication in chickens and mice, and lethality in mice. Different influenza strains were used, such as the wt and *ca* H5N1 and H7N7, and seasonal strains (Fluenz). Overall these studies showed that the cold attenuated vaccine virus is not able to replicate in the respiratory tract and brain, and was not pathogenic.

Specifically the vaccine virus was non-pathogenic for chickens after intravenous administration. In mice, the vaccine virus replication in the upper and lower respiratory tract was significantly lower than that of the wild-type H5N1 virus, with no vaccine virus being detected in the brain of mice. In ferrets, the vaccine virus can replicate in the upper respiratory tract, but was not detected in the lower respiratory tract or brain of this animal species.

No other safety pharmacology studies have been conducted. However, some relevant endpoints (heart and respiration rate) have been included in the toxicological studies with repeat dosing. No vaccine-related adverse changes to these parameters have been reported. Even though the measured endpoints are limited, the safety pharmacology evaluation is considered sufficient, given the extensive clinical data available with the seasonal live attenuated influenza vaccines (Fluenz and Fluenz Tetra), which have a similar construct to P/LAIV.

Pharmacodynamic drug interactions

Pharmacodynamic drug interaction studies have not been conducted with P/LAIV, in accordance with "Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines" (CPMP/SWP/465/95) and with "Guideline on Adjuvants in Vaccines for human use" (CHMP/VEG/134716/2004). Clinical studies are available to confirm the safety, tolerability and immunogenicity of Fluenz administered concurrently with measles, mumps, rubella (MMR), varicella and oral polio vaccines in young children.

2.3.3. Pharmacokinetics

Typical pharmacokinetic studies including absorption, metabolism, and excretion do not pertain to live vaccines and are therefore not provided. Local deposition and distribution studies have been performed in humans with Fluenz. The characteristics of the intranasal spray were also evaluated in a series of studies which evaluated properties such as density, viscosity, surface tension, droplet size and spray pattern. Together, these studies define the pharmacokinetic profile of Fluenz and the results are considered of value also for P/LAIV. Moreover the live attenuated vaccine construct does not contain an adjuvant or new excipients which would require other pharmacokinetics studies.

2.3.4. Toxicology

One GLP-compliant repeat dose toxicology study was conducted in ferrets to support safety of *ca* A/Vietnam/1203/2004 (H5N1) vaccine. This study also evaluated local tolerance and toxicity after a single vaccination.

In addition, 8 GLP-compliant and 2 non-GLP toxicology studies conducted with other P/LAIV strains, or with trivalent LAIV (T/LAIV) and quadrivalent LAIV (Q/LAIV), are provided in this application:

- Five repeat-dose toxicity studies in ferrets; three reproductive and developmental toxicity studies (two in rats and one in ferrets), and two eye irritation studies in rabbits;
- All P/LAIV, the Q/LAIV, and the FluMist/Fluenz were administered IN and at the intended human dose (exceptions: in eye irritation studies FluMist/Fluenz was instilled into the conjunctival sac).

A list of the nonclinical toxicology studies is provided in Table 7.

Table 2. List of nonclinical toxicology studies supporting *ca* A/Vietnam/1203/2004 (H5N1) P/LAIV

Study Number	Study Title	Species/ROA	Test Article	GLP
SVT06-11	Repeat Dose Toxicology Testing of Candidate H5N1 A/Vietnam/1203/2004 Vaccine in Ferrets – 35 Days	Ferret/Intranasal	H5N1 VN 2004/AA	Yes
SVT08-10	Repeat Intranasal Safety Study of Candidate Pandemic Influenza Vaccine H2N2 A/Ann Arbor/6/60 and H6N1 A/Teal/Hong Kong/W312/97 in Ferrets – 56 Days	Ferret/Intranasal	H2N2 A/Ann Arbor/6/60 and H6N1 A/Teal/Hong Kong/W312/97	Yes
077-09-001	Non-GLP Repeat Dose Intranasal Safety Study of Candidate Pandemic Influenza Vaccine (H2N3 Swine MO 06/AA *ca*) in Ferrets – 35 Days	Ferret/Intranasal	H2N3 Swine MO 06/AA *ca*	No
077-08-001	Non-GLP Repeat Dose Intranasal Safety Study of Candidate Pandemic Influenza Vaccine H7N7 NL 03/AA *ca* in Ferrets – 28 Days (MEDI-550)	Ferret/Intranasal	H7N7 NL 03/AA	No
SVT08-18	Repeat Intranasal Dose Toxicology Study of MEDI3250 (CAIV-Q) Influenza Vaccine in Ferrets	Ferret/Intranasal	Q/LAIV	Yes
SVT01-18	Intranasal Toxicity of CAIV-T Liquid in Ferrets	Ferret/Intranasal	Refrigerated FluMist	Yes
20001854	Reproductive and Developmental Toxicology Study of MEDI3250 in Rats	Rat/Intranasal	Q/LAIV	Yes
3113-001	Intranasal Instillation Reproductive Toxicity Study of FluMist (Influenza Virus Vaccine™ Live, Intranasal) in Rats	Rat/Intranasal	Frozen FluMist	Yes
SVT01-19	Intranasal Inoculation Developmental Toxicity Study of CAIV-T Vaccine in Ferrets	Ferret/Intranasal	Refrigerated FluMist	Yes
80102730	Primary Eye Irritation Study with Cold Adapted Influenza Vaccine (Trivalent Blend/Filled Trivalent Vaccine) in Rabbits	Rabbit/Intraocular	Frozen FluMist	Yes
SVT02-10	A Primary Eye Irritation Study In New Zealand White Rabbits with CAIV-T Vaccine	Rabbit/Intraocular	Refrigerated FluMist	Yes

Single dose toxicity and Repeat dose toxicity

Formal single-dose toxicity studies with H5N1 or other strains of pandemic potential were evaluated as part of repeat-dose toxicity studies in ferrets (Studies SVT08-10, 077-09-001, SVT06-11, 077-08-001, SVT08-18, SVT01-18).

One pivotal GLP-compliant repeat-dose toxicity study was conducted to assess local and systemic toxicity of the proposed 2-dose regime of P/LAIV H5N1 VN04 in ferrets over a 35 day period. A higher dosage (10^9 $TCID_{50}$) was also tested. In this study, the vaccine was administered in 0.5 ml, i.e. 0.25 ml/nare. Overall, the candidate vaccine was safe and well tolerated. There were no vaccine-related toxicities in any of the parameters measured, with the exception of a dose-dependent bronchointerstitial inflammation in the lungs of vaccinated animals at Day 3. The severity of inflammation decreased by Day 35, which is suggestive of an ongoing resolving process. The cause for this observation might be an inappropriate vaccine dose volume (0.5 mL) for the ferret species, which led to vaccine virus deposition into the lungs, since subsequent investigation revealed that administration of the same vaccine titre in a smaller volume (0.025 mL/nare) elicited only a minimal inflammatory response in a fewer number of ferrets.

An additional vaccine-related histopathology finding was an acute inflammation in nasal turbinates at Day 3, resolving by Day 35, as well as a prominent lymphocytic hyperplasia in cervical lymph nodes noted at Day 35 but not Day 3. These changes should be considered as a reflection of immune stimulation following administration of a live virus, and were not considered unexpected.

A number of non-clinical safety studies conducted with other P/LAIV candidates and seasonal LAIVs, including Q/LAIV and Fluenz, were submitted as supportive evidence. A similar safe and well-tolerated profile was consistently seen in these supportive studies. Histopathology findings in some studies (related to H7N7, H2N2 and H6N1) included inflammation of the basal turbinate which initially was considered as vaccine-related. However, a retrospective detailed microscopical evaluation in control animals suggested that nasal turbinate enlargement is a normal developmental feature of the growing ferrets, irrespective of inflammation in nasal tissues or inflammatory exudate in the nasal cavity.

Genotoxicity and carcinogenicity

Studies evaluating mutagenic or carcinogenic potential of P/LAIV were not conducted as they are not normally required for viral vaccines in line with the available guidelines (EMA guideline CPMP/SWP/465/95 and EMA draft Non-clinical and Clinical Module of the Influenza Guideline; WHO Guidelines on nonclinical evaluation of vaccines (WHO Technical Report Series, No. 927, 2005). That is because vaccine antigens, in general, are not considered to have genotoxicity potential or carcinogenic effect based on their chemical structure, mechanism of action and lack of repeated chronic administration. Specifically for LAIVs, wild-type or *att* influenza viruses are not considered to be mutagenic or carcinogenic based on observation from naturally occurring cases of influenza and on previous experience with LAIVs. No mutagenic or carcinogenic effects of LAIVs have been observed in any of the nonclinical or clinical studies conducted to date with LAIVs.

Reproduction Toxicity

Dedicated developmental toxicity studies with P/LAIV H5N1 VN04 or other pandemic vaccine candidates were not performed. This is consistent with previous discussions with the Applicant and is considered acceptable in light of the existing evidence. Existing data from reproductive and developmental toxicology studies generated with Fluenz tetra and Fluenz in rats and ferrets was submitted as supportive evidence and did not reveal maternal toxicities or teratogenic effects. The two influenza vaccines did not affect reproduction and development in rats and ferrets. Further, the F0 generation females and the F1 generation offspring were unaffected by Fluenz Tetra and Fluenz. These studies were assessed and are considered relevant for P/LAIV.

As noted in the Note for Guidance on Preclinical Pharmacological and Toxicological testing of Vaccines (CPMP/SWP/465/95) testing in juvenile animals is not required for vaccines. Repeated dose toxicity studies were performed in prepubertal animals. Appropriate clinical data are available.

Toxicokinetic data

Not applicable

Local Tolerance

No dedicated local tolerance studies have been conducted with P/LAIV. However the site of vaccination with *ca* Vietnam/1203/2004 (H5N1) was evaluated in ferrets in repeat-dose toxicity study (Study SVT06-11). Additionally, evaluation of the site of vaccination was performed in studies with other P/LAIVs and Q/LAIV as part of repeat-dose toxicity studies. There were no adverse vaccine-related findings.

In addition, evaluation of local tolerance at the administration site was included in the Fluenz repeated dose toxicity study with the evaluation of the nasal mucosa with similar results. Furthermore the

potential for ocular toxicity resulting from the inadvertent instillation into the eye was evaluated in two Fluenz ocular toxicity studies performed in rabbits. A standard Draize test was performed in two separate studies using the frozen and refrigerated formulations of Fluenz. Neither study elicited results consistent with ocular toxicity.

Other toxicity studies

No other toxicity studies were performed.

2.3.5. Ecotoxicity / environmental risk assessment

The Environmental Risk Assessment (ERA) submitted for P/LAIV is built on the ERA that was submitted in the MAA for Fluenz Tetra (Q/LAIV), which was in itself based on the ERA submitted in the MAA for Fluenz (T/LAIV). The majority of the studies included in the ERA were performed with Fluenz. All these vaccine formulations are prepared by reverse genetics techniques and are therefore considered GMOs.

A series of environmental safety studies with Fluenz designed to evaluate the tropism of the vaccine for nonhuman species were conducted in 21 animal species.

Study Number	Study Title	Species/ROA	Test Article	GLP
51123, 51124, 51125, 51126, 51127, 51158, 51159, 51173, 51192, 51193, 51194, 51195, 51196, 51197, 51198, 51199, 51200, 51201, 51202, 51203, 51204	Environmental safety studies of Cold-adapted Influenza Vaccine, trivalent (CAIV-T) in ferrets, hamsters, guinea pigs, mice, rabbits, dogs, cats, horses, sheep, goats, cows, pigs, canaries, quail, pigeons, pheasants, parakeets, geese, turkeys, ducks, chickens	Ferret, hamster, guinea pig, mouse, rabbit, dog, cat, horse, sheep, goat, cow, pig, canary, quail, pigeon, pheasant, parakeet, goose, turkey, duck, chicken Intranasal or oral	Refrigerated FluMist	No

Replication of vaccine viruses was measured in respiratory tissues. The vaccine viruses did not replicate in any bird species, which is consistent with the *ts* phenotype of vaccine viruses and the relatively high body temperature of birds. In mammals, replication of the vaccine viruses was only noted in hamsters, guinea pigs, and ferrets. These species have been shown previously to be experimentally infected with human influenza virus. Collectively, the results of these studies demonstrated that the vaccine viruses do not have novel tropism for nonhuman species. Evaluation of a number of experimentally created reassortants (genetic reassortment between wt and vaccine strains) in a ferret model indicated that such reassortment is not likely to create viruses with new properties compared with either progenitor and is more likely that the reassortant would also be attenuated. The overall risk posed by Fluenz or Fluenz tetra to human health and the environment was considered low or negligible. These vaccines do not replicate freely in the environment and moreover they are specific to humans and a few mammalian species (see above); they do not carry a toxic transgene, do not integrate in the genome and therefore it is very unlikely that they could transfer genes to any other species.

Based on the similarities between the authorised seasonal LAIV (Fluenz and Fluenz Tetra) and P/LAIV, and the fact that all three products are manufactured, transported, and administered under the same controlled conditions, and the genetic stability is comparable (see the quality section), no increased environmental risk is anticipated for P/LAIV compared to Fluenz and Fluenz Tetra.

Human-adapted seasonal or pandemic influenza virus strains are characterised by a marked restriction to the human host, i.e. effective transmission to other species is unlikely or limited due to lack of receptor specificity for the HA ligand in hosts other than humans.

The same host restriction applies to LAIVs derived from either seasonal or pandemic human wild-type influenza strains. Thus, health threats for humans exposed to these vaccines within the approved indication (age and epidemiological context) are predictable and minimal.

Risks for non-human species through the massive use of LAIVs are also negligible, provided that these vaccines are correctly used within a seasonal or pandemic context as per respective indication.

LAIVs that contain HA/NA combinations not occurring in circulating human influenza viruses must not be used outside of a well-controlled setting.

LAIVs that contain pandemic HA/NA surface antigens which will be used during a duly recognised pandemic have the same low risk factors as seasonal LAIVs.

Based on all the above elements and considerations, the pandemic LAIV construct evaluated in this dossier is acceptable from an environmental safety point of view.

At the time of the assessment of the MAA for Fluenz Tetra, the initial environmental risk assessment (ERA) was considered to remain relevant for future seasonal strains of Fluenz tetra, since the simple change in strain to be included in the vaccine is not expected to alter the ERA profile of the vaccine. This rational and conclusion is considered relevant also for P/LAIV, hence submission of an ERA at each strain update procedure following pandemic declaration is not required for the same reason expressed above for Fluenz Tetra, with all reserves of new scientific information publication on the ERA for this kind of vaccine.

2.3.6. Discussion on non-clinical aspects

The proof-of-protection for P/LAIV H5N1 VN04 candidate vaccine has been adequately demonstrated in different animal models, including ferrets and African Green Monkeys. The consistent demonstration of protection in several P/LAIV studies and supportive studies with other strains provides reasonable reassurance about the overall performance of this LAIV-based pandemic vaccine.

The immunogenicity of P/LAIV H5N1 VN04 was explored more broadly in AGMs than in ferrets, with a correlation observed in AGMs between serum antibody levels (especially neutralising antibodies) and level of protection. In the ferret model it was noted that in the absence of detectable HAI titre induced by H1N1 vaccine, the vaccine provided no protection, and lower HAI titres induced by the P/LAIV H5N1 vaccine provided full protection.

The general toxicity of P/LAIV H5N1 VN04 and other candidates was studied in ferrets. These studies consistently showed that the safety profile of P/LAIV candidates was similar and did not differ from that of the seasonal LAIVs (Fluenz and Fluenz Tetra).

The neurovirulence potential of P/LAIV H5N1 VN04 candidate appears unlikely, based on the existing non-clinical data available and the completed phase I clinical trials.

The formulation of a pandemic monovalent vaccine does not change the risk to the environment with respect to the seasonal Fluenz or Fluenz Tetra formulations, which were already evaluated. The manufacturing procedures are identical for the 3 vaccine constructs and the genetic stability is comparable.

In summary, it is considered that the non-clinical pharmacological and toxicological testing is extensive and complete.

2.3.7. Conclusion on the non-clinical aspects

The pharmacological program for *ca* Vietnam/1203/2004 P/LAIV H5N1 is considered adequate in terms of study conduct and quality of results, and the studies have provided proof-of-concept for protective efficacy of this candidate vaccine. The non-clinical toxicology data did not reveal any specific safety issues. The non-clinical investigation is overall satisfactory and therefore the application is approvable from a non-clinical perspective.

2.4. Clinical aspects

2.4.1. Introduction

The purpose of this Marketing Authorization Application (MAA) is to seek approval of a Pandemic Live Attenuated Influenza Vaccine (P/LAIV manufactured by MedImmune) for the prophylaxis of influenza in children and adolescents from 12 months to less than 18 years of age. P/LAIV is an intranasally administered vaccine that contains a live reassortant A/H5N1 strain of influenza virus. 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.

P/LAIV contains the same type of components as currently included in the EU approved vaccine for seasonal influenza, Fluenz Tetra, and differs only in the fact that it is a monovalent formulation containing a single type A influenza virus strain with pandemic potential. Otherwise the two vaccines are produced by the same manufacturing process, use the same attenuated master donor virus and excipients, are blended with the same potency specification: $10^7.0 \pm 0.5$ fluorescent focus units (FFU) per strain, and are administered intranasally using the same Becton Dickinson (BD) Accuspray™ device.

Immunogenicity or safety paediatric studies of pandemic candidate vaccines may not be conducted in an interpandemic period due to feasibility and ethical reasons. Paediatric studies foreseen in the paediatric investigational plan for P/LAIV have been deferred until the time of the next pandemic. Therefore, efficacy and safety of the P/LAIV H5N1 VN04 preparedness vaccine is to be predicted based on immunogenicity and safety data generated in naïve adults, with the support of additional non-clinical and clinical data generated with other P/LAIV candidates, and efficacy, safety and effectiveness data of already authorised seasonal and H1N1 pandemic LAIV vaccines. The seasonal LAIVs are Fluenz, the trivalent seasonal formulation (T/LAIV) which was withdrawn in 2013, and the new quadrivalent formulation Fluenz Tetra (Q/LAIV), currently authorised. The H1N1 pandemic LAIV was authorised in the US and used in the 2009 pandemic.

Indeed, also given the limited understanding of how immunity works for LAIVs, the data bridging for this dossier is indeed supported by the large body of safety and efficacy data generated with the seasonal influenza vaccines and on the clinical experience (effectiveness data) generated with the seasonal formulations and with the H1N1-based P/LAIV (see also section 2.5).

GCP

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

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

- Tabular overview of clinical studies

Study Identifier	Population enrolled	Vaccine strain & dose level (subtype)	Study design	Study objectives
Pivotal P/LAIV H5N1 studies (0.5 mL IN delivered by Accuspray device as a divided dose into two nostrils)				
CIR 217 (CRADA)	Adults (n=42)* 21-49 years	*ca* A/Vietnam/1203/2004, $10^{6.7}$ or $10^{7.5}$ TCID50	phase I, open-label, inpatient study	safety, infectivity, immunogenicity
CIR 239 (CRADA)	Adults (n=17) 18-49 years	*ca* A/HK/213/2003, $10^{7.5}$ TCID50	phase I, open-label, inpatient study	safety, infectivity, immunogenicity
CIR 277 (NIH) [prime-boost]	Adults (n=69) 22-54 years	H5N1 pIIV; 45 µg IM	phase I open-label, outpatient study	Immunogenicity,safety of a H5N1 pIIV in P/LAIV H5N1-primed subjects
Supportive P/LAIV studies (0.5 mL IN delivered by Accuspray device as a divided dose into two nostrils)				
CIR 247 (CRADA)	Adults (n=21) 18-39 years	*ca* A/Ann Arbor/6/60, 10^{7} TCID50 (H2N2)	phase I, open-label, inpatient study,	safety, infectivity, immunogenicity
URMC 10-004 (CRADA)	Adults (n=19) 18-39 years	*ca* A/swine/MO/4296424/2006 $10^{7.5}$ TCID50 (H2N3)	phase I, open-label, inpatient study	safety, infectivity, immunogenicity
CIR 251 (CRADA)	Adults (n=22) 18-49 years	*ca* A/Teal/HK/W312/1997, $10^{7.0}$ TCID50 (H6N1)	phase I open-label, inpatient study	safety, infectivity, immunogenicity
CIR 241 (CRADA)	Adults (n=21) 18-49 years	*ca* A/chicken/British Columbia/CN-6/2004, $10^{7.5}$ TCID50 (H7N3)	phase I open-label, inpatient study	safety, infectivity, immunogenicity
URMC 10-002 (CRADA)	Adults (n=20) 18-49 years	*ca* A/chicken/British Columbia/CN-6/2004, $10^{7.5}$ TCID50 (H7N3)	phase I, open-label, inpatient study	safety, infectivity, immunogenicity
URMC 10-003 (CRADA)	Adults (n=25) 18-49 years	*ca* A/Netherlands/219/03, $10^{7.5}$ TCID50 (H7N7)	phase I open-label, inpatient study	safety, infectivity, immunogenicity
CIR 293 (NIH) [prime-boost]	Adults (n=99) 18-49 years	*ca* A/Anhui/1/2013, $10^{7.0}$ FFU (H7N9) H7N9 pIIV, 30 µg IM	phase I, open-label, inpatient study	safety, infectivity, immunogenicity of P/LAIV H7N9 followed by a H7N9 pIIV
CIR 211 (CRADA)	Adults (n=50) born after 1968	*ca* A/chicken/HK/G9/97, $10^{7.0}$ TCID50 (H9N2)	phase I, open-label, inpatient study	safety, infectivity, immunogenicity
URMC 11-001 (NIH) [prime boost]	Adults (n=39) 18-50 years	H7N7 pIIV; 45 µg IM	phase I open-label, outpatient study	Immunogenicity,safety of a H7N7 pIIV in P/LAIV H7N7- or H7N3-primed subjects
URMC 13-001 (NIH) [prime-boost]	Adults (n=32) 18-49 years	*ca* A/Anhui/1/2013, $10^{7.0}$ FFU (H7N9) H7N9 pIIV, 30 µg IM	phase I, open-label, inpatient study	safety, infectivity, immunogenicity of P/LAIV H7N9 followed by a H7N9 pIIV

CIR: Center for Immunization Research; URMC: University of Rochester Medical Center; pIIV: pandemic inactivated influenza vaccine; *: number of subjects enrolled or randomised

Only simplified study reports including study protocols, published literatures and study narratives were submitted for the 3 pivotal P/LAIV H5N1 studies. For other supportive P/LAIV studies, only study

narratives without published literatures were available, as agreed upfront with the Applicant. The CIR 293 study is still on-going, and interim immunogenicity data were submitted during the procedure.

2.4.2. Pharmacokinetics

P/LAIV is a monovalent live attenuated virus vaccine composed of a H5N1 reassortant influenza virus strain that is expected to replicate locally in the mucosa of the upper respiratory tract and to induce both localized and systemic immune responses. Since classical pharmacokinetic studies do not pertain to this type of product, clinical pharmacology studies have included assessment of vaccine-induced immune responses and characterization of the in vivo deposition and distribution of intranasally administered vaccine vehicle.

Study PPL-1014

The Applicant provided data from one biodistribution study (Study PPL-1014) of two formulations and volumes of FluMist-like vaccine applied by Becton Dickinson Accuspray to demonstrate tissue deposition and clearance.

The two formulations were:

- FluMist placebo: 2* 0.25 mL of frozen vehicle formulation with sucrose-phosphate-glutamate (SPG) buffer
- CAIV placebo: 2* 0.1 mL of refrigerated vehicle formulation with SPG buffer, arginine and partially hydrolyzed porcine gelatin

Vehicle formulations were mixed with the radiolabelled marker 99mTechnetium diethylenetriaminepentaacetic acid (99mTc-DTPA).

The regions of interest regarding deposition and clearance were:

- Nasal cavity and adjacent tissue
- Brain
- Lower respiratory tract

In vivo distribution was determined using standard 2-dimensional gamma scintigraphy nuclear imaging as commonly used for ventilation scans.

The study was a randomized, open-label, 2-way crossover study in 21 adults.

The primary objective was assessment and comparison of the deposition patterns in the nasal cavity; the secondary objective was the quantification of the clearance over a 4 hour period after application. A minimal wash-out time of 44h was allowed before the cross-over. MRI scans were done before the application to provide clear anatomical information before the scintigraphy.

For distribution evaluations, scintigraphic data were presented as percentage of the delivered dose. The delivered dose was defined as the formulation that had left the device (and any attachments) and been deposited in the subjects (i.e. it does not include any residual material remaining in the sprayer after administration).

Results

In summary, the majority of the dose of a radiolabelled vaccine vehicle delivered by the same device used to deliver P/LAIV or FluMist was deposited in the nasal cavity with little or no measurable

deposition in the lower airways and lungs, which is consistent with the relatively large droplet size of the spray material.

2.4.3. Pharmacodynamics

The pharmacodynamics of a vaccine relate to its interaction with the immune system.

Mechanism of action

The influenza virus strain in Pandemic influenza vaccine H5N1 MedImmune is (a) cold adapted (*ca*); (b) temperature sensitive (*ts*); and (c) attenuated (*att*). The live attenuated virus included in the vaccine must infect and replicate in cells lining the nasopharynx of the vaccine recipient in order to induce protective immunity.

Natural immunity to wild-type influenza results primarily from both serum (primarily IgG) and mucosal (primarily secretory IgA) antibodies, produced in response to virus exposure. Serum antibodies are primarily responsible for lower respiratory tract protection and are the most commonly measured correlate of protection from illness. Local mucosal antibodies are critical for protection of the upper respiratory tract and may be more important to overall protection against infection. In addition to humoral immunity, cytotoxic T-cell responses play a significant role in recovery from illness and viral clearance, and innate immune responses, such as the production of interferon, also contribute to protection from influenza.

P/LAIV is designed to induce an immune response that resembles the response generated by wild-type influenza infection, without causing influenza disease. In contrast, inactivated influenza virus vaccines (IV) primarily stimulate a serum antibody response to specific viral surface antigens contained in the vaccine. The greatest response is seen in already primed individuals who have had previous immunologic exposure to wild-type influenza virus.

Immunological correlates of protection have not been established for LAIVs. The most extensive immunogenicity data relate to serum haemagglutination inhibition (HAI) antibody responses. These responses seem to vary significantly depending on age and prior experience with influenza, with more robust responses typically observed in very young, seronaïve, unprimed children. Although the presence of a serum antibody response has been shown to be predictive for protection, the absence of an antibody response following LAIV like FluMist/Fluenz vaccination does not reflect the absence of protection, as clinical efficacy studies have shown protection in the absence of significant antibody responses (Belshe, March 2000). It is likely that immune mechanisms other than influenza-specific serum antibodies contribute to protection conferred by LAIVs (e.g. mucosal antibodies, cell-mediated immunity). Additionally, the protective immune responses may be different in young, unprimed, seronegative children compared to older children and adults.

P/LAIV is produced in the same way as Fluenz Tetra and Fluenz/Flumist, but with a single virus strain. Because of the similarity with these other LAIVs, the current MAA is to a large degree based on efficacy data from Fluenz/Flumist in addition to data obtained from studies using H1N1v LAIV, as well as data from candidate vaccines with a range of different Influenza A subtypes with pandemic potential. A general problem with using immunogenicity (HAI, NAI, neutralisation assays) for bridging of data for LAIVs is the lack of correlation with protection against disease.

2.4.4. Discussion on clinical pharmacology

Classical pharmacokinetic and pharmacodynamic studies do not pertain to P/LAIV, because the mechanism of action involves local and systemic immune responses induced by vaccine virus

replication in the mucosa of the upper respiratory tract. The immunogenicity studies that form the basis for this application are included in the Clinical Efficacy section together with the data on clinical efficacy from the other LAIVs. Therefore also the methodology used to evaluate immunogenicity of P/LAIV is discussed in the Clinical efficacy section.

A biodistribution study (Study PPL-1014) of intranasally administered radiolabelled vaccine buffer was submitted. In brief, the initial deposition and clearance of frozen and refrigerated vehicle (i.e., excipient only) formulations of FluMist that were mixed with a radiolabelled 99mtechnetium diethylenetriaminepentaacetic acid (99mTc-DTPA) marker were evaluated by gamma scintigraphy nuclear imaging in a randomized, open-label, 2-way crossover study in 21 adults. In summary, the majority of the dose of a radiolabelled vaccine vehicle delivered by the same device used to deliver P/LAIV or FluMist was deposited in the nasal cavity with little or no measurable deposition in the lower airways and lungs, which is consistent with the relatively large droplet size of the spray material.

2.4.5. Conclusions on clinical pharmacology

A tissue deposition and clearance study has been performed with FluMist/Fluenz, but the results are equally relevant for the deposition and clearance of P/LAIV in the nasal cavity. The majority of the refrigerated dose of a radiolabelled vaccine vehicle was deposited in the nasal cavity, with little or no measurable deposition in the lower airways and lungs, consistent with the relatively large droplet size of the spray material. This pattern of deposition is acceptable because is in line with the intended mechanism of action of the vaccine, i.e. replication restricted to the upper respiratory tract, and the lungs are not reached by the vaccine virus.

No other pharmacology studies have been conducted with P/LAIV; the immunogenicity studies are considered in the following section. This is acceptable for vaccines and more specifically is in line with what has been done for other similar vaccines (Flumist/Fluenz and Fluenz Tetra).

2.5. Clinical efficacy

Data demonstrating formal clinical efficacy of P/LAIV could not be generated since efficacy of a pandemic vaccine cannot be tested in the absence of a circulating pandemic virus. Instead, the expected efficacy of P/LAIV in the claimed indication against pandemic influenza is inferred based on the following data:

1. Immunogenicity data gathered from adult clinical studies performed with candidate P/LAIVs;
2. Immunogenicity data gathered from adult clinical studies in which an inactivated pandemic vaccine was administered to unmask the long-lasting immunity induced by prior receipt of P/LAIV;
3. Effectiveness data in children gathered with the H1N1pdm P/LAIV during the 2009 Pandemic;
4. Clinical efficacy data obtained with Fluenz in immunologically naïve young children.

Concerning immunogenicity studies in adults, in the context of pandemic preparedness activities, the Applicant has participated in a Collaborative Research and Development agreement (CRADA) with the US National Institutes of Health (NIH) since 2005. Under this agreement the candidate vaccines were manufactured by the Applicant and assessed in immunogenicity clinical trials for influenza subtypes by NIH. Subtypes with a potential to cause pandemic, including H5, H2, H6, H7, and H9, were investigated and subsequently submitted as pivotal evidence for the current application.

Therefore the pandemic clinical development program of P/LAIV is based on 3 pivotal adult studies with H5N1 strains (CIR217, CIR239 and CIR 277) and 10 supportive adult studies conducted with other potential pandemic strains (H2N2, H2N3, H6N1, H7N3 (2 studies), H7N7 (2 studies), H7N9 (2 studies), H9N2).

Given i) the known lack for LAIVs of a clear correlation between immunogenicity and protection, and ii) the restriction in replication and immune responses of the pandemic vaccine viruses, which may be attributable to the fact that these viruses are derived from pandemic strains not optimally adapted for human transmission, it was considered that a subsequent administration of an inactivated antigen could reveal whether the primary series of LAIV had induced robust and long-lasting B-cell memory response. Such memory B-cell response would allow for a rapid increase in high quality antibody titres upon re-challenge with an inactivated pandemic antigen even for those vaccines for which immune responses were difficult to detect at all in the original studies (i.e. H5N1, H7N7 and H7N9). These strains were therefore tested in prime-boost studies, as further clarified below.

Concerning the pivotal studies, the A/Vietnam/1203/2004 (H5N1) isolate was used in study CIR 217, and the A/Hong Kong/213/2003 (H5N1) isolate in study CIR 239. To prove the ability of the H5N1 P/LAIV to prime naïve subjects, subjects that received P/LAIV in clinical trials CIR 217 and CIR 239 were subsequently enrolled in Study CIR 277, where they were vaccinated with an inactivated non-adjuvanted H5N1 vaccine. The immune responses to the inactivated vaccine provide useful information on the ability of P/LAIV to prime various age groups against a poorly immunogenic strain to which most, if not all, are naive. This study design was agreed in preliminary discussions with the Applicant as it is considered useful as an indirect proof of the potential for protection of a pandemic LAIV in the absence of efficacy data in the interpandemic period. It should not be considered as indicative for the definition of the LAIV posology in pandemic settings.

Concerning the supportive 10 additional adult studies, three of these followed the prime-boost concept mentioned above (URMC11-001, URMC13-001 and CIR293), i.e. to evaluate the immunogenicity response following the administration of an inactivated pandemic vaccine in subjects previously vaccinated with a P/LAIV candidate vaccine (only H7N7 and H7N9 strains). The study CIR293 is still ongoing.

In addition, the P/LAIV body of evidence benefits from the effectiveness data gathered from two safety and immunogenicity placebo-controlled trials with the 2009 pandemic LAIV (H1N1 A/California/7/2009), which were conducted in 2009 with adults and children and were submitted with this application as supportive. In addition effectiveness data gathered during the last pandemic with the 2009 pandemic LAIV was considered supportive of this application.

Finally, the many efficacy and safety studies conducted in children with the seasonal T/LAIV are also considered supportive of the P/LAIV application because all these vaccines (including the P/LAIV 2009) are manufactured using the same process, administered through the same route, and studied primarily in naïve individuals.

Pre-authorisation immunogenicity paediatric studies of pandemic candidate vaccines are not required to be conducted in an interpandemic period due to feasibility and ethical reasons.

Summary of analytical methods used in the immunogenicity studies

A variety of assays were used in the CRADA studies in adults to assess the immunogenicity of the candidate vaccines. All studies evaluated serum haemagglutination-inhibiting (HAI) antibodies and microneutralisation (MN) antibody responses. In most studies antibody responses were also measured by enzyme-linked immunosorbent assay (ELISA) to the homologous HA, to measure serum IgG and IgA, and nasal wash IgA and by detection of antibody secreting cells (ASCs) to vaccine virus. Some studies also looked at markers of B-cell activation (cluster of differentiation [CD]27+CD38+). Antibody

responses were measured prior to vaccination and after the first and second doses of vaccine (for studies evaluating a 2-dose regimen). Serum was obtained for HAI, MN, IgG and IgA approximately 1 month after each dose while ASCs were evaluated approximately 7 days after dosing.

All the immunoassays were performed by the laboratories of study sites or contracted research organisations. No centralised laboratory was employed for the P/LAIV study programme. Analysis of samples from clinical studies conducted under the CRADA with the NIH were performed at two academic clinical study sites that have extensive experience in conducting influenza studies: 1) The Centre for Immunization Research (CIR) at the Johns Hopkins Bloomberg School for Public Health, in Baltimore, and 2) the University of Rochester Medical Centre (URMC), Rochester, NY.

Assessment of Viral Shedding

For studies performed at the CIR at the Johns Hopkins University, nasal washes were obtained prior to vaccination and then daily from the day of vaccination until the day of discharge. Daily throat swabs were also obtained in studies of the low dose ($10^{6.7}$ $TCID_{50}$) of the *ca* A/Vietnam/1203/2004 (H5N1) vaccine. Specimens were tested for the presence of vaccine virus by quantitative viral culture on MDCK cells and using a rRT-PCR assay that amplified a portion of the influenza type A M2 gene, as previously described (Karron et al, 2009). The sensitivity of the rRT-PCR assay for vaccine virus was approximately $10^{0.5}$ $TCID_{50}$/mL of nasal wash. For studies performed at URMC, nasal swab specimens were tested for vaccine viruses by quantitative viral culture in MDCK cells at 33°C and by quantitative reverse transcription polymerase chain reaction (qRT-PCR) amplification (Shchrebik et al, 2014). The limit of viral detection was $10^{0.6}$ $TCID_{50}$/mL for virus culture and $10^{0.4}$ $TCID_{50}$/mL for qRT-PCR.

Serum Hemagglutination Inhibition Assay (HAI)

Sera were tested for HAI antibodies to H5N1 virus using horse red blood cells (RBC) for the *ca* A/Vietnam/1203/2004 (H5N1) (Study CIR 217) and turkey RBC for the *ca* A/Hong Kong/213/2003 (H5N1) (Study CIR 239), as previously described (Stephenson et al, 2004; Clements et al, 1983). For Study CIR 277, in which subjects received H5N1 P/LAIV followed by inactivated H5N1 vaccine, the HAI assay was performed at the Southern Research Institute (Birmingham, AL), using wt influenza *ca* A/Vietnam/1203/2004 (H5N1) in an enhanced biosafety level 3 containment laboratory. HAI assays were performed according to standard procedures (Stephenson et al, 2004), using horse erythrocytes. Use of the wild-type homologous and/or heterologous pandemic influenza viruses was only noted in two prime-boost studies (CIR277, URMC11-001).

A serum HAI or MN response (seroconversion) was defined as ≥4-fold increase in titres from the baseline for all of the P/LAIV studies. The same criterion was also used for a serum IgG or IgA or a nasal IgA response for all studies, but URMC 10-002 and URMC 10-003 which used ≥2-fold rise for serum IgG and/or nasal IgA.

Microneutralisation Assay

For the *ca* A/Vietnam/1203/2004 (H5N1) and the *ca* A/Hong Kong/213/2003 (H5N1) studies (CIR 217 and CIR 239, respectively), sera were tested for neutralizing antibodies using a modified version of a previously described microneutralisation (MN) assay, in which titres of neutralizing antibody to vaccine virus were assessed (Karron et al, 2009). For Study CIR 277, in which subjects received H5N1 P/LAIV followed by inactivated vaccine, the MN assay was performed at Southern Research Institute (Birmingham, AL), using wt A/Vietnam/1203/2004 (H5N1) in an enhanced biosafety level 3 containment laboratory. MN assays were performed according to previously described methods (Rowe et al, 1999; Walls et al, 1986). Neutralizing antibody activity against different clades of influenza A/H5N1 was analysed by MN based on the methods of the pandemic influenza reference laboratory of the CDC (Rowe et al, 1999). Reverse genetics (RG)-derived influenza A/H5N1 representing different clades of the highly pathogenic avian influenza H5N1 A/ goose/Guangdong/1/96 lineage, engineered to

delete the virulence motif, were obtained from St. Jude's Children's Research Hospital (SJCRH; Memphis, TN), the CDC, and the National Institute for Biological Standards and Control (Potters Bar, United Kingdom): A/ Vietnam/1203/2004 (SJCRH, clade 1), A/Indonesia/5/2005 (PR8-IBCDC-RG2; clade 2.1.3.2), A/turkey/Turkey/1/05 (NIBRG-23; clade 2.2.1), A/Anhui/1/05 (IBCDC-RG5, clade 2.3.4), and A/Egypt/3072/2010 (IBCDC-RG29; clade 2.2.1). The assays were conducted with 3 replicates of each serum sample and were performed at least twice.

Assays validation

Serum haemagglutination-inhibiting (HAI) and microneutralisation (MN) assays were the basic measure of vaccine immunogenicity for all the P/LAIV studies. No validated assays were used in clinical development programme. Extensively qualified HAI and MN assays were used for CIR277, but not for CIR217 and CIR239. It was noted that the same qualified assays are used for the prime-boost H7N9 CIR293 study to evaluate booster response to wild-type antigens.

While qualified assays were not run for pivotal Studies CIR 217 and CIR 239, HAI and MN seroconversion rates in both of these studies were low ranging, from 0% to 10%; as a result, despite the known variability of these assays across testing sites, it is unlikely that spuriously elevated results were obtained or that use of a qualified assay would have yielded different results.

For the supportive URMC 11-001 study (H7N7 prime-boost study) no qualification report was submitted, however the Applicant was able to confirm that the US CDC, who performed the MN serology, used an MN assay that was fully qualified with rigorous quality control measures and performance monitoring.

The CHMP deemed that the overall evidence of assay performance was acceptable for the current application (see the discussion on clinical efficacy for further details), and further recommended for completeness and confirmation that the Applicant submits validation reports of the HAI and MN assays for study CIR 277 as a post-marketing commitment, which was agreed.

Neuraminidase Inhibition Assay

Neuraminidase-specific antibodies were measured by a previously described miniaturized neuraminidase inhibition (NAI) assay (Sandbulte et al, 2009). Measurement of NAI titres requires the use of a virus with an irrelevant HA so that anti-HA antibodies do not interfere with the detection of anti-NA antibodies. Therefore, two H6N2 reassortant viruses were generated, containing the HA from an A/Teal/W312/HK/97 (H6N1) virus and the N2 NA of the A/Uruguay/716/007 (H3N2) virus that was representative of viruses circulating at the time of the clinical trial or the N2 NA of the *ca* A/Ann Arbor/6/60 (H2N2) virus. The A/Uruguay/716/2007 (H3N2) strain was the A/Brisbane/10/2007-like H3N2 component of the seasonal LAIV in 2008–2009. Briefly, the NA activity of each virus was standardized by colorimetric analysis of sialic acid released from the substrate fetuin. NAI activity in the sera was determined by comparing the NA activity of the virus alone with the activity measured following incubation of the virus with serially diluted sera. The dilution of serum that resulted in a 50% reduction in NA activity of the virus without serum was recorded as the NAI titre.

Serum IgG and Nasal IgA Enzyme-linked Immunosorbent Assays

For the *ca* A/Vietnam/1203/2004 (H5N1) and the *ca* A/Hong Kong/213/2003 (H5N1) studies (CIR 217 and CIR 239, respectively), sera were tested for IgG and IgA antibody to the *ca* A/Vietnam/1203/2004 (H5N1) HA by ELISA. Immulon 2 plates were coated with 30 ng/well of recombinant baculovirus-expressed H5 VN2004 HA (Protein Sciences, Meriden, CT), and the ELISA was performed using endpoint titration (Clements et al, 1983). A subset of sera was also tested for IgA antibody to recombinant baculovirus-expressed H1 A/New Caledonia/20/99 and H7 A/Netherlands/219/03 HA (Protein Sciences, Meriden, CT). Nasal wash specimens were concentrated and were tested using the

same antigen to measure vaccine-specific IgA, expressed as a percent of total IgA (Clements et al, 1983).

Antibody-secreting Cells

In some studies, blood circulating IgGs and/or IgA antibody-secreting cells (ASCs) specific for vaccine virus were measured 7 days after dosing.

For the *ca* A/Vietnam/1203/2004 (H5N1) and the *ca* A/Hong Kong/213/2003 (H5N1) studies (CIR 217 and CIR 239, respectively), total and influenza vaccine-specific IgG and IgA ASCs were measured using an enzyme-linked immunospot (ELISPOT) assay (Sasaki et al, 2007). Briefly, the assay differed from the published assay in that the wells were coated with one of the following: 1) rH5 A/Vietnam/1203/2004 HA protein diluted to 10 µg/mL in Dulbecco's PBS (D-PBS; Invitrogen); 2) beta-propiolactone treated H5N1/AA *ca* vaccine virus; 3) beta-propiolactone-treated *ca* A/AnnArbor/6/60 (H2N2) virus diluted to $5x10^3$ haemagglutinating units (HAU)/mL; or 4) purified goat antihuman IgA + IgG + IgM (Kirkegaard & Perry Laboratories, Gaithersburg, MD) at a concentration of 5 µg/mL in D-PBS. PBS alone and human CCRF-CEM cells (human T-cell lymphoblast-like cell line; American Type Culture collection [ATCC], Manassas, VA) were used as negative controls; human IM9 cells (human IgG+ lymphoblasts; ATCC) were used as a positive control. Plate images were recorded and counted using ImmunoSpot 4 software (Cellular Technologies Ltd., Shaker Heights, OH). Human IgA ASCs were visualized as red spots and IgG ASCs were visualized as blue spots. The numbers of specific ASCs were expressed as total IgG or IgA ASCs per 10^6 PBMSc.

Assay antigens in cross-reactivity and antibody affinity evaluations

The viruses used for the cross-reactive antibody assays were reverse genetics-derived reassortant viruses on the PR8 backbone representing different clades of the A(H5N1)/goose/Guangdong/1/96 lineage. The viruses were engineered to remove the multibasic cleavage motif from the H5 hemagglutinin (HA) (Talaat et al, 2014).

The viruses used were as follows:

• A/Vietnam/1203/2004 (St. Jude's Children's Research Hospital; clade 1)

• A/Indonesia/5/2005 (PR8-IBCDC-RG2; US CDC; clade 2.1.3.2)

• A/turkey/Turkey/1/05 (National Institute for Biological Standards and Control-23; Potters Bar, United Kingdom; clade 2.2.1)

• A/Anhui/1/05 (IBCDC-RG5; US CDC; clade 2.3.4)

• A/Egypt/3072/2010 (IBCDC-RG29; US CDC; clade 2.2.1)

The assays to measure antibody affinity were performed with recombinant HA1 (rHA1, amino acids 1-300) and recombinant HA2 (rHA2, amino acids 331-480) domains from A/Vietnam/1203/2004 (H5N1).

2.5.1. Dose response studies

No dedicated dose-response studies were conducted for P/LAIV.

Each 0.2 mL dose of P/LAIV contains $10^{7.0 \pm 0.5}$ FFU of a *ca*, *ts*, *att*, 6:2 reassortant influenza strain. The proposed potency specification for P/LAIV is based on potency specifications established for the trivalent vaccine FluMist/Fluenz (see below for Fluenz dose-response studies). Whereas study CIR 217 is not formally a dose-response study, CIR217 study subjects received one or two doses of the $10^{6.7}$ $TCID_{50}$ potency or the $10^{7.5}$ $TCID_{50}$ potency. The results from this study and possible recommendations for dosing are discussed in the efficacy section.

FluMist/Fluenz dose-response studies (immunogenicity studies)

Two studies (D153-P513 and AV002/AV002-2) provide data regarding the dose-dependent immunogenicity of FluMist/Fluenz in paediatric and adult subjects.

Study D153-P513 was a prospective, randomized, double-blind, placebo controlled, multicentre study to compare the safety, immunogenicity, and efficacy of FluMist 10^7, 10^6, and 10^5 in subjects 6 to < 36 months of age. An IFN-γ ELISPOT assay was used to discriminate between levels of immune response elicited at each dosage level in subjects 6 to < 36 months of age. This assay measured the number of PBMCs secreting IFN-γ pre-vaccination and post-vaccination in response to stimulation with monovalent inactivated antigens identical to those in the vaccine. In summary, a dosage level response was observed in cellular immune responses in the periphery following vaccination as measured by IFN-γ responses in vitro to A/H1N1 and A/H3N2 test stimuli. Study D153-P513 also showed that a 2-dose regimen of FluMist at a dosage level of 10^7 FFU per strain was statistically significantly more protective against culture-confirmed influenza illness in subjects 6 to < 36 months of age than a 2-dose regimen at a dosage level of 10^6 FFU or 10^5 FFU per strain.

Study AV002/AV002-2 was a randomized, double-blind, placebo controlled, dose escalation study to evaluate the safety and immunogenicity of frozen FluMist administered by drops or spray in subjects 18 through 71 months of age. The primary objective of this study was to assess the safety of FluMist in subjects 18 through 71 months of age. In addition, this study was designed to identify a dose of FluMist that is both safe and immunogenic. AV002 subjects received a single dose of FluMist or placebo either as an intranasal spray or as nose drops; AV002-2 subjects received FluMist or placebo as spray only. In summary, in subjects 18 to 71 months of age given only a single dose of FluMist, dosage levels of 10^6 or 10^7 $TCID_{50}$ were highly immunogenic for the A/H3N2 and B strains based on rises in serum HAI titre levels. Serological immune responses to A/H1N1 following a single dose of FluMist were limited and increasing the dose of FluMist did not result in substantially increased immunogenicity in this age group.

FluMist/Fluenz - One Dose vs Two Doses (efficacy studies)

Two studies (AV006 Year 1 and D153-P504) provide data regarding the efficacy of 1 dose vs 2 doses of FluMist in paediatric subjects. In subjects 15 to 71 and 6 to 35 months of age, a single dose of FluMist was found to be efficacious against culture-confirmed influenza illness; estimates of efficacy were 88.8% and 57.7% respectively. However, the efficacy associated with a 2-dose primary series was numerically higher, and in most of the comparisons made within the studies, statistically higher than the efficacy of a single dose.

Overall, previous results with Fluenz/FluMist demonstrate the benefit of 2 doses compared to a single dose in younger children, confirming the selection of dosage also for P/LAIV. They also confirm the variability in immunological responses according to age groups and influenza strain. However, a clear limitation regarding dosing is the lack of data on doses above $10^{7.0}$ FFU. While for some influenza strains $10^{7.0}$ FFU or even $10^{6.0}$ FFU may provide optimal protection, it is not clear that this will be the case for other influenza strains. The fact that H1N1 P/LAIV showed effectiveness in children despite low increase in HAI GMT clearly demonstrates the lack of correlation between protection and HAI titres.

2.5.2. Main studies

Title of Studies

Study CIR 217

Study CIR 217 was an open-label, inpatient Phase I study conducted in 2006-2007 in healthy adults 18 to 49 years of age at a single site in the USA. The objectives of the study were to assess the

safety, infectivity, and immunogenicity of a live attenuated, vaccine virus based upon the A/Vietnam/1203/2004 (H5N1) influenza isolate (conducted between April and December of 2006).

Study CIR 239

Study CIR 239 was an open-label, inpatient Phase I study conducted in 2007 in healthy adults 18 to 49 years of age at a single site in the USA. The objectives of the study were to assess the safety, infectivity, and immunogenicity of a live attenuated, vaccine virus based upon the A/Hong Kong/213/2003 (H5N1) influenza isolate (conducted between April and December of 2006). Subjects were H5N1-seronegative (H5 HAI ≤ 1:8) at baseline).

Study CIR 277

A prime-boost Immunogenicity and Safety Study of a 45 Microgram Dose of inactivated, non-adjuvanted H5N1 Vaccine in H5N1 and H7N3 LAIV Recipients and LAIV Naive Individuals. Study CIR 277 was an open-label, outpatient study conducted in 2011-2012 in healthy adults 22 to 54 years of age at a single site in the USA. The objective of the study was to assess whether prior receipt of pandemic live attenuated influenza H5N1 vaccines primed or established long-lasting immunity that could be detected by the administration of an inactivated H5N1 vaccine (conducted in 2011-2012).

One of the goals of the prime-boost studies has been to evaluate the time course over which immunological priming occurs. The first of such studies, the CIR 277 H5N1 study, brought subjects enrolled in the original H5N1 P/LAIV studies back to the clinical site approximately 4 to 5 years later for receipt of the inactivated vaccine. This interval was shortened in subsequent studies; subjects enrolled in the H7N7 study (URMC 11-001) returned to the site 18 to 24 months after their initial P/LAIV vaccinations while subjects in the H7N9 study (URMC 13-001) returned after an interval of 3 months (see section on supportive studies).

Methods

Study Participants

In general, the P/LAIV studies enrolled healthy male and non-pregnant female adults approximately 18 to 49 years of age. This age range was selected as it reflects the indicated age range of the seasonal live attenuated vaccine approved in the USA. The age range for Study CIR 277 was slightly older (22 to 54 years) as subjects previously enrolled in Studies CIR 217 and CIR 239 were brought back to clinical site 4-5 years after completion of the initial studies.

For the pivotal H5N1 studies the main inclusion and exclusion criteria are listed below.

Inclusion criteria:

- General good health, without medical significant illness, physical examination findings illness, or significant laboratory abnormalities;
- Female subjects must agree to use effective birth control methods for the duration of the study.

Exclusion criteria:

- Pregnancy as determined by a positive beta-human chorionic gonadotropin test.
- Currently breast-feeding.
- Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, rheumatologic, autoimmune, or renal disease by history, physical examination, and/or laboratory studies including urine testing.

- History of anaphylaxis or history of life threatening reaction to prior influenza vaccine.
- Current diagnosis of asthma or reactive airway disease (within the past 2 years).

Inclusion and exclusion criteria were chosen adequately for a nasal live attenuated pandemic influenza vaccine.

Treatments

In clinical trial CIR 217 the H5N1 A/Vietnam/1203/2004 *ca* vaccine was evaluated at doses of $10^{6.7}$ $TCID_{50}$ (low dose) and $10^{7.5}$ $TCID_{50}$ (high dose) administered 4 to 8 weeks apart. The table below summarises the number of subjects who received the H5N1 vaccines.

Table 3. Summary of subjects and dosages in study CIR 217

Group		Planned Enrollment	Actual Enrollment	Completed	Discontinued/ Withdrew*	Deaths
		10^{7} $TCID_{50}$ (Actual Dose = $10^{6.7}$ $TCID_{50}$)				
1	Test subjects	16-22	21 (1st dose)	21	0	0
			18* (2nd dose)	17	1§	
		~$10^{7.5}$ $TCID_{50}$				
2	Test subjects	16-22	21 (1st dose)	19 (1st Dose)	0	0
			19* (2nd dose)	19 (2nd Dose)	0	

Three subjects in Group 1 and two subjects in Group 2 were not re-admitted for a second dose due to persistent elevated ALT value, pre-existing wheeze, missed visit, possible contraction of an infectious agent, and sample clotting of platelet. One subject discontinued the study in the follow-up period due to a gunshot wound in the head.

In clinical trial CIR 239 16 to 22 subjects were planned to receive twice $10^{7.5}$ $TCID_{50}$ the H5N1 A/Hong Kong/213/2003 vaccine. The table below provides the enrolled subjects of Study CIR 239.

Table 4. Summary of subjects in study CIR 239

Group		Planned Enrollment	Actual Enrollment	Completed	Discontinued/ Withdrew*	Deaths
		~$10^{7.5}$ $TCID_{50}$ (Actual Dose = $10^{7.6}$ $TCID_{50}$)				
1	Test subjects	16-22	17 (1st Dose)	17 (1st dose)	1**	0
			16 (2nd Dose)	16 (2nd dose)	0	0

One subject withdrew the participation to the trial, because subject was offered immediate, full-time employment.

Nasal washes for viral detection were obtained daily from the day of admission through the day of discharge.

The study CIR 277 enrolled 69 subjects in 5 groups: Group 1 enrolled 11 subjects who had previously received 2 doses of the A/H5N1/Vietnam/1203/2004 P/LAIV in 2006-2007; Group 2 enrolled 10 subjects who had previously received 2 doses of the A/H5N1/Hong Kong/213/2003 P/LAIV in 2007; Group 3 enrolled 8 subjects who had previously received 2 doses of the A/British Columbia/CN-6/2004

P/LAIV in 2010 (as a P/LAIV control group); Groups 4 and 5 each enrolled 20 subjects who had not been previously vaccinated with LAIV and were influenza H5-naive. Subjects in Groups 1 to 4 received a single 45 µg dose of an inactivated A/H5N1/Vietnam/1203/2004 vaccine (P/IIV) while subjects in Group 5 received 2 doses, approximately 28 days apart. The purpose of Study 277 was to evaluate whether the dose of inactivated vaccine could unmask memory B-cell responses that had been induced by P/LAIV in Studies CIR 217 and CIR 239. The following table gives an overview on planned subjects in the different vaccination groups.

Table 5. Immunization schedule of Study CIR 277

Group	Number of Subjects	Previous Vaccine	Number of doses of inactivated H5N1 vaccine
1	10-19	2 doses of $10^{7.5}$ $TCID_{50}$ of H5N1 A/VN/1203/04 x A/AA/6/60 *ca* LAIV (H5N1 VN 04 *ca*)	1
2	10-16	2 doses of $10^{7.5}$ $TCID_{50}$ of H5N1 A/HK/213/03 x A/AA/6/60 *ca* LAIV (H5N1 HK 03 *ca*)	1
3	10-17	2 doses of $10^{7.5}$ $TCID_{50}$ of H7N3 A/ck/BC/CN-6/04 x A/AA/6/60 *ca* LAIV (H7N3 *ca*)	1
4	~20	No previous LAIV of any kind	1
5	~20	No previous LAIV of any kind	2

The below table summarises how many subjects were enrolled and withdrew in the clinical trial CIR 277.

Table 6. Subject Disposition, Study CIR 277

Subject Status	Cohort 1 N (%)	Cohort 2 N (%)	Cohort 3 N (%)	Cohort 4 N (%)	Cohort 5 N (%)	Overall N (%)
	Inactivated A/Vietnam/1203/2004 (H5N1)					
Number of subjects enrolled	11 (100)	10 (100)	8 (100)	20 (100)	20 (100)	69 (100)
Number of subjects who received Dose 1	11 (100)	10 (100)	8 (100)	20 (100)	20 (100)	69 (100)
Number of subjects who received Dose 2	NA	NA	NA	NA	20 (100)	20 (100)
Number of subjects who completed the study	10 (90.9)	10 (100)	8 (100)	19 (95)	19 (95)	66 (95.6)
Number of subjects who withdrew for reasons other than AE	1 (9.1)	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.0)	3 (4.3)

The 5 groups were essentially comparable, with the exception of age. The 69 enrolled subjects were mainly male (61 %) and black (88 %). 3 subjects discontinued due to relocation and two woman became pregnant. Both pregnancies were terminated for reasons unrelated to vaccine.

Overall the dropout rate of subjects between dose 1 and dose 2 in studies CIR 217 and CIR239 was very low for Phase I trials, which requested an inpatient phase of almost 14 days. Also in study CIR 277 the drop-out rate of less than 5% is considered very low.

Objectives

Study CIR 217

To investigate the safety, infectivity and immunogenicity of H5N1 VN 2004/AA *ca* recombinant vaccine by:

- determining the frequency of vaccine-related reactogenicity events and other AEs for each dose
- quantifying the amount of vaccine virus shed by each recipient
- determining the amount of serum and nasal wash antibody induced by the vaccine

Study CIR 239

To determine the safety, infectivity and immunogenicity of the H5N1 HK2003/AA *ca* recombinant vaccine by:

- determining the frequency of vaccine-related reactogenicity events and other AEs for each dose
- quantifying the amount of vaccine virus shed by each recipient
- determining the amount of serum and nasal wash antibody induced by the vaccine

Study CIR 277

To evaluate the reactogenicity and immunogenicity of the inactivated H5N1 vaccine in subject who previously received a live attenuated H5N1 vaccine, as measured by HAI and MN assays. The study also assessed whether prior receipt of pandemic live attenuated influenza H5N1 vaccines in Studies CIR 217 or CIR 239 primed or established long-lasting immunity that could be detected by the administration of an inactivated H5N1 vaccine.

Overall the objectives of all three pivotal studies were chosen adequately for Phase I trials.

Outcomes/endpoints

In P/LAIV studies CIR 217 (*ca* A/Vietnam/1203/2004 [H5N1] vaccine) and CIR 239 (*ca* A/Hong Kong/213/2003 [H5N1] vaccine), antibody responses were measured by assays for serum haemagglutination inhibition (HAI), microneutralisation (MN), immunoglobulin G (IgG), and immunoglobulin A (IgA) and nasal IgA assays. In addition, for several studies including Study CIR 217, antibody secreting cells (ASCs) were also evaluated. For Study CIR 277, in which subjects who were previously enrolled in Studies CIR 217 and CIR 239 and thereby vaccinated with H5N1 P/LAIV received an inactivated H5N1 vaccine, antibody responses were measured by HAI and MN assays. HAI antibodies were assayed using horse or Turkey red blood cells, whereas antibody neutralising activity was titrated on MDCK cells, mostly using a modified method.

Other immunoassays included ELISAs for serum IgG and IgA and nasal wash IgA against recombinant homologous HA, and in few cases, also the vaccine virus, in most of the CRADA/NIH studies. In some studies, blood circulating IgG and/or IgA antibody-secreting cells (ASCs) vaccine virus specific were measured 7 days after dosing. The numbers of specific ASCs were measured using ELISPOT assay and expressed as total IgG or IgA ASCs per 10^6 PBMSc.

A serum HAI or MN response (seroconversion) was defined as ≥4-fold increase in titres from the baseline for all of the P/LAIV studies. The same criterion was also used for a serum IgG or IgA or a nasal IgA response for all studies, but URMC 10-002 and URMC 10-003 which used ≥2-fold rise for serum IgG and/or nasal IgA.

All these immunoassays were performed by the laboratories of study sites or contracted research organisations. No centralised laboratory was employed for the P/LAIV study programme.

Sample size

The sample size of the pivotal studies CIR217, CIR239, CIR277 was not based on statistical considerations and was overall limited.

Randomisation

Studies CIR217 and CIR239 were not randomized. In study CIR277 block randomisation was applied to randomize H5 and LAIV naïve subjects to receive either 1 or 2 doses of the H5N1 vaccine.

Blinding (masking)

The pivotal studies were open label studies.

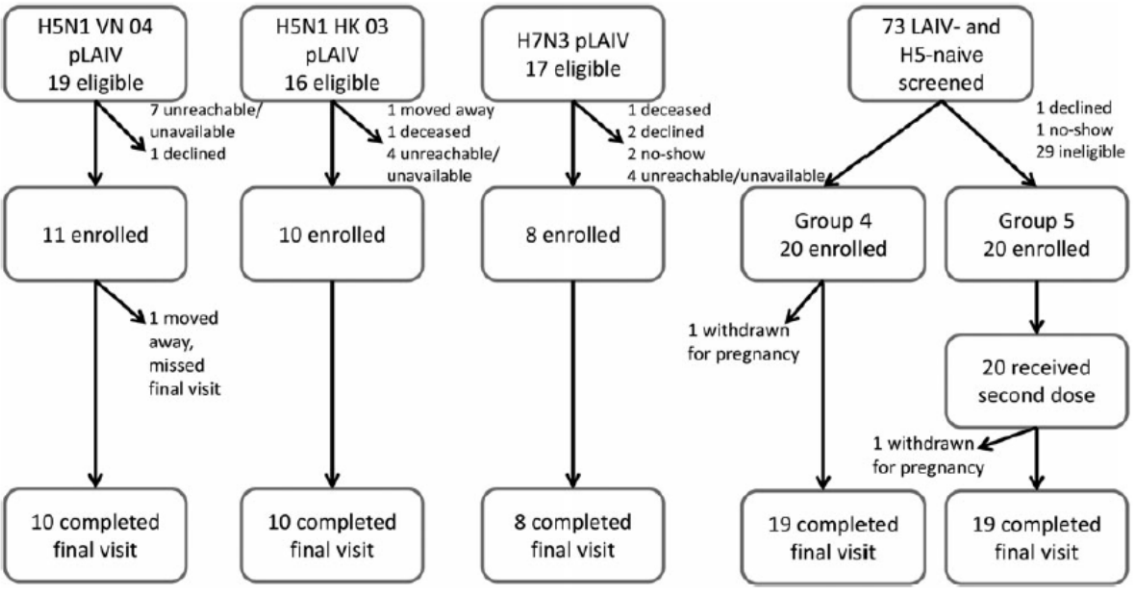
Statistical methods

For the pivotal studies statistical characteristics (continuous data: mean, SD, median, minimum, maximum; categorical data: absolute and relative frequencies) stratified by group and time (where appropriate) were used to describe the observed parameter. The geometric mean values including their 95%-CIs were used to describe immunogenicity parameter. Where appropriate, dichotomic outcomes (i.e. AE's) were compared between groups by Fisher's exact test; continuous variables (e.g. immunogenicity parameter) were compared by means of non-parametric tests. No type I error control was employed.

Results

Participant flow (study CIR277)

The CIR 277 study enrolled 69 subjects in 5 groups: Group 1 enrolled 11 subjects who had previously received 2 doses of the *ca* A/Vietnam/1203/2004 H5N1 P/LAIV in 2006-2007 (CIR217, $10^{7.5}$ $TCID_{50}$ dose); Group 2 enrolled 10 subjects who had previously received 2 doses of the *ca* A/Hong Kong/213/2003 H5N1 P/LAIV in 2007 (CIR239); Group 3 enrolled 8 subjects who had previously received 2 doses of the *ca* A/British Columbia/CN-6/2004 H7N3 P/LAIV in 2010 (as a P/LAIV control group); Groups 4 and 5 each enrolled 20 subjects who had not been previously vaccinated with LAIV and were influenza H5-naïve. Subjects in Groups 1 to 4 received a single 45-µg dose of an inactivated A/Vietnam/1203/2004 vaccine (pandemic inactivated influenza vaccine [P/IIV]) while subjects in Group 5 received 2 doses, approximately 28 days apart.



To determine antibody responses blood draws were taken at baseline, Day 7, Day 28, Day 35 (Group 5 only), Day 84 (Group 5 only), Day 180 Groups 1-4), and Day 208 (Group 5 only).

Enrolled subjects were carefully screened before enrolment into the study. Also study conduct within the isolation unit was very safe for the enrolled subjects.

Recruitment

Healthy men and non-pregnant women, 18-49 years of age, were enrolled in the clinical trials CIR 217 and CIR 239 conducted in Baltimore if they met the eligibility criteria and were willing to remain on the isolation unit for the duration of the inpatient portion of the trials. Based upon the pattern of vaccine virus shedding observed with the low dose H5N1 A/Vietnam/2004 *ca* vaccine, the duration of the inpatient stay was shortened from a total of 14 days (3 before vaccination and 11 days following vaccination) to 12 days (3 days before vaccination and 9 days following vaccination) for group 2 of CIR 217 and CIR 239 study, providing that vaccine virus was not detected by rRT-PCR from nasal washes obtained for three consecutive days prior to discharge day. Subjects enrolled in Study CIR 277 were slightly older, because the received prior two doses of P/LAIVs 4-5 years before.

The CHMP noted that subjects vaccinated with a live attenuated vaccine were monitored very carefully in the isolation units by rRT-PCR from nasal washes daily and prior to vaccination. Daily throat swabs were also obtained for the low dose group 1 in study CIR 217.

Conduct of the study

Studies CIR 217 and CIR 239 were conducted between April and December in the years 2006 and 2007, when *wt* human influenza viruses would be unlikely to be circulating in the local community. Additionally the *wt* influenza viruses in the community were monitored in order to minimise the risk or reassortment between a naturally occurring *wt* influenza virus and the vaccine virus. Participants would not have been enrolled if there were more than 3 influenza hospitalisations in the week preceding the planned vaccination.

Potential participants were screened for tuberculosis, viral hepatitis, HIV infection, and antibodies for H5N1 (high dose group in CIR 217 and complete trial CIR 239) using serological assays. Haematology, biochemistry for blood specimens and dipstick analyses were performed. Pregnancy tests for female subjects were performed to exclude pregnancies.

Subjects were admitted to the isolation unit. Vaccine was administered at day 0 and the enrolled subjects received 0.5 mL of vaccine via nasal spray. Nasal washes were collected daily during the inpatient portion of the study and were tested for vaccine virus.

In the event of a respiratory or febrile illness, nasal wash specimens were also cultured for adventitious respiratory viruses. Laboratory findings of patients with severe avian influenza H5N1 include leucopenia, lymphopenia, impaired liver function with elevated liver enzymes, prolonged clotting times, and renal impairment. The lymphocyte count appears to be the most valuable parameter for identification of patients who are at risk of progression to severe illness. Therefore serum alanine aminotransferase (ALT) levels and complete blood counts were determined before vaccination and on day 7 following vaccination. Any abnormal results were followed until resolution.

After discharge from the isolation unit, participants were asked for outpatient visits on study day 28 (+7 days) following each dose of vaccine. At each visit, staff obtained vital signs, reviewed interim histories and obtained blood and nasal wash samples for antibody testing.

Baseline data

Study CIR 217 and CIR 239 recruited primarily African Americans. Sex and race of enrolled subjects in these studies were: 32% were female, 85% black and 8% white.

Study 277 was a follow-up of CIR217 and CIR239. The baseline demographics in Groups 1-5 subjects were essentially comparable, with the exception of age. The subjects were mainly male (61%) and black (88%). Of the 69 enrolled, 66 completed the final study visit.

Table 7. Demographic characteristics, by study Group

Characteristic	1 (n = 11)	2 (n = 10)	3 (n = 8)	4 (n = 20)	5 (n = 20)	Overall (n = 69)
Dose 1	H5N1 VN04 pLAIV	H5N1 HK03 pLAIV	H7N3 pLAIV	None	ISIV	
Dose 2	ISIV	ISIV	ISIV	ISIV	ISIV	
Age, y, median (range)	44 (30–53)	34 (24–49)	35.5 (23–54)	35 (23–47)	34 (22–53)	37 (22–54)
Female sex	3 (27)	5 (50)	2 (25)	9 (45)	8 (40)	27 (39)
Race						
Black	9 (82)	9 (90)	8 (100)	18 (90)	17 (85)	61 (88)
White	1 (9)	1 (10)	0	1 (5)	2 (10)	5 (7)
Multiracial	1 (9)	0	0	1 (5)	1 (5)	3 (4)

Data are no. (%) of subjects, unless otherwise indicated.
Abbreviations: H5N1, influenza A(H5N1); H7N3, influenza A(H7N3); ISIV, inactivated subvirion influenza vaccine; pLAIV, pandemic live attenuated influenza vaccine.

The Applicant was asked to justify whether the demographics of the pivotal studies could have implications for validity of data with regards to the ethnical composition of the EU population. It was clarified that studies CIR 217, CIR 239, and CIR 277 were conducted in Baltimore, MD, United States of America (USA). As a result, the subjects enrolled primarily reflect the demographics of this urban city. Previous studies conducted with FluMist have shown, however, that the safety and efficacy of the vaccine are not affected by the race and ethnicity of the vaccine recipients; the results of the P/LAIV studies can therefore be applied to the European Union (EU) population. Also, additional studies conducted at the University of Rochester and the studies of the 2009 H1N1pdm vaccine sponsored by MedImmune enrolled a racially and ethnically more diverse set of subjects. While a demographic composition more similar to the European situation would have been preferable, the practical limitations linked to study design (i.e. involving isolation of study subjects) is acknowledged, and the argumentation in support of the validity of the data generated in the pivotal studies for the EU population were deemed acceptable.

Numbers analysed

194 potential participants aged 18 to 49 years were screened for the H5N1 P/LAIV vaccine trials and 59 participants were enrolled. Tables 9 and 10 provided the actual number of subjects and dosages in the trials.

CIR 217:

- 21 in group 1, who received $10^{6.7}$ $TCID_{50}$ of H5N1 A/Vietnam/2004 (18 received a second dose)
- 21 in group 2, who received $10^{7.5}$ $TCID_{50}$ of H5N1 A/Vietnam/2004 (19 received a second dose)

CIR 239:

- 17 in the cohort that received $10^{7.5}$ $TCID_{50}$ of H5N1 A/Hong Kong/213/2003 vaccine (16 received a second dose)

Participants were not equally balanced across the intended age group. Of the 59 participants 19 (32%) were female, 50 (85%) black, 5 (8%) white, 3 (6%) Asian, and 1 described herself as other. For study CIR 239 122 potential participants were screened for H5 HA antibody. 8 of these individuals had titres ≥1:8 and were excluded from participation.

CIR 277

The study was a follow-up of CIR217 and CIR239. Only about half of the original subjects in CIR217 and CIR239 were enrolled into this study. See the participant flow. The CIR 277 study only included subjects which had received two $10^{7.5}$ $TCID_{50}$ doses of H5N1 VN 04 in the previous CIR 217 study and excluded subjects which had received the lower $10^{6.7}$ $TCID_{50}$ doses. At the time that Study CIR 277 was planned it was unclear whether subjects enrolled in Study CIR 217 had mounted meaningful immune responses to the P/LAIV H5N1 Vietnam/1203/2004 vaccine, as subjects in both the lower dose and higher dose cohorts had minimal antibody responses to the vaccine. A decision was made to re-enrol the subjects in the higher dose cohort in order to maximize the likelihood of revealing any priming responses that might have been induced approximately 5 years previously.

Outcomes and estimation

Results of study CIR217

The study was initiated in 2006 at a single site in the USA. Enrolment details were reported together with study CIR239 in the same publication. Pre-screening test for H5 HAI antibody was undertaken only for the $10^{7.5}$ $TCID_{50}$ dosage groups of H5N1 VN04, but not for $10^{6.7}$ $TCID_{50}$ dosage group of H5N1 VN04.

For CIR217, a total of 42 subjects were eventually enrolled (21 in $10^{6.7}$, 21 in $10^{7.5}$ group) and evaluable for the safety, infectivity and immunogenicity. Subject age ranged from 21 to 49 years.

Virologic responses

Vaccine virus was undetected by culture in $10^{6.7}$ $TCID_{50}$ dosage group but detected by rRT-PCR in 2 subjects post dose 1 and in 3 subjects post dose 2. In $10^{7.5}$ group, vaccine virus was recovered by culture in 2 subjects following dose 2, and detected by rRT-PCR in 3 subjects post dose 1, and in 15 subjects post dose 2.

Antibody responses

A serum HAI or IgA antibody response was each detected in 2 subjects (10%) after 1 or 2 doses of $10^{6.7}$ $TCID_{50}$ vaccine (i.e. after any dose). Nasal wash IgA responses were detected in 5 subjects (24%) after any dose. No MN or serum IgG responses were detected.

For recipients of 1 or 2 doses of $10^{7.5}$ $TCID_{50}$ vaccine, 2 (10%) and 1 (5%) subjects developed a HAI and MN seroresponse, respectively, after any dose, and 11 (52%) subjects had a serum IgA response.

T cell-mediated immune response

Cell-mediated immunity was measured after 2 doses of P/LAIV H5N1 VN in 21 vaccine recipients. It was found that T cell responses to conserved internal proteins M and NP were boosted by vaccination. In addition, H5N1 pLAIV appeared to preferentially stimulate and boost pre-existing seasonal influenza virus HA-specific T cell responses that showed low cross-reactivity with the H5 HA. No evidence that pre-existing T cells prevented pLAIV replication and take was found. Furthermore, it was demonstrated that cross-reactive T cell responses could be boosted by pLAIV regardless of the induction of antibody.

The publication by Peng et al 2015 has been submitted.

Results of study CIR239

The study was initiated in 2007 at a single site in the USA. Pre-screening test for H5 HAI antibody of potential participants was undertaken for the $10^{7.5}$ $TCID_{50}$ dosage group of H5N1 HK03. As a result, 17 subjects were enrolled in this study, all received the first dose and 16 of them received the second dose of vaccine. Participants ranged in age from 18 to 49 years.

Virologic responses

After the first dose, vaccine virus was detected in 1 (6%) subject by culture and in 8 (47%) by rRT-PCR. After the second dose, vaccine virus was undetected by virus culture but detected by rRT-PCR in 9 (56%) subjects.

Antibody responses

No HAI or MN seroresponse was detected in any study subjects after any dose of vaccine. Serum IgG response was detected in 1 (6%) and serum IgA and nasal wash IgA response each in 3 (18%) subjects, after 1 or 2 doses of vaccine.

Results of CIR277

HAI and MN responses

All subjects in Groups 1-5 had H5 HAI titres of ≤1:8 prior to immunisation, which means they were H5N1 seronegative. As shown in the table below, 28 days following receipt of a single 45µg dose of inactivated H5N1 VN04 vaccine (ISIV: inactivated subvirion influenza vaccine), 73% of subjects in Group 1 developed HAI and MN seroresponses to the wild-type H5N1 VN04. At day 56, seroconversion rates increased to 82% in HAI assay but decreased to 55% in MN assay. In contrast, the frequencies and magnitudes of antibody responses were minimal in Group 3 (P/LAIV H7N3-primed) and Group 4 (unprimed subjects, 1 dose). Subjects in Group 5 (unprimed subjects, 2 doses) also developed lower HAI and MN antibody responses at Day 28, which gradually increased by Day 56 showing no statistically significant difference with Group 1 in MN response at Day 56.

Table 8. Serum HAI and MN antibody responses following immunisation

Ab Assay, Study Group	pLAIV	VN04 ISIV Doses, No.	Subjects, No.	28 d After ISIV[a]				56 d After ISIV[a]			
						Subjects With 4-fold Ab Rise[b]				Subjects With 4-fold Ab Rise[b]	
				Range	GMT	Percentage	GMT (95% CI)	Range	GMT	Percentage	GMT (95% CI)
HAI assay											
1	H5N1 VN 04	1	11	5–1280	**87**[c]	73	222 (115–426)	5–960	66	82	112 (54–233)
2	H5N1 HK 03	1	10	5–480	**29**[d]	50	146 (57–374)	5–480	21	40	120 (20–727)
3	H7N3	1	8[e]	5–160	8	14	160 (0[f])	5	5	0	5
4	None	1	20	5–640	8	10	277	5–240	8	10	120
5	None	2	20[e]	5–640	15	40	81 (36–181)	5–120	**21**[c,d]	50	76 (43–133)
MN assay											
1	H5N1 VN 04	1	11	5–1280	**48**[g]	73	89 (31–253)	5–1280	25	55	61 (12–318)
2	H5N1 HK 03	1	10	5–160	**31**[h]	60	61 (29–127)	5–160	22	60	43 (24–76)
3	H7N3	1	8[e]	5–60	8	14	60 (0)	5	5	0	5
4	None	1	20	5–60	7	10	35	5–40	4	10	35 (6–215)
5	None	2	20[e]	5–480	11	30	46 (11–190)	5–160	**19**[g,h]	56	35 (19–64)

Abbreviations: CI, confidence interval; GMT, geometric mean titer; HAI, hemagglutination inhibition; MN, microneutralization; pLAIV, pandemic live attenuated influenza vaccine

[a] Days are counted relative to the only ISIV dose for groups 1–4 and after the first of 2 ISIV doses for group 5.

[b] Serological response defined as a ≥4-fold rise in Ab titer (≥1:20).

[c] Group 1 day 28 vs group 5 day 56: $P = .04$ (*t* test).

[d] Group 2 day 28 vs group 5 day 56: $P = .62$ (*t* test).

[e] Serum samples were available from 7 subjects in group 3 on day 28 and from 18 subjects in group 5 on day 56.

[f] A single subject in this group had antibody detected.

[g] Group 1 day 28 vs group 5 day 56: $P = .08$ (*t* test).

[h] Group 2 day 28 vs group 5 day 56: $P = .22$ (*t* test).

Subjects in Group 2, who were previously primed by P/LAIV H5N1 HK03 candidate, also responded to a single heterologous boosting dose, but their Day 28 responses did not differ significantly from Day 56 responses of Group 5 subjects.

Overall, subjects who were primed with either the A/Vietnam/1203/2004 P/LAIV or the A/Hong Kong/213/2003 P/LAIV had a significantly better response to a single dose of inactivated H5N1 vaccine than P/LAIV-naïve subjects. The antibody response in A/Vietnam/1203/2004 P/LAIV-primed subjects also exceeded that observed after 2 doses of inactivated vaccine in P/LAIV-naïve subjects.

Although only the cohort with the higher dose from study CIR 217 was enrolled in study CIR 277, it is likely that subjects enrolled in the lower, $10^{6.7}$ $TCID_{50}$, cohort in Study CIR 217 would also have demonstrated significant booster responses.

Kinetics of serum antibody responses

Upon re-vaccination, subjects of Group 1 rapidly developed serum HAI response: 7/11 (64%) had a 4-fold rise in HAI titre (Figure 4A), with a GMT of 165 and a titre range from 20 to 1280 in responding subjects (Figure 4B) by day 7. Longevity of the antibody response was witnessed at later time points (Figure 4 A and C), but GMT of HAI antibodies in all groups declined 6 months after dosing. Fewer subjects had detectable antibodies by 6 months after the last vaccine dose (Figure 4A). Of the P/LAIV-naïve subjects only 10% had a 4-fold rise in HAI titre by Day 7.

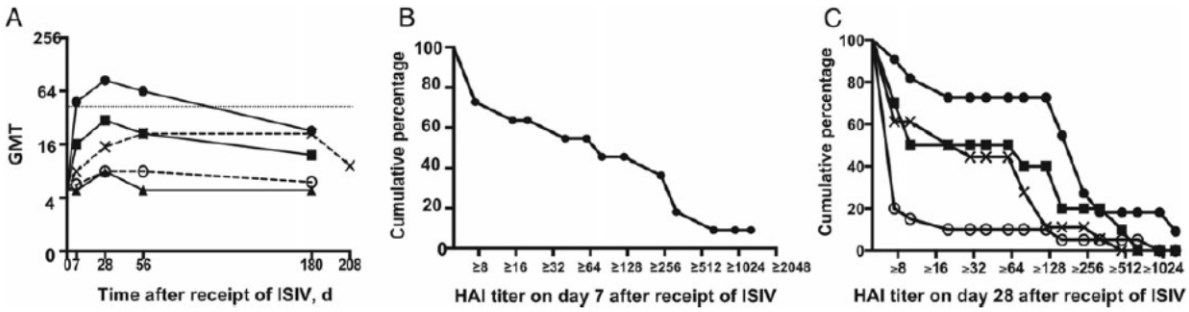
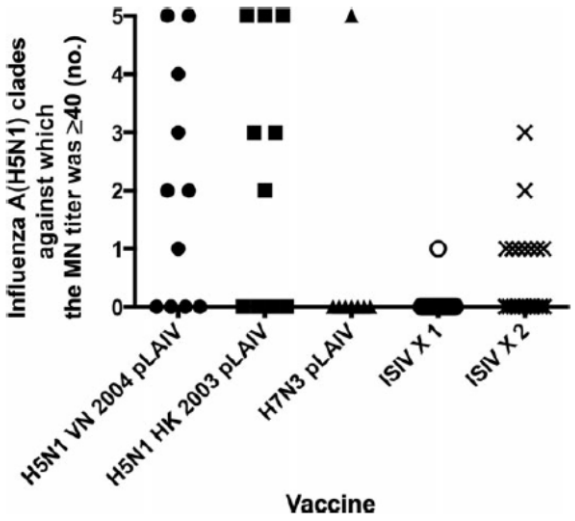


Figure 3. A, Kinetics of HAI antibody response to wild-type H5N1 VN04 virus, assayed using horse red blood cells. Dotted line indicates a HAI GMT of 1:40. Filled circle, group 1 (H5N1 VN04 P/LAIV); square, group 2 (H5N1 HK03 P/LAIV); triangle, group 3 (H7N3 P/LAIV); open circle, group 4 (one dose); and X, group 5 (2 doses). B, Reverse cumulative distribution of HAI titres for group 1 on day 7 after dosing. C, Reverse cumulative distribution of HAI titres from groups 1, 2, 4, and 5 on day 28 after last dose of inactivated vaccine.

Cross-Clade reactivity

Thirteen of 21 subjects in Groups 1 and 2 had MN titres of ≥1:40. Of these, sera from all but 1 H5N1 P/LAIV-primed subject neutralised ≥2 clades of H5N1 viruses from the A/Goose/Guangdong/1/96 H5N1 lineage (Figure 5). In Group 5, serum from only 1 subject neutralised >1 clade, and the receipt of a second dose of inactivated vaccine did not increase the breadth of antibody response.

Figure 4. Cross-reactivity against antigenically distinct clades.



The proportion of subjects from each group with a MN titre of ≥1:40 against any one of five H5N1 viruses from 4 antigenically distinct clades: A/VietNam/1203/2004 (clade 1), A/Indonesia/5/2005 (clade 2.1.3), A/Anhui/1/05 (clade 2.3.4), A/turkey/Turkey/1/05 (clade 2.2.1), and A/Egypt/3072/2101 (clade 2.2.1).

At Day 28 the cross-neutralizing antibodies against Indonesia/5/2005, Turkey/Turkey/1/05, Anhui/1/05, and Egypt/3072/2010 H5N1 strains (all reverse genetics-derived reassortant viruses) were in 27%, 18%, 45%, and 45%, respectively, of previous recipients of P/LAIV H5N1 VN04 (Cohort 1). A somewhat better seroconversion rate (SCR) against each heterologous strain was observed in Cohort 2 subjects who previously received the P/LAIV H5N1 HK candidate. No such advantage was observed in GMTs for Cohort 2.

The 95% confidence intervals of these data were not presented. However, the overall small sample size in each cohort suggests that these data should not be over interpreted.

Antibody affinity

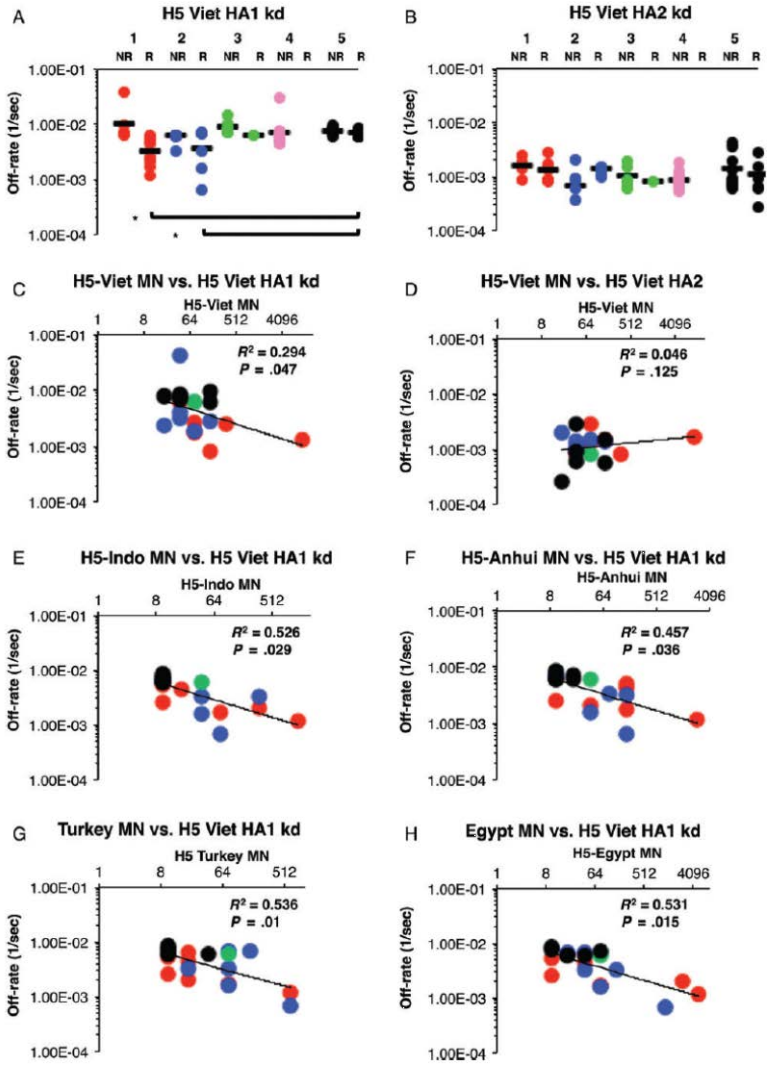
Antibody affinity to recombinant HA1 (1-330) and HA2 (331-480) protein was assayed via monitoring steady-state equilibrium binding of post-vaccination sera at 25^{0}C, for responders (titres of ≥1:40) and non-responders (titres < 1:40) of Groups 1-5. The antibody off-rate against rHA1 and rHA2 were plotted against MN titre to homologous H5N1 VN04 (vaccine strain) or heterologous H5N1 clades and subclades mentioned above (Figure 6).

In Group 1, the average off-rates for antibody bound to rHA1 were significantly slower in responders than non-responders (P =0.0083). A similar pattern was for group 2 but did not reach statistical significance. Compared with Group 5, Groups 1 and 2 responders showed 2–3-fold stronger (slower off-rates) antibody affinity against rHA1 (P =0.0249 and 0.0013, respectively) but not against rHA2 (Figure 6A, 6B).

Inverse correlations were observed between antibody off-rates against rHA1 (Figure 6C and 6E–H) but not rHA2 (Figure 6D) and the MN titres against all H5N1 strains tested.

In summary the affinity of antibodies against the HA1 domain of the H5 HA in the H5N1 P/LAIV-primed groups was significantly higher than the 2-dose inactivated vaccine group, which correlated with cross-clade H5N1 neutralization.

Figure 5. Antibody affinity to H5N1 recombinant HA1 and HA2 of different Clades.



Surface plasmon resonance analysis of post-vaccination sera from all responders (R; MN ≥1:40) and non-responders (NR; MN <1:40) from all 5 study Groups was performed. Each symbol represents 1 individual. Groups are represented by the coloured circles as follows: Group 1, red; Group 2, blue

Ancillary analyses

Not applicable

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

Not applicable

Clinical studies in special populations

Not applicable

Supportive studies

P/LAIV studies

Ten P/LAIV studies (CIR247, URMC10-004, CIR251, CIR241, URMC10-002, URMC11-001, URMC10-003, CIR293, URMC13-001 and CIR211) evaluated the safety, infectivity and immunogenicity of 2 doses of P/LAIV candidates developed for conducted with other potential pandemic strains, H2N2, H2N3, H6N1, H7N3 (2 studies), H7N7 (2 studies), H7N9 (2 studies) and H9N2, respectively. Study URMC10-002 assessed a single dose of P/LAIV H7N3. The time points for serum and nasal wash antibodies measurements were prior to vaccination and at Days 21, 28, and 42 after each dose in CIR211, or at baseline and Day 28 after each dose in CIR247.

Three studies were based on a prime-boost design and these are described more in detail below.

Study URMC11-001

The study was conducted in 2013 at a single site in the USA, to investigate the boosting effect of the pIIV in 18-50 years old subjects immunised 18-24 months earlier with the P/LAIV H7N7 candidate (A/H7N7 A/Netherlands/219/03 vaccine, study URMC10-003). In total the study enrolled 39 subjects in 3 groups: 14 subjects (58%) including 12 recipients of 2 P/LAIV doses and 2 recipients of 1 P/LAIV dose from URMC10-003 entered Group 1; Group 2 enrolled 5 subjects prior recipients of P/LAIV H7N3 (A/H7N3 A/chicken/British Columbia/CN-6/04) as a P/LAIV control group); and Group 3 enrolled 20 subjects who were seronegative to H7 (HAI titre ≤1:8) and who never received a P/LAIV. All subjects received a single 45-µg dose of the inactivated H7N7 vaccine.

In general, there was no substantial difference in demographic aspects between the group receiving P/LAIV H7N7 and the subset that was boosted with H7N7 pIIV, or between primed and unprimed recipients of pIIV.

Antibody responses

All subjects in Group 1 did not develop detectable HAI and MN seroresponses in precursor study URMC10-003. After receipt of a single 45µg dose of inactivated H7N7 vaccine, 9/13 (69%) of Group 1 subjects had a serum HAI titre of ≥1:40 and 10/13 (77%) had a serum MN response to the H7N7 vaccine virus (Table 14), including the 2 subjects who had received only 1 dose of H7N7 P/LAIV. Serum IgG and IgA antibody responses to homologous HA were also detected by ELISA in most of the Group 1 subjects. In contrast, none of subjects in Groups 2 and 3 had a detectable serum HAI or MN response (Table 14).

Table 9. Serum antibody response to inactivated H7N7 vaccine in naïve subjects or prior recipients of P/LAIV H7N7 or P/LAIV H7N3

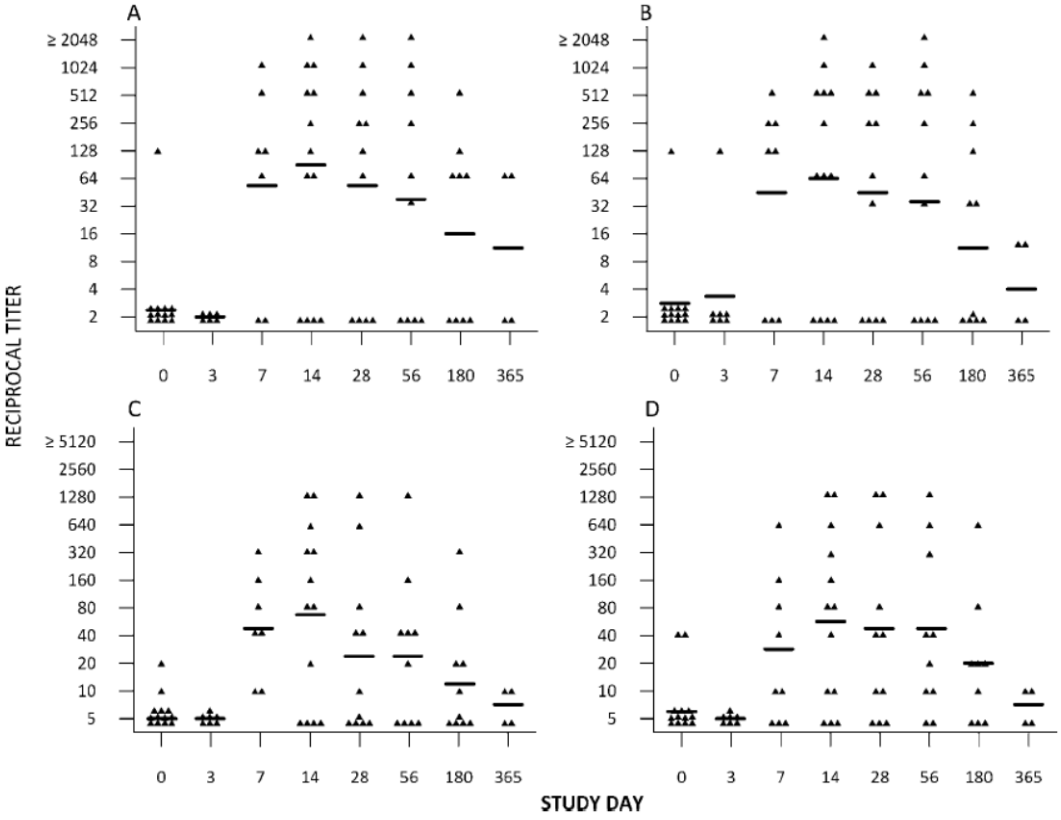
Priming pLAIV	*N*	MN	HAI	Serum HA-specific antibody response* by ELISA				
		Responding*	Titer ≥ 1:40	Responding*	Titer ≥ 1:40	IgG ELISA	IgA ELISA	IgM ELISA*
Number (%) of subjects who responded, and who achieved the indicated post-vaccination titer by the following assays								
H7N7	13	10.(77)**	9.(69)**	9.(69)**	9.(69)**	12.(92)**	8 (62)	0
H7N3	5	0	0	0	0	1 (20)	NT	NT
None	20	0	0	0	0	11 (55)	NT	NT

NT, not tested.
* Response defined as a four fold increase in titer above baseline at any time point after receipt of H7N7 pIIV.
** $P < .05$ compared to either H7N7 primed or naïve subjects, Fisher Exact Test.

Kinetics of antibody responses

Upon re-vaccination of P/LAIV H7N7-primed subjects, antibody responses were rapid, with the GMTs of HAI and MN at > 32 and > 40, respectively, by Day 7. Peak titres were reached on Day 14, and then declined over time (Figure 7). One year after dosing, 2 of 4 subjects tested still had detectable titres against H7N7, although the levels were low.

Figure 6. Kinetics of (A) HAI against H7N7, (B) HAI against H7N3, (C) MN against H7N7, (D) MN against H7N3 for each subject following re-vaccination in Group 1 subjects.



GMTs are displayed by the horizontal line. Assays were done using P/LAIV H7N7 virus or P/LAIV H7N3 as test antigen. Samples with a HAI titre of <4 are assigned a value of 2, and samples with a MN titre of <10 are assigned a value of 5.

The reverse cumulative distribution data for subjects in Cohort 1 at day 14 revealed that approximately 20% or less of subjects developed higher HAI or MN titres ≥1:1000, either against H7N7 (homologous) or H7N3 (heterologous) strain.

Cross-reactivity

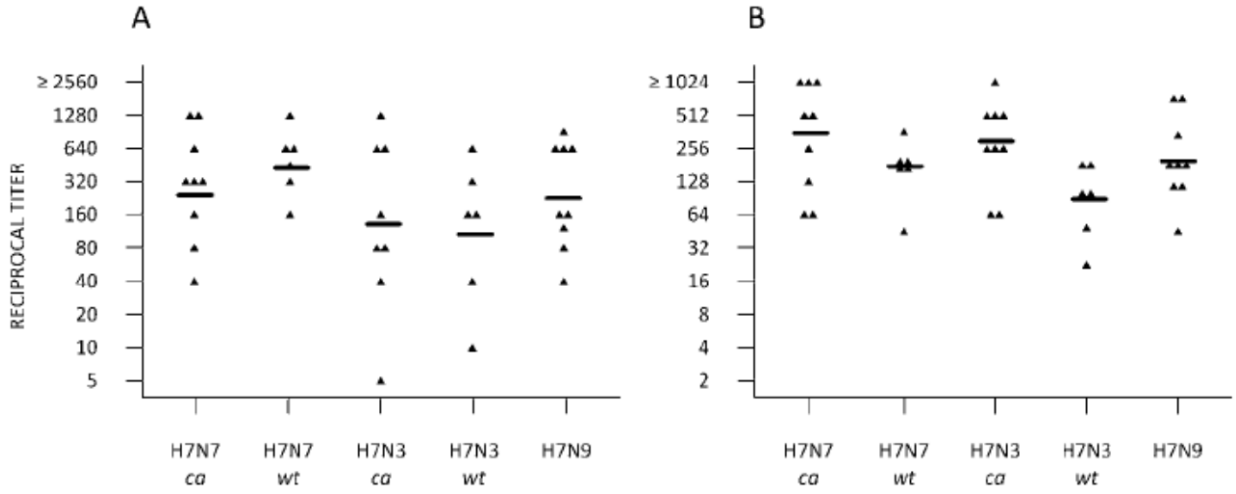
Sera were also tested for reactivity against other H7 variant viruses, including the ca A/chicken/British Columbia/CN- 6/2004 derived from the wild-type virus (H7N3, North American lineage), the

A/mallard/Netherlands/12/2000 wild type virus (H7N3, Eurasian lineage and donor of the HA gene for the inactivated vaccine) and the human isolate A/Anhui/1/2013 (H7N9, Eurasian lineage).

Among the responders in Group 1 who developed MN and HAI responses following re-vaccination, broadly cross-reactive antibodies were detected at Day 14, both against vaccine viruses and the antigenically distinct wild-type viruses mentioned above (Figure 8).

Interestingly, antibody response induced by inactivated H7N7 vaccine in P/LAIV H7N7-primed subjects also recognised H7N3 virus, while subjects primed with P/LAIV H7N3 did not generate antibody to H7N3 virus following re-vaccination with inactivated H7N7 vaccine (data not shown).

Figure 7. Cross reactivity of Day 14 sera from Group 1 individual responders showing MN (A) and HAI (B) antibody responses.



Shown are individual titres and the GMTs of antibody responses to A/Netherlands/219/2003 P/LAIV (H7N7 *ca*), A/Netherlands/219/2003 wild-type virus (H7N7 wt), A/chicken/British Columbia/CN-6/2004 P/LAIV (H7N3 *ca*), A/mallard/Netherlands/12/2000 (H7N3 wt), and A/Anhui/1/2013 (H7N9 wt).

At Day 14 post-booster immunization in group 1, cross-reactive HAI antibodies against H7N3 P/LAIV *ca*, H7N3 wt, and H7N9 wt developed in 69%, 46% and 69% of subjects, respectively, and the MN antibodies in 62%, 38% and 69% of subjects, respectively. The lowest GMT titres in both assays were seen for the H7N3 wt strain. The differential responses in GMTs to the H7N3 wt virus (HAI 74.2; MN 113.1) versus the H7N9 wt virus (HAI 172.8; MN 244.6) might suggest difference in antigenicity and closeness to the H7N7 P/LAIV strain (HAI 348.4; MN 296.3).

Study URMC13-001

Initiated in October 2013 at a single site in the USA, this inpatient study enrolled 32 healthy adults 18 to 49 years of age, divided into 2 groups: 16 received a single dose of P/LAIV H7N9 candidate (10^7 FFU), and 16 received 2 doses administered 28 to 42 days apart. Of these 32, 30 including 14 in the 1-dose group and 16 in the 2-doses group were re-vaccinated, approximately 3 months later, with a single 30µg dose of inactivated H7N9 vaccine.

Concerning demographic aspects of the study population, more male subjects (75%) and more White people (43.8%) were enrolled in this supportive study, compared with other prime-boost studies such as CIR 277.

Virological responses

2 subjects shed vaccine virus as detected by viral culture in nasal swabs, after the first dose or the second dose of P/LAIV H7N9 vaccine. By rRT-PCR, vaccine virus shedding was detected in 18 subjects after the first vaccine dose, and in 10 subjects after the second dose.

Antibody responses

All subjects showed H7N9-specific serum HAI titre ≤1:8 at baseline, which means that they were all seronegative for H7N9 prior to vaccination. Following receipt of P/LAIV H7N9 candidate, serum HAI and MN antibodies were detected in 2 subjects, both in the 2-doses group.

Re-vaccination with inactivated H7N9 vaccine rapidly elicited HAI antibodies at Day 7 against P/LAIV H7N9 in 8/14 (57%) of prior recipients of 1 dose, and in 13/16 (81%) of prior recipients of 2 doses of P/LAIV H7N9 candidate. Corresponding HAI GMTs in the two cohorts were 128 and 351, respectively, at Day 7. MN seroresponse rates at Day 7 were 64% in prior recipients of 1 dose of P/LAIV H7N9 candidate and 94% in prior recipients of 2 doses.

Antibody responses following receipt of the inactivated H7N9 antigen were evaluated up to Day 82, demonstrating notable HAI and MN antibody responses to the vaccine virus in H7N9 IIV recipients (see tables 15 and 16). Thus, the H7N9 P/LAIV candidate vaccine primed for a rapid, robust antibody response to H7N9 IIV.

Table 10. HAI antibody titres against H7N9, H7N7 and H7N3 P/LAIVs in recipients of H7N9 P/LAIV following receipt of H7N9 inactivated influenza vaccine (IIV)

P/LAIV	No. of Doses	Number of Subjects	Number of Responders[a] (%)	GMT HAI Titer in Responders on Indicated Day Post Receipt of IIV [b]				
				Day 7	Day 14	Day 28	Day 56	Day 82
H7N9	1	14	8 (57)	128	235	215	141	116
H7N9	2	16	13 (81)	351	512	460	192	144
H7N7	1	14	8 (57)	72	59	59	58	78
H7N7	2	16	13 (81)	69	64	68	51	76
H7N3	1	14	8 (57)	128	113	140	144	91
H7N3	2	16	13 (81)	104	102	115	85	108

GMT = geometric mean titre; HAI = haemagglutination inhibition; pIIV = pandemic inactivated influenza vaccine; No. = number; P/LAIV = pandemic live attenuated influenza vaccine. [a] Response defined as ≥ 4-fold rise in titre. [b] HAI assay performed with horse red blood cells

In addition, HAI titres ranging from 69 to 104 GMTs were detected at day 7 in around 60 to 80% of vaccinees against antigenically distinct H7N7 and H7N3 P/LAIVs (table 10).

Table 11. Neutralising antibody responses against H7N9 P/LAIV in recipients of H7N9 P/LAIV following receipt of H7N9 inactivated influenza vaccine

P/LAIV	No. of Doses	No. of Subjects	No of Responders [a] (%)	GMT Neutralizing Antibody Titer in Responders on Indicated Day Post Receipt of IIV				
				Day 7	Day 14	Day 28	Day 56	Day 82
H7N9	1	14	9 (64)	95	59	187	95	67
H7N9	2	16	15 (95)	127	103	221	136	151

GMT = geometric mean titre; IIV = inactivated influenza vaccine; No. = number; P/LAIV = pandemic live attenuated influenza vaccine; a Response is defined as ≥4-fold rise in titre compared to day of receipt of IIV.

Study CIR 293

This is the second study evaluating boosting effect in P/LAIV H7N9-primed healthy adults in addition to URMC 13-001 (also prime-boost). Study CIR293 began in 2014 and is still ongoing. The Interim results

of this study were submitted during the procedure. The study enrolled 5 cohorts as detailed in Table 17.

Table 12. Design of study CIR 293

Cohort	No. of Doses of P/LAIV[a]	No. of Months Between Receipt of P/LAIV and pIIV	No. of Doses of pIIV [b]
1	2	1	1
2	2	2	1
3	1	2	1
4	1	1	1
5	0	NA	2

FFU = fluorescent focus units; NA = not applicable; No. = number; pIIV = pandemic inactivated influenza vaccine; P/LAIV = pandemic live attenuated influenza vaccine; [a] ~107.0 FFU dose level; [b] 30 µg dose level

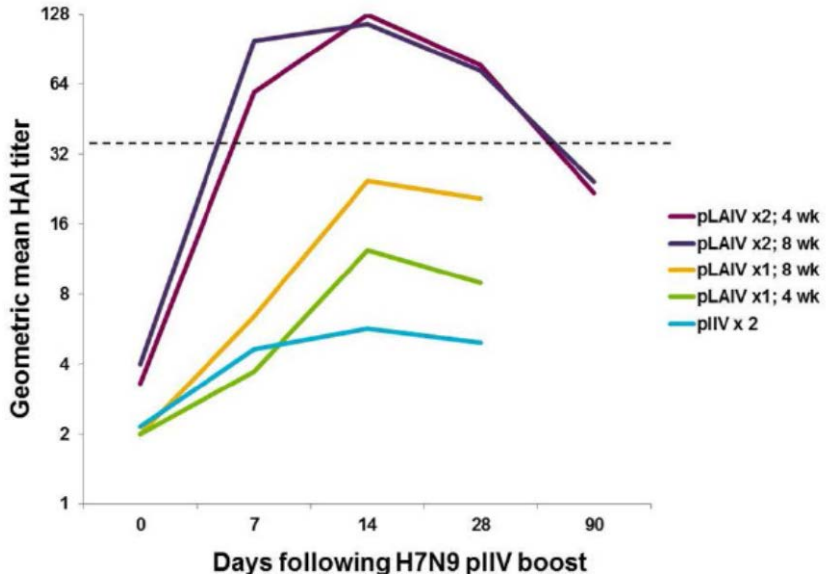
A total of 39 subjects in Cohorts 1 and 2 received 2 doses of P/LAIV and a total of 40 subjects in Cohorts 3 and 4 received a single dose of P/LAIV. Twenty subjects in Cohort 5 were not vaccinated with P/LAIV and received 2 doses of pIIV.

A total of 92 of the 99 subjects completed the study:

- Cohort 1: 18 subjects received a single dose of pIIV 4 weeks post receipt of P/LAIV and all 18 subjects completed the study
- Cohort 2: 17 subjects received a single dose of pIIV 8 weeks post receipt of P/LAIV and 16 subjects completed the study
- Cohort 3: 20 subjects received a single dose of pIIV 8 weeks post receipt of P/LAIV and 19 subjects completed the study
- Cohort 4: 19 subjects received a single dose of pIIV 4 weeks post receipt of P/LAIV and all 19 subjects completed the study
- Cohort 5: All 20 subjects completed the study

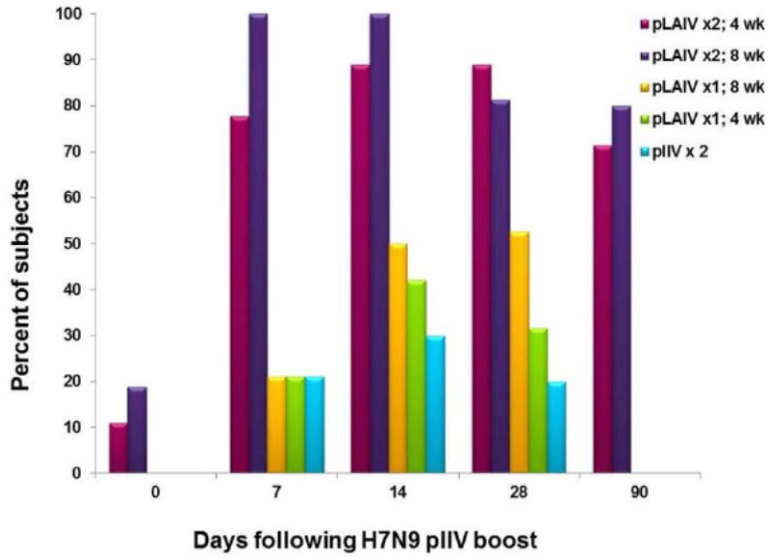
Figure 9, Figure 10, and Figure 11 provide summary information for HAI responses against the live attenuated virus; testing against wild-type viruses is planned to be conducted.

Figure 8. Figure 3.18-1 Geometric Mean HAI Titres Following Receipt of Inactivated H7N9 Vaccine in Study CIR 293, by Dosing Cohort



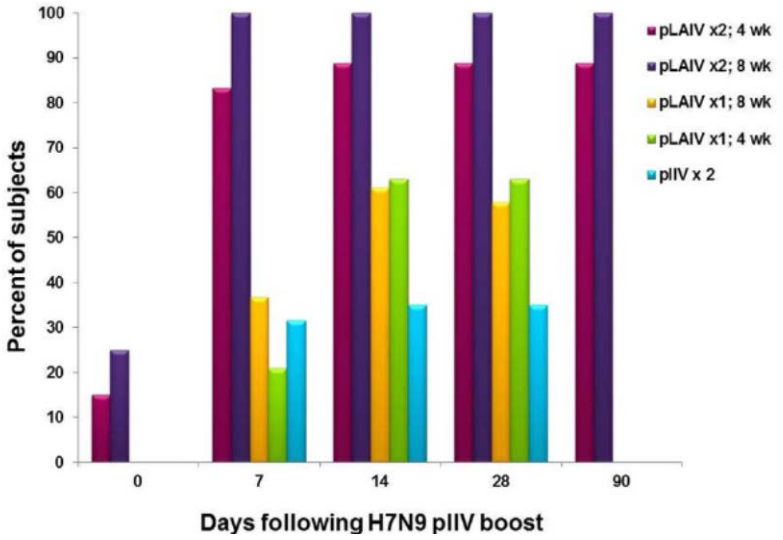
HAI = haemagglutination inhibition; pLAIV = pandemic live attenuated influenza vaccine; pIIV = pandemic inactivated influenza vaccine; wk = week

Figure 9. Figure 3.18-2 Percentage of Subjects With a Greater Than or Equal to 4-fold Increase in HAI Titre From Baseline Following Receipt of an Inactivated H7N9 Vaccine in Study CIR 293, by Dosing Cohort



HAI = haemagglutination inhibition; pLAIV = pandemic live attenuated influenza vaccine; pIIV = pandemic inactivated influenza vaccine; wk = week

Figure 10. Figure 3.18-3 Percentage of Subjects With HAI Titres Greater Than or Equal to 40 Following Receipt of an Inactivated H7N9 Vaccine in Study CIR 293, by Dosing Cohort



HAI = haemagglutination inhibition; pLAIV = pandemic live attenuated influenza vaccine; pIIV = pandemic inactivated influenza vaccine; wk = week

The data indicate that P/LAIV priming led to robust responses to H7N9 pIIV. The magnitude and frequency of HAI responses were greater in subjects primed with 2 doses of P/LAIV than in subjects primed with 1 dose P/LAIV or unprimed subjects. However, subjects primed with a single dose of P/LAIV had more robust responses by Day 14 than unprimed subjects who received 2 doses of inactivated vaccine. In addition, a dosing interval as short as 4 weeks still resulted in robust immune responses for subjects who previously received 2 doses of P/LAIV. Indeed > 80% of subjects seroconverted by HAI on Day 7 following receipt of the inactivated H7N9 antigen 4 weeks post-priming and there was no significant difference between the 4- and 8-week dosing intervals between P/LAIV prime and pIIV boost doses.

Other supportive P/LAIV studies

Virologic and immunogenicity data generated from 8 additional P/LAIV studies are summarised in Table 18.

Overall, individual candidate vaccine viruses could be recovered from nasal secretion of vaccine recipients following inoculation, but at low level, in a small proportion of subjects and detected primarily by rRT-PCR tests. Overall the incidence of shedding appears slightly lower compared to shedding of vaccine virus in adult subjects who received T/LAIV. The serum HAI and MN antibody responses were minimal to modest in subjects primed with 1 or 2 doses of these P/LAIV candidates.

Table 13. Overview of virologic and immunological responses in 8 supportive P/LAIV studies

Study	Treatment	No. of subjects	No. of subjects with virus shedding detected by rRT-PCR	% Seroresponse (≥ 4-fold rise in titres from baseline)				% nasal IgA response (≥ 4-fold rise in titres)	ASCs* (No. of subjects)
				MN	HAI	IgG	IgA		
CIR247 (H2N2)	Dose 1	21	6	0%	0%	14%	10%	0%	
	Dose 2	18	5	0%	12%	11%	6%	6%	
URMC10-004 (H2N3)	Dose 1	19	11 in total after either dose	No seroresponse detected after either dose				21% in total after either dose	1
	Dose 2	15							3
CIR251 (H6N1)	Dose 1	22	8	5%	5%	19%	14%	0%	
	Dose 2	18	6	0%	0%	11%	17%	6%	
CIR241*** (H7N3)	Dose 1	21	17	10%	14%	29%	52%	24%	12
	Dose 2	17	0	41%	41%	24%	29%	12%	far less frequent
URMC10-002 (H7N3)	One dose	20	13	0%	0%			0%	9
URMC10-003** (H7N7)	Dose 1	24	14	0%	0%	33% together		0%	1
	Dose 2	22	5	0%	0%	0%		0%	2
CIR293 (H7N9)priming	Dose 1	40	23	Final immunogenicity data not available (see text for interim results)					
	Dose 2	39	5						
CIR211 (H9N2) - seropositive	Dose 1	9	2	11%	0%	0%		11%	
	Dose 2	3	1	0%	0%	33%		0%	
- seronegative	Dose 1	41	15	24%	29%	12%		2%	
	Dose 2	24	2	50%	58%	13%		13%	

*IgG or IgA vaccine-specific ASCs (≥ 5 cell increases/106 PBMCs) at day 7, ***using modified HAI assay with 2 HA units/well, **serum IgG and IgA response defined using ≥ 2-fold increase and only measured in first 15 subjects enrolled. Within these 15 subjects , 7 (47%) also showed elevated B cell levels defined by > 2% CD27+CD28+ B cells on days 6, 7, and 8 post dose 1.

An additional study, URMC 14-004, is currently recruiting subjects. In this study, subjects 50 to 70 years of age will receive 2 doses (28 days apart) of P/LAIV H7N9 followed by one dose of an H7N9 pIIV 70 days after the second P/LAIV dose. While this study will provide some additional information on immune response following a boosting interval of 70 days, the primary goal of the study is to evaluate how successful P/LAIV is in generating immune responses in older subjects who have traditionally responded poorly to inactivated pandemic vaccines.

Pandemic H1N1pdm09 LAIV studies

Methodological aspects of studies MI-CP215 and MI-CP217

The sample size of the supportive H1N1 studies was based on safety considerations: with 300 evaluable subjects (240 vaccine, 60 placebo) and a true fever rate in placebo recipients between 0.5% and 2%, study MI-CP215 had at least 99.9% power to rule out a rate increase of 10 percentage points assuming the true difference between the treatment groups was zero and the true fever rate was ≤3%. With 300 evaluable subjects (240 vaccine, 60 placebo), a true fever rate in vaccine recipients between 3% and 8% and a 0% to 3% lower fever rate in placebo recipients, study MI-CP217 had between 66% and 99.9% power to rule out a rate increase of 10 percentage points.

An IVRS (Interactive Voice Response System) was used to randomize subjects in a 4:1 ratio to receive either 2 doses of active 2009 H1N1 vaccine or placebo. Randomisation was stratified by site. The studies were double blinded; to blind study participants, the active vaccine and placebo were identically labelled and indistinguishable in appearance.

The difference in proportions between treatment groups including their two-sided exact 95% confidence intervals (CIs) were constructed using the exact method proposed by Chan and Zhang. Geometric mean titres and geometric mean fold rises (GMFRs) were summarized for baseline seronegative subjects and all subjects by treatment group and by visit. The 95% CIs for GMFRs were constructed using a percentile-based bootstrap method.

MI-CP215

A randomised, double-blind, placebo-controlled study conducted from 03 August 2009 through 22 March 2010 in healthy adults 18 to 49 years of age at 5 sites in USA. The primary safety endpoint was the occurrence of fever ≥101°F during Days 1 to 8 after the first dose; the primary immunogenicity endpoint was the proportion of subjects experiencing a post-dose seroresponse (≥4-fold increase in HAI titres from baseline).

Of the 300 subjects enrolled, 240 received a first dose of H1N1pdm09 P/LAIV on day 1, and 228 received the second dose on day 29.

Regardless of baseline serostatus, seroresponse rates after receipt of H1N1 P/LAIV were 2.5% and 6.1% for Days 15 and 29, respectively, and 14.9% on Day 57. For placebo recipients, regardless of baseline serostatus, seroresponse rates were 0% on Days 15 and 29 and 5.6% on Day 57. Seroresponse rates were slightly higher among subjects who were seronegative at baseline.

MI-CP217

A randomised, double-blind, placebo-controlled study conducted from 03 August 2009 through 23March 2010 in children 2 to 17 years of age at 16 sites in the USA. The primary safety endpoint and the primary immunogenicity endpoint were the same as MI-CP215 study above.

326 subjects were randomized and 259 received the monovalent vaccine on day 1 and 256 of them received the second dose on day 29.

Regardless of baseline serostatus, seroresponse rates after receipt of monovalent vaccine were 7.8% and 11.1% for Days 15 and 29, respectively, and 32.0% on Day 57. For placebo recipients, regardless of baseline serostatus, seroresponse rate was 6.3% on Days 15 and 29 and 14.5% on Day 57. Seroresponse rates were slightly higher among subjects who were seronegative at baseline.

Effectiveness of pandemic H1N1pdm09 LAIV vaccine

The US CDC estimated the effectiveness of monovalent H1N1pdm09 LAIV vaccine using a test-negative study design. 7-days after a single dose of vaccine, vaccine effectiveness against influenza cases reported during this period was 60.6% (95% CI: 12, 82) among those aged 2-49 years, and 81.9% (95% CI: 14, 96) among those aged 2 to 9 years (Griffin, et al, 2011). Vaccine effectiveness for those 10 to 49 years of age was 26.4% (95% CI: -91.3, 71.7), however, due to the small number of cases the study was not adequately powered to assess effectiveness in this age group.

In school-aged children (approximately 5 to 14 years of age), vaccine effectiveness was estimated to be 81% (95% CI: -37, 97) against rRT-PCR–confirmed H1N1 infection vs. 58% for inactivated H1N1 vaccine (Uzicanin et al, 2012), and in children 2-9 years of age was 100% (95% CI: < 0, 100) against hospitalisation vs. 66% for inactivated vaccine (aged 3-9years) (Hadler et al, 2012).

Seasonal LAIV studies

Since very young children are generally seronaïve to circulating wild-type seasonal influenza strains, efficacy data gathered from the paediatric population with seasonal LAIV studies, including children less than 24 months of age, are of relevance for predicting the efficacy of P/LAIV H5N1 VN04 in this population.

In children, placebo-controlled studies with T/LAIV enrolling more than 12,000 subjects were conducted over 5 influenza seasons from 1996 through 2003 in Europe, Latin America, Africa, Asia/Oceania, and USA. In these studies, Fluenz consistently demonstrated protective efficacy against laboratory-confirmed influenza illness, following 2 primary doses of administration or a single dose re-vaccination in the second season (Table 19).

Table 14. T/LAIV efficacy in placebo controlled paediatric studies

Study number	Region	Age range[a]	Number of study participants[b]	Influenza season	Efficacy (95% CI)[c] matched strains	Efficacy (95% CI)[c] all strains regardless of match
D153-P502	Europe	6 to 35 M	1,616	2000-2001	85.4% (74.3, 92.2)	85.9% (76.3, 92.0)
			1,090	2001-2002	88.7% (82.0, 93.2)	85.8% (78.6, 90.9)
D153-P504	Africa, Latin America	6 to 35 M	1,886	2001	73.5% (63.6, 81.0)[d]	72.0% (61.9, 79.8)[d]
			680	2002	73.6% (33.3, 91.2)	46.6% (14.9, 67.2)
D153-P513	Asia/ Oceania	6 to 35 M	1,041	2002	62.2% (43.6, 75.2)	48.6% (28.8, 63.3)
D153-P522	Europe, Asia/ Oceania, Latin America	11 to 24 M	1,150	2002-2003	78.4% (50.9, 91.3)	63.8% (36.2, 79.8)
D153-P501	Asia/ Oceania	12 to 35 M	2,764	2000-2001	72.9% (62.8, 80.5)	70.1% (60.9, 77.3)
			1,265	2001-2002	84.3% (70.1, 92.4)[e]	64.2% (44.2, 77.3)[e]
AV006	USA	15 to 71 M	1,259	1996-1997	93.4% (87.5, 96.5)	93.4% (87.5, 96.5)
			1.358	1997-1998	100% (63.1, 100)	87.1% (77.7, 92.6)[f]

[a]M = months
[b]Number of study participants for year 1 or year 2 primary efficacy analysis.
[c]Reduction in culture-confirmed influenza illness relative to placebo.
[d]Data presented for clinical trial D153-P504 are for study participants who received two doses of study vaccine or placebo. In previously unvaccinated study participants who received one dose in year 1, efficacy was 57.7% (95% CI: 44.7, 67.9) against matched strains and 56.3% (95% CI: 43.1, 66.7) against all strains regardless of match, respectively, thus supporting the need for two doses of vaccine in previously unvaccinated children.
[e]In study participants who received 2 doses in year 1 and placebo in year 2, efficacy in year 2 was 56.2% (95% CI: 30.5, 72.7) against matched strains and 44.8% (95% CI: 18.2, 62.9) against all strains regardless of match, respectively, in D153-P501, thus supporting the need for second-season revaccination.
[f]The primary circulating strain was antigenically dissimilar from the H3N2 strain represented in the vaccine; efficacy against the mismatched A/H3N2 strain was 85.9% (95% CI: 75.3, 91.9).

Table 15. T/LAIV relative efficacy in active controlled paediatric studies with seasonal injectable influenza vaccine

Study number	Region	Age range[a]	Number of study participants	Influenza season	Improved efficacy (95% CI)[b] matched strains	Improved efficacy (95% CI)[b] all strains regardless of match
MI-CP111	USA, Europe, Asia/Oceania	6 to 59 M	7,852	2004-2005	44.5% (22.4, 60.6) fewer cases than injectable	54.9% (45.4, 62.9)[c] fewer cases than injectable
D153-P514	Europe	6 to 71 M	2,085	2002-2003	52.7% (21.6, 72.2) fewer cases than injectable	52.4% (24.6, 70.5)[d] fewer cases than injectable
D153-P515	Europe	6 to 17 Y	2,211	2002-2003	34.7% (3.9, 56.0) fewer cases than injectable	31.9% (1.1, 53.5) fewer cases than injectable

[a]M = months. Y = years. Age range as described in the protocol for the study.
[b]Reduction in culture-confirmed influenza illness relative to injectable influenza vaccine.
[c]T/LAIV demonstrated 55.7% (39.9, 67.6) fewer cases than injectable influenza vaccine in 3,686 infants and toddlers 6-23 months of age and 54.4% (41.8, 64.5) fewer cases in 4,166 children 24-59 months of age.
[d]T/LAIV demonstrated 64.4% (1.4, 88.8) fewer cases than injectable influenza vaccine in 476 infants and toddlers 6-23 months of age and 48.2% (12.7, 70.0) fewer cases in 1,609 children 24-71 months of age.

Following receipt of a single dose of Fluenz, 88.8% (95% CI 64.5, 96.5) of previously unvaccinated children in AV006 1996-1997 season and 57.7% (95% CI: 44.7, 67.9) in D153-P504 2001 season were protected from culture-confirmed influenza illness. Efficacy associated with a single dose was numerically or statistically lower than that of 2-dose regime.

Results of D153-P501 showed that, prior recipients of 2 primary doses of Fluenz in 2000-2001 without re-vaccination in 2001-2002 were still protected from laboratory confirmed influenza illness. Such a persistent efficacy into the second season was estimated to be 56.2% (95% CI: 30.5, 72.7) against matched strains and 44.8% (95% CI: 18.2, 62.9) against all strains regardless of match. A very similar finding was seen in D153-P504, with estimated persistent efficacy of 57% (95%CI: 6, 82) against matched strains and 35% (95% CI: -0.3, 59) against all strains regardless of match. Both studies showed that re-vaccination with a single dose of Fluenz in the second season provided additional benefit.

No immunological correlate of protection has been identified for Fluenz or LAIVs in general. Whereas the presence of a serum antibody response has been associated with protection from influenza illness, the absence of a significant serum antibody response following FluMist/Fluenz vaccination does not necessarily indicate absence of protection.

Co-Administration with other live vaccines

The co-administration of Fluenz with other live attenuated vaccines (measles, mumps, rubella, varicella, and orally administered poliovirus) has been studied in 3 studies with children 6 to < 36

months of age. All 3 studies assessed whether concomitant Fluenz would interfere with the immune responses to the other vaccines. No clinically meaningful changes in immune responses to measles, mumps, varicella, orally administered poliovirus or influenza vaccines have been observed. The immune response to a single dose of rubella vaccine was significantly altered. However, this alteration might not be of clinical relevance with the two dose immunisation schedule of the rubella vaccine. This observation with the seasonal T/LAIV vaccine is relevant to the use of Pandemic influenza vaccine H5N1 MedImmune, because Pandemic influenza vaccine H5N1 MedImmune and T/LAIV are manufactured by the same process.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The studies conducted with P/LAIV were open-labelled, small sample size, uncontrolled, and enrolled healthy adults but not the target group of vaccination based on the indication (i.e. paediatric subjects). These clinical studies had been conducted using the proposed commercial vaccine formulation and a dose regimen for which efficacy and effectiveness have been established for seasonal and pandemic H1N1pdm09 LAIVs. Serving as scientific proof of concept for predicting efficacy in paediatrics, there are no concerns related to study design. A statement by the Applicant that all clinical studies were performed in compliance with GCP or equivalent ethical standards was provided.

No validated assays were used and no blinding of laboratory personnel was undertaken for the 3 pivotal studies. However, qualified HAI and MN assays were used for CIR277 and for CIR293, the key supportive study. The extensive testing of qualification parameters, especially for HAI assay, supports the conclusion that the booster data generated in CIR277 are of satisfactory credibility. The use of the ISO 9001 certified laboratory also alleviates the concern of bias due to the absence of blinding measure in CIR277. The Applicant was recommended and agreed to submit post-authorisation the validation reports of HI and MN assays used in study CIR 277.

In pivotal studies CIR 217 and CIR 239 as well as in other P/LAIV studies, a predominantly Black American male population was enrolled. As such, this study population would not reflect the demographic situation in the EU. However, previous studies conducted with FluMist/Fluenz revealed that the safety and efficacy of the vaccine are not affected by the race and ethnicity of the vaccine recipients. Additional studies conducted at the University of Rochester and studies with the 2009 H1N1pdm vaccine sponsored by MedImmune enrolled a racially and ethnically more diverse set of subjects.

Efficacy data

The available data from CIR217 (P/LAIV H5N1 VN04), CIR239 (P/LAIV H5N1 HK03), URMC11-001 (P/LAIV H7N7), URMC13-001 (P/LAIV H7N9) and other 3 P/LAIV studies showed that, following 2-doses of these P/LAIV candidates in naive adults, the primed serological responses were commonly undetectable by the currently available immunoassays, which is consistent with previous observations made for seasonal and H1N1pdm09 LAIVs. Some exception to this were the low -up to moderate-immune responses against H7N3 and H9N2 vaccine viruses, measured by HAI or MN assay, in studies CIR241 and CIR211. This finding supports the assumption about the heterogeneity of serological immunogenicity of different P/LAIV candidates.

Therefore, for P/LAIV H5N1 VN04, the option that was favoured was to show its ability to effectively prime naïve subjects in a booster setting, that is to demonstrate notable serological responses in prior P/LAIV recipients upon re-vaccination with an inactivated H5N1 VN04 vaccine. Ideally the effect of this re-exposure should be assessed shortly after priming, e.g. in an interval of weeks or a few months.

The presented data from the prime-boost study CIR277 illustrated serological responses to re-vaccination approximately 5 years following priming in prior P/LAIV H5N1 recipients. Against wild-type H5N1 VN04 virus the functional HAI and MN antibodies were detected in 73% (8/11 seroresponders, i.e. subjects with at least a 4 fold increase in antibody titre vs. baseline values, or a titre of ≥ 1:20) of the re-vaccinated subjects at Day 28, after re-exposure to an inactivated vaccine. Of the P/LAIV naïve subjects, only 10% (1 dose of IIV) or 40% (2 doses of IIV) had seroconverted by day 28. The proportion of HAI seroresponders was 64% by Day 7, with a GMT of 165 and a titre range from 20 to 1280 in responding subjects. Of the P/LAIV-naive subjects, only 10% had ≥ 4-fold rises by Day 7 after 1 dose of IIV (GMT 8). The GMTs of HAI and MN antibodies peaked at Day 28, starting to decline at Day 56 and gradually reached undetectable level by Month 6. These serological responses are clearly characteristic of memory B cell responses. Since control cohorts of subjects (not primed by P/LAIV) commonly failed to develop such rapid and significant responses, it can be reasonably concluded that these memory responses can be ascribed to the original priming with P/LAIV H5N1 VN04. Preliminary data from study CIR 293 indicate that a similar or greater recall response could be boosted after a shorter interval (see below).

Against heterologous strains the serum antibodies of responding subjects with a MN titre of ≥1:40 neutralised H5N1 viruses of clade 2.1.3, clade 2.3.4, clade 2.2.1, and clade 2.2.1, demonstrating broadly neutralising activity of these antibodies. Consistent with this finding was the observation of increased antibody affinity in responding subjects.

Booster responses in prior recipients of only 1-dose P/LAIV H5N1 VN04 vaccine were not evaluated in study CIR277. Therefore, it remains to be fully elucidated whether a 1-dose regimen of P/LAIV has sufficient priming capacity. However, existing data from seasonal trivalent LAIV vaccine did show additional benefit of the second dose in very young children. In the monkey model, a single $2x10^6$ $TCID_{50}$ dose was ineffective in protecting against homologous wild-type virus challenge, in contrast to complete protection offered by 2 doses of vaccine. Interim results from study CIR 293 (further down) seems to be consistent with this conclusion. It may be argued that doses higher than $10^{7.5}$ $TCID_{50}$ could lead to more efficient immune responses. However the Applicant concluded to maintain for P/LAIV the dose previously established based on substantial safety and efficacy data generated with the seasonal and H1N1 LAIVs.

The ability of H5N1 P/LAIV to induce T cell responses was demonstrated in study CIR217. After 2 doses of P/LAIV H5N1 VN, influenza-specific T-cell responses to internal viral proteins (M and NP) were detected in most vaccinees. T-cell responses comprised both CD4+ and CD8+ T cells that were specific for influenza M and NP antigens and showed cytotoxic activities. Concerning HA, T-cell responses displayed lower responses vs. the H5 HA antigen, as compared to other HA proteins of seasonal influenza viruses. This finding indicates that P/LAIV may preferentially boost pre-existing specific T cell responses to the HA of the seasonal influenza virus (H1 and H3) rather than H5N1 HA.

The data of study URMC11-001 similarly showed the nature of a recalled memory responses in prior recipients of 2 doses of P/LAIV H7N7 candidate, upon re-exposure to H7N7 antigen via an inactivated vaccine. In this setting where re-exposure occurred 18-24 months later, the boosted responses peaked at Day 14 post-booster immunization: the cross-reactive HAI antibodies against H7N3 P/LAIV *ca*, H7N3 wt, and H7N9 wt developed in 69%, 46% and 69% of the subjects respectively, and the MN antibodies in 62%, 38% and 69% respectively.

Data up to Day 82 were submitted for URMC13-001. In this study setting where prime-boost interval was approximately 3 months, a robust response was detected on Day 7 in prior recipients of 2 doses of P/LAIV H7N9 candidate, upon re-exposure to H7N9 antigen in an inactivated vaccine (H7N9IIV). Thus, the H7N9 P/LAIV candidate vaccine primed for a rapid, robust antibody response to H7N9 IIV.

The interim results of CIR293 were included in the Day 120 responses. The data indicate that P/LAIV priming led to robust booster responses to H7N9 pIIV. The magnitude and frequency of HAI responses were greater in subjects primed with 2 doses of P/LAIV than in subjects primed with 1 dose P/LAIV or unprimed subjects (~100% of subjects vs. ~50%, respectively, had a ≥ 4 fold increase in HAI titre from baseline at day 14 post IIV (when administered 8 weeks post P/LAIV)). In addition, subjects primed with a single dose of P/LAIV had more robust responses by Day 14 than unprimed subjects who received 2 doses of inactivated vaccine (~50% responders and GMT ~24 vs. ~30% responders and GMT <8, respectively). The boosted HAI response followed typical kinetic as expected, with rapid onset as early as at Day 7, peaked at Day 14, and gradually declined thereafter. A dosing interval as short as 4 weeks appeared successful as there was no significant difference between the 4- and 8-week dosing intervals between P/LAIV prime and pIIV boost doses. Data from this study are of highly supportive value because of the relevance of prime-boost interval to re-exposure in a pandemic situation. Indeed these data suggest that P/LAIV H7N9 primed naïve subjects for a HAI response that could be significantly boosted upon antigen re-exposure shortly after priming. In addition, the advantage of a 2 doses-regimen over 1-dose regimen of P/LAIV H7N9 in priming was evidenced. Final results of study CIR293 should be submitted as soon as they are available.

It has to be reiterated that the immune response to the inactivated pandemic vaccine used for boosting in the prime-boost studies provides useful information on the ability of P/LAIV to prime various age groups against a poorly immunogenic influenza strain to which most humans, if not all, are naive. This study design including a boost dose by an inactivated vaccine is considered useful as an indirect proof of the potential for protection of a pandemic LAIV in the absence of efficacy data in the interpandemic period. Therefore the posology in the P/LAIV SmPC reflects the use of P/LAIV only as a protective vaccine following administration of 2 doses.

Assessment of paediatric data on clinical efficacy

Effectiveness and/or efficacy of pandemic/H1N1pdm09 and seasonal LAIVs

Supportive data gathered in the US during the 2009 H1N1 pandemic indicate that H1N1pdm09 LAIV is highly effective, especially in young children. A large difference in effectiveness was observed between the 10-49 year and the 2-9 year age strata (26.4% vs. 81.9% with a 7 day interval, from Griffin et al, 2011). Without additional stratified analysis in the 10-49 year group, the lower efficacy in this age stratum cannot be considered indicative of how well the LAIV actually performs in subjects 10-17 years of age. In addition, due to the small number of cases the study was not adequately powered to assess effectiveness in this age group. Similar results were obtained from 2 other effectiveness studies in children.

Supportive data from placebo-controlled paediatric studies comprising over 20,000 infants, toddlers, children and adolescents demonstrate vaccine efficacy for seasonal LAIVs from 62-93% for matched strains and 47-93% for all strains regardless of match.

Additional efficacy data to be provided in the context of a preparedness pandemic vaccine

Data demonstrating formal clinical efficacy of P/LAIV could not be generated since efficacy of a pandemic vaccine cannot be tested in the absence of a circulating pandemic virus. Instead, the expected efficacy of P/LAIV against pandemic influenza is inferred based on the following data:

1. Immunogenicity data gathered from adult clinical studies performed with candidate P/LAIVs;
2. Immunogenicity data gathered from adult clinical studies in which an inactivated pandemic vaccine was administered to unmask the long-lasting immunity induced by prior receipt of P/LAIV;
3. Effectiveness data in children gathered with the H1N1pdm P/LAIV during the 2009 Pandemic;

4. Clinical efficacy data obtained with Fluenz in immunologically naïve young children.

This approach is usually followed for pandemic preparedness vaccines, such as P/LAIV, whereby the vaccine is authorised in advance of a pandemic based on a core dossier that includes a minimum set of data needed to define the benefit risk balance in an emergency situation. For P/LAIV the core dossier is summarised by points 1 to 4 above, and, in addition, appropriate specific obligations have been identified in the context of a conditional marketing authorisation, in order to address the gaps in knowledge post-approval. P/LAIV can only be used after a pandemic is duly recognised in the EU and after a variation application is submitted to include in the vaccine the declared pandemic strain. Such specific obligations for P/LAIV include studies which aim at confirming vaccine performance with the actual pandemic strain, i.e. safety (see relevant section) and effectiveness in the intended target population. The CHMP requested that as soon as the vaccine is deployed during the next pandemic the MAH should conduct an observational effectiveness study in community dwelling children and adolescents to identify breakthrough cases of influenza by laboratory confirmation. Effectiveness data is expected to provide confirmation on the efficacy of the actual pandemic vaccine in the intended target population.

Further details of the effectiveness study have been considered by the PRAC during the MAA evaluation and are included in the agreed RMP.

In addition, as per agreed PIP, a single arm clinical trial in children and adolescent will investigate the safety and reactogenicity of the pandemic vaccine (see safety section), but also will explore as secondary endpoint the immunogenicity of the actual pandemic strain. This will provide supportive data on immunogenicity aspects of the actual pandemic strain in naïve children. For further details see the safety section.

2.5.4. Conclusions on clinical efficacy

The presented data are consistent and demonstrate that the proposed 2-dose regime of P/LAIV H5N1 is able to prime naïve adults and to elicit a memory response lasting at least 4-5 years, which is considered relevant for the claimed indication in children and adolescents 1-18 years of age. Pivotal immunogenicity data from CIR277 are considered sufficient for initial assessment of immunogenicity profile of P/LAIV H5N1 candidate vaccine and, in addition to the large body of evidence on efficacy in children with relevant seasonal and pandemic strains included in the same LAIV platform, are deemed sufficient to establish the benefits of P/LAIV and establish a dosing recommendation in the context of pandemic preparedness activities for future pandemics in the claimed paediatric indication.

The CHMP considers the following measures necessary to address the missing efficacy data in the context of a pandemic preparedness vaccine:

Description	Background
1. In order to further corroborate the efficacy of P/LAIV, the MAH should conduct an observational effectiveness study in community dwelling children and adolescents from 12 months to less than 18 years of age against laboratory confirmed influenza during the next declared pandemic. The MAH should submit the results of this study.	Effectiveness data generated during the pandemic will provide confirmation of the efficacy of the vaccine with the actual pandemic strain.

The CHMP considers that it would be relevant to follow-up on aspects related to efficacy that were

discussed in this application, and thus recommends the following measures, to which the Applicant agreed:

1. Submit the validation of HAI and MN assays performed for study CIR277, pending availability of the sera.
2. For study CIR293, provide the results of the serological testing performed at Southern Research against wild-type viruses as soon as they are available.

2.6. Clinical safety

Patient exposure

Tables 21 and 22 summarise the patient exposure to the H5N1 P/LAIV and to the other P/LAIVs tested for this application (i.e. H2N2, H2N3, H6N1, H7N3, H7N7, H7N9, and H9N2 P/LAIV). 59 subjects received at least one dose of H5N1 P/LAIV based on two different strains (*ca* A/Vietnam/1203/2004 and *ca* A/Hong Kong/213/2003) and 288 were exposed to the P/LAIVs based on the other potential pandemic strains. Only healthy adults from 18 to 49 years of age were enrolled in the studies.

Safety data from 300 adults 18 to 49 years of age and 324 children 2 to 17 years of age who received at least one dose of H1N1pdm LAIV in two clinical trials conducted during the pandemic situation in 2009 are considered supportive.

Table 16. Investigational Product Exposure, Pivotal H5N1 P/LAIV Studies

Study	Vaccine Strain (Subtype)	Dose Level	Number of Subjects Receiving at Least 1 Dose	Number of Subjects Receiving at Least 2 Doses
CIR 217	*ca* A/Vietnam/1203/2004 (H5N1)	$10^{6.7}$ $TCID_{50}$	21	18
CIR 217	*ca* A/Vietnam/1203/2004 (H5N1)	$10^{7.5}$ $TCID_{50}$	21	19
CIR 239	*ca* A/Hong Kong/213/2003 (H5N1)	$10^{7.5}$ $TCID_{50}$	17	16
Total			59	53

ca = cold adapted; CIR = Center for Immunization Research; P/LAIV = pandemic live attenuated influenza vaccine; $TCID_{50}$ = tissue culture median infectious dose.

Table 17. Number of Subjects Exposed to H2N2, H2N3, H6N1, H7N3, H7N7, H7N9, or H9N2 P/LAIV by Dose Level and Number of Doses, Supportive P/LAIV Studies

Study	Vaccine Strain (Subtype)	Dose Level	Number of Subjects Receiving at Least One Dose	Number of Subjects Receiving at Least Two Doses
CIR 247	*ca* A/Ann Arbor/6/60 (H2N2)	10^{7} $TCID_{50}$	21	18
URMC 10-004	*ca* A/swine/Missouri/4296424/2006 (H2N3)	$10^{7.5}$ $TCID_{50}$	19	15
CIR 251	*ca* A/Teal/Hong Kong/W312/1997 (H6N1)	$10^{7.0}$ $TCID_{50}$	22	18

Study	Vaccine Strain (Subtype)	Dose Level	Number of Subjects Receiving at Least One Dose	Number of Subjects Receiving at Least Two Doses
CIR 241	*ca* A/chicken/British Columbia/CN-6/2004 (H7N3)	$10^{7.5}$ $TCID_{50}$	21	17
URMC 10-002	*ca* A/chicken/British Columbia/CN-6/2004 (H7N3)	$10^{7.5}$ $TCID_{50}$	20	NA
URMC 10-003	*ca* A/Netherlands/219/03 (H7N7)	$10^{7.5}$ $TCID_{50}$	24	22
URMC 13-001	*ca* A/Anhui/1/2013 (H7N9)	$10^{7.0}$ FFU	32	16
CIR 293	*ca* A/Anhui/1/2013 (H7N9)	$10^{7.0}$ FFU	40	NA
	ca A/Anhui/1/2013 (H7N9)	$10^{7.0}$ FFU	39	39
CIR 211	*ca* A/chicken/Hong Kong/G9/97 (H9N2)	$10^{7.0}$ $TCID_{50}$	50	27
Total Number of Subjects			288	172

ca = cold adapted; CIR = Center for Immunization Research; FFU = fluorescent focus unit; NA = not applicable; P/LAIV = Pandemic Live Attenuated Influenza Vaccine; $TCID_{50}$ = tissue culture median infectious dose; URMC = University of Rochester Medical Center.

The exposure to seasonal Q/LAIV and T/LAIV is presented as safety data gathered from the seasonal LAIVs and are also considered supportive.

Q/LAIV exposure

A total of 3,783 subjects received at least 1 dose of Q/LAIV in 3 clinical studies, including 1,199 subjects who received Q/LAIV by a different delivery system that was not further developed. A total of 1, 386 Q/LAIV recipients were 2 years to less than 18 years of age.

Table 18. Q/LAIV Exposure by Age Group and by Gender - All Q/LAIV Studies

Age Group [a]	Q/LAIV Total			Q/LAIV in Accuspray			Q/LAIV-BFS		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
2 to < 18 years	679	707	1,386	679	707	1,386	0	0	0
2 to < 9 years	542	545	1,087	542	545	1,087	0	0	0
9 to < 18 years	137	162	299	137	162	299	0	0	0
≥ 18 years	1,041	1,355	2,397	541	657	1,198	501	698	1,199
Total	1,721	2,062	3,783	1220	1364	2,584	501	698	1,199

BFS: blow-fill-seal; a: age at first dose

T/LAIV exposure

A total of 51,393 subjects have received T/LAIV (frozen or refrigerated formulation) in clinical studies (excluding post marketing studies). 39,116 subjects where 12 months to less than 18 years of age. Additionally, to date over 80 million doses of T/LAIV have been distributed commercially.

Table 19. T/LAIV Exposure by Age Group and by Gender - All Clinical Safety Studies except Post marketing Studies as of 16 Dec 2011

Age Group [a]	Frozen/Refrigerated FluMist [b]				Refrigerated FluMist Subset		
	Male	Female	Missing	Total	Male	Female	Total
< 1 year	1,003	956	0	1,959	1,003	955	1,958
1 to < 2 years	4,952	4,706	0	9,658	3,335	3,070	6,405
2 to < 18 years	14,930	14,528	5	29,463	5,246	4,736	9,982
2 to < 9 years	10,308	10,045	1	20,354	4,352	4,032	8,384
9 to < 18 years	4,622	4,483	4	9,109	894	704	1,598
≥ 18 years	5,037	5,276	0	10,313	1,929	2,765	4,694
18 to < 50 years	2,374	2,996	0	5,370	664	824	1,488
50 to < 64 years	924	803	0	1,727	355	526	881
≥ 64 years	1,739	1,477	0	3,216	910	1,415	2,325
Total	25,922	25,466	5	51,393	11,513	11,526	23,039

[a] Age at first dose.

[b] Only subjects who received the 10^7 per strain dosage level of trivalent frozen or refrigerated FluMist were included.

Note: Data as of 16Dec2011

Adverse events

Pivotal H5N1 P/LAIV studies

Study reports, protocols, publications and study narratives were submitted for the 3 pivotal H5N1 studies. For the supportive P/LAIV studies with other vaccine candidates of pandemic potential only study narratives with or without publications were submitted. This was agreed upon during preliminary discussions with the Applicant.

In summary, based on the review of the limited safety data from the pivotal H5N1 P/LAIV studies and the supportive P/LAIV studies conducted with other pandemic vaccine candidates, the solicited symptoms reflect the mechanism of action of LAIVs and are consistent with those previously observed with seasonal LAIVs (Fluenz and Fluenz tetra) and with the monovalent H1N1pdm LAIV evaluated in the setting of the influenza pandemic in 2009. The new safety data evaluated in this dossier are derived from adults from 18 to 49 years of age.

CIR 217

The most frequently reported AE considered to be possibly related to the study vaccine in all subjects following any dose was headache (12 subjects), followed by AEs affecting the upper respiratory tract, like rhinorrhoea (3 subjects), nasal congestion and sore throat (1 subject each). Other AEs possibly related to the study vaccine were fever, myalgia, stomach cramps, diaphoresis and diarrhoea (one subject each). Other events for which relatedness could not be totally excluded (“remote”) were cough, conjunctival erythema, indigestion, nosebleed, pharyngitis and sinus congestion. All events were of mild or moderate intensity and all resolved. One participant with a history of wheezing had transient asymptomatic wheezing noted by auscultation on Day 2 and on Day 7 following Dose 1. The subject did not receive a second vaccine dose.

CIR 239

The most frequently reported RE/AE possibly related to the study vaccine in all subjects following any dose was headache experienced by 9 subjects, followed by nasal congestion in 4 subjects. Other REs/AEs possibly related to the study vaccine were rhinorrhoea, upper respiratory tract infection, lymphadenopathy cervical, chills, sore throat and throat pain (reported by one subject each). The events were of mild to moderate intensity. All events resolved.

CIR 277 (prime-boost study)

No P/LAIV was administered in this study. The administered inactivated H5N1 influenza vaccine was generally well tolerated in all groups. None of the previously P/LAIV recipients reported local AEs.

In the overall study population, the most common systemic solicited AE in the study was headache (4 events) followed by fatigue (3 events), nausea (3 events), vomiting (2 events) and myalgia (1 event). All systemic solicited AEs were deemed to be mild. The most common AEs in all groups were injection site pain (15.9%), respiratory rate (8.7%), contusion (5.8%), diastolic hypertension (5.8%), headache (4.3%), fatigue (4.3%), and blood pressure increased (4.3%).

The Applicant claims that the P/LAIV generally exhibited a safety profile similar to that of seasonal LAIV, with reports of a few minor illnesses. For the H5N1 P/LAIV this is based on comparison of AEs from 59 subjects in two studies with several tens of thousands in Fluenz/Fluenz Tetra subjects.

The CHMP agrees with this assessment, although the H5N1 data are limited.

Supportive P/LAIV studies

The assessment is based on publications and study narratives.

288 adult healthy subjects were exposed to P/LAIVs based on other vaccine strains with pandemic potential. In comparison to subjects in the H5N1 studies, the most frequently reported RE/AEs were headache and nasal congestion. Overall, the P/LAIVs were well tolerated and the safety profile was comparable to that of seasonal T/LAIV and Q/LAIV.

Safety of H1N1 P/LAIV

The US FDA approved all of the Influenza A (H1N1) 2009 vaccines as a strain change to each manufacturer's approved seasonal influenza vaccine. In support of this strain change monovalent H1N1 P/LAIV vaccine prepared from the new A/California/7/2009 strain was evaluated in two studies (MI-CP215 and MI-CP217). Both studies were randomized, double-blind, placebo-controlled and were conducted at multiple sites in the United States from August 2009 through March 2010. The study objectives were to evaluate the safety and immunogenicity of 2 doses of H1N1 LAIV in either healthy children aged 2 to 17 years or healthy adults aged 18 to 49 years.

The primary safety endpoint was the occurrence of fever ≥ 38.3°C (101° F) during Day 1 to Day 8 after Dose 1. Additional safety endpoints included solicited events, AEs, and antipyretic and analgesic use from Day 1 through Day 8 and from Day 1 through Day 15 following each vaccination. SAEs and new onset chronic diseases (NOCDs) were collected through Day 180 following the final dose. Solicited symptoms included fever (temperature was recorded daily), runny nose (adults) or runny/stuffy nose (children), sore throat, cough, vomiting (adults), muscle aches, chills (adults), decreased activity, decreased appetite (children), and headache.

Generally the two studies demonstrated that 2 doses of the H1N1 P/LAIV are safe in children from 2 to 17 years of age and in adults from 18 to 49 years of age.

Safety data were collected from 324 children following dose 1 (259 received H1N1 P/LAIV and 65 received placebo) and 318 children following dose 2 (255 received vaccine and 63 placebo), and from 300 adults following Dose 1 (240 received vaccine and 60 received placebo) and 283 adults following dose 2 (228 received vaccine and 55 received placebo).

There was no statistical difference between treatment groups for the primary endpoint in children or adults. Among children, fever ≥38.3 °C occurred in 1.5% (n= 4) of vaccine recipients and in 1.5% (n= 1) of placebo recipients following Dose 1 (95% CI: –6.4%, 3.1%), and in 1.2% (n= 3) and 0% following Dose 2 (rate difference, 1.2%; 95% CI: –4.1%, 3.7%) respectively. Fever was not reported among adult subjects following Dose 1 but was reported in 0.4% (n= 1) and in 1.8% (n= 1) of vaccine and placebo recipients respectively following Dose 2 (rate difference, –1.4%; 95% CI: –8.7%, 1.4%).

The percentage of individuals reporting solicited symptoms decreased in both adults and children following Dose 2. Also antipyretic and/or analgesic use following Dose 1 and 2 was not significantly different among vaccine and placebo recipients in both studies.

In conclusion, the adverse event profile of the 2009 H1N1 vaccine was comparable to that of the seasonal T/LAIV and Q/LAIV. The Applicant has described the background for using fever as the safety endpoint in the two pandemic H1N1 studies in children and adults. The starting point was the annual safety studies performed in the US in adults, which were used as models for the paediatric and adults H1N1 safety studies. In those studies, a vaccinated group is compared to a placebo group regarding the rate of fever. The equivalence criteria used in these studies is 5% for the upper limit of 95% CI of the difference (FluMist minus placebo). Based on a different risk-benefit consideration in the case of a pandemic vs. a normal influenza season (a vaccine with a slightly higher rate of fever than placebo would still have the potential to provide substantial overall benefit), the Applicant has argued for a higher limit of demonstrating equivalence, i.e. criterion of 10%, in the pandemic H1N1 studies. This is agreed. Concerning the use of the same equivalence criterion of 10% difference for children and adults, a higher rate of fever in children could be expected compared to adults in the vaccine group - and therefore a higher difference. However for both age groups the difference in rate of fever was 0.0%. In the case of children the upper limit of 95% CI of the difference was 3.1 and for adults it was 1.9. As no subjects reported fever in the adult group, the higher limit for equivalence compared to seasonal vaccine could have been also set at 5%. However, the equivalence criteria are justified based on vaccine strain and target population.

MI-CP 215

The most common solicited symptom reported by adults through Day 8 post Dose 1 was headache. The rate of headache did not significantly differ between vaccine recipients and placebo recipients (25.4% versus 20.0% of subjects respectively). Significantly more vaccine recipients than placebo recipients experienced runny nose (15.4% versus 5.0%) and muscle aches (6.7% versus 0.0%).

Adverse events considered by the investigator to be related to investigational product were reported in 7.9% of subjects in the vaccine and 8.3% placebo group through 15 days post Dose 1. The only vaccine related AEs ≥1% in the vaccine group were nasal congestion (1.3%), throat irritation (1.3%) and rash (1.3%).

MI-CP 217

Like in adults the most common solicited symptom in the paediatric population through Day 8 post dose 1 was headache (16.6% of subjects receiving vaccine and 15.4% of subjects receiving placebo; rate difference, 1.2%; 95% CI: –10.2%, 10.2%). Runny nose/nasal congestion was the solicited symptom reported more frequently by vaccine recipients with the highest rate difference compared to placebo recipients (15.8% versus 12.3%, RD3.5 % [95% CI: -7.2, 11.8]).

The most common AEs in the paediatric vaccine group, reported in a frequency ≥ 1.0% through Day 15 post Dose 1 were vomiting (2.7%), nausea (1.9%), diarrhoea (1.5%), abdominal pain upper (1.5%) and ear pain (1.2%).

Post-marketing safety assessment of H1N1 P/LAIV

The safety of monovalent H1N1 LAIV was evaluated using data generated from the Vaccine Safety Datalink (VSD) Database (Lee et al, 2011). Eleven potential neurologic, allergic, and cardiac AEs were monitored. No significant associations were noted during sequential analyses for the monitored events. As of May 2010, a total of 267,715 monovalent H1N1 P/LAIV doses were administered.

Safety of Q/LAIV

CSRs from 3 clinical studies (MI-CP208, MI-CP185 and MI-CP206) evaluating the immunologic non inferiority of Q/LAIV compared to T/LAIV provide supportive safety data for the approval of the pandemic preparedness vaccine P/LAIV. The 3 studies were previously submitted to the EMA and were reviewed to approve Fluenz Tetra in the EU.

The studies evaluated the safety profile of Q/LAIV directly compared to that of T/LAIV in 2 pivotal studies. Study MI-CP208 was conducted in paediatric subjects (2 to 17 years of age) and study MI-CP185 in adult subjects (18 to 49 years of age). In an additional study (MI-CP 206) Q/LAIV was administered to adults 18 to 49 years of age by a novel blow-fill seal device (BFS) as a stream in one nostril as opposed to a spray into two.

In summary, the safety profile of Fluenz Tetra was similar to that of the trivalent Fluenz.

The proportion of subjects reporting any solicited symptom following Dose 1 in the paediatric population of study MI-CP208 was comparable between the Q/LAIV subjects and all T/LAIV subjects (overall 47.9% versus 47.4%) following Dose 1. Fever ≥ 38.5°C was more often reported by Q/LAIV subjects compared to T/LAIV subjects (5.7% versus 3.9% of subjects) following Dose 1. Fever was the only solicited AE reported with a rate difference ≥ 1.0 %. The overall rates of fever were low and fever was generally mild or moderate. Runny nose/nasal congestion was the most commonly reported solicited AE in both vaccine groups (32.3% of Q/LAIV subjects versus 32.0% of all T/LAIV subjects). The proportion of subjects reporting any solicited AE in the 2 dose groups decreased from Dose 1 to Dose 2 in both vaccine arms. AEs were balanced with 21.0% of subjects in the Q/LAIV and 20.7% in the T/LAIV reporting any AE. The most commonly reported AE was vomiting in both groups.

In the adult population enrolled in study MI-CP185 the rates of solicited symptoms was comparable between Q/LAIV and T/LAIV recipients (59.6% in the Q/LAIV and 60.0% in the T/LAIV vaccine group). Runny/stuffy nose, reported by 4.1% more Q/LAIV recipients than T/LAIV recipients, was the most commonly reported solicited symptom (43.6% of subjects in the Q/LAIV and 39.5% in the T/LAIV vaccine group), followed by headache (28.2% versus 27.5%), sore throat (19.0% versus 19.8%), lethargy (17.6% versus 17.8%) and cough (13.6% versus 12.6%).

The most common AEs reported in the study through Day 28 were sneezing, oropharyngeal pain, rhinorrhoea and upper respiratory tract infections.

Safety of T/LAIV

T/LAIV was approved in June 2003 under the trade name of FluMist in the US and 2011 in the EU under the trade name of Fluenz. Since December 2013, the QLAIV, Fluenz Tetra, has replaced the T/LAIV, Fluenz, in the EU. Safety data to support the approval of Fluenz in the EU derived from over 141,000 subjects who received the frozen or refrigerated liquid formulation of Fluenz in 73 clinical and post marketing studies that were conducted from 1994 to 2008 in multiple regions of the world. 39 studies included more than 39,000 children aged 7 weeks to 17 years of age. Additionally, to date over 80 million doses of T/LAIV have been distributed commercially.

Overall, based on the review of clinical data during the assessment of the Fluenz MAA, T/LAIV was considered safe and well tolerated in adults and children with a safety profile similar to that of TIV.

To support the proposed age indication for the pandemic preparedness vaccine (i.e. 12 months of age through less than 18 years of age), pooled safety data for solicited events (SEs) and AEs derived from TIV- and placebo-controlled studies with more than 37,000 subjects 1 to 17 years of age were included in the application dossier. The pooled safety data were previously submitted to the EMA and reviewed to support approval of Fluenz in the EU.

Of the 37,968 subjects 1 to 17 years of age who received either refrigerated or frozen T/LAIV and contributed data to the pooled analysis for Year 1, 17,922 received 2 doses. Of the 15,322 subjects who received refrigerated T/LAIV, 10,466 of subjects received 2 doses.

As mentioned, T/LAIV was considered safe and well tolerated in adults and children with a safety profile similar to that of TIV. In subjects <18 years of age, runny/stuff nose was more commonly observed in the T/LAIV group than the TIV groups (59.3% versus 47.0%). Other solicited AEs through Day 10 occurred only in a slightly higher rate in Fluenz recipients compared with the TIV group: decreased appetite (16.8% in T/LAIV recipients versus 15.9% in TIV recipients), irritability (16.9% versus 15.5%, respectively), headache (10.5 versus 9.5%) and fever ≥ 38.0° C (10.9% versus 9.9%). High fever (≥39.5° C) was no more common in T/LAIV subjects than in subjects who received placebo or TIV. The most frequently reported AE that occurred at a higher rate in T/LAIV than TIV or placebo subjects was pyrexia.

The incidence of solicited AEs was lower post Dose 2 compared with the incidence post Dose 1 in T/LAIV studies.

Wheezing in individuals below 24 months of age for T/LAIV

A major identified safety signal for the T/LAIV was the increased risk of wheezing through 42 days post vaccination in children below 24 months of age. Study AV019, a Phase 3, placebo-controlled study conducted in 9,689 children 1 to 17 years of age indicated a signal for asthma/reactive airway disease in children 18 to 35 months of age. Based on this finding in the subgroup analysis, a pivotal study (MI-CP 111) was conducted to evaluate the safety in children 6 to 59 months of age. MI-CP111 was a large randomised, double-blind, multicentre trial conducted in children 6 to 59 month of age in the US, Asia, Europe and Middle East. The study evaluated safety and relative efficacy of T/LAIV compared to TIV. 4243 subjects were randomised in the LAIV group, and 4232 in the TIV group. Children who had previously received any influenza vaccine were to receive one dose of either T/LAIV or TIV and children who never had previously received any influenza vaccine received two doses of vaccine.

An increased rate of medical significant wheezing (MSW) up to 42 days after vaccination was seen in infants and toddlers from 6 to 23 months of age (5.9% of subjects in the in T/LAIV versus 3.8% in the TIV group, p-value 0.002). The rate of wheezing was not higher in children 24 months and older receiving T/LAIV compared TIV.

The increased incidence of MSW was particularly seen in children with a history of wheezing/asthma: 11% of subjects below 24 months of age with a history of wheezing in the one dose LAIV group reported the wheezing through Day 28 following dose one, in the TIV group it was 2.0%, respectively. In subjects of the same age range without a prior history of wheezing, AEs were reported by 3.7% of subjects in the LAIV group through Day 28 and 1.4% in the TIV group.

The majority of medically significant wheezing events from T/LAIV clinical Study MI-CP111 were treated on an outpatient basis. No deaths, intensive care unit admissions, or need for mechanical ventilation occurred. The majority of subjects below 24 months of age in the 2 dose group who had MWS reported post dose 1 met the criteria for medically significant wheezing based solely on a new bronchodilator prescription (40 of 55 in the LAIV group, 73%; and 25 of 34 in the TIV group,74%). The rates of respiratory distress in the LAIV and TIV groups were 27% and 21%, respectively, and the corresponding rates of hypoxemia were 5% and 12% respectively. Through Day 42 post dosing only 0.3% of all subjects in the LAIV and 0.2% in the TIV group were hospitalised in association with MSW. In the age subgroup below 24 months of age a total of 9 subjects 0.22%) in the LAIV and 3 subjects in the TIV vaccine group (0.07%) were hospitalised. No deaths resulted from these events, and none of the hospitalized children required mechanical ventilation or admission to an intensive care unit. MSW occurred mainly during weeks 2, 3 and 4.

Hospitalisation in subjects below 12 months of age with T/LAIV

A statistically significantly increased rate of hospitalizations through 180 days after the receipt of T/LAIV compared to the receipt of TIV was observed in subjects 6 to 11 months of age (6.1% versus 2.6%). Most of the excess hospitalizations in this subset of younger children were due to late events, were not temporally clustered, and were due to diagnoses commonly expected to occur in a young paediatric population. Most hospitalisations were due to gastrointestinal and respiratory tract infections and occurred more than 6 weeks post vaccination. The rate of hospitalizations was not increased in Fluenz recipients of ages 12 months and older.

Vaccine virus shedding

In general, the P/LAIV vaccines were highly restricted in replication with vaccine virus detected in only a small proportion of subjects. The peak incidence of shedding generally occurred on Day 1. Vaccine virus was more often detected by rRT-PCR than by culture. The incidence of shedding appears slightly lower compared to shedding of vaccine virus in adult subjects who received T/LAIV.

The Applicant discussed the potential variation in the safety profile of potential pandemic strains according to the different properties to replicate in humans. Replication of the seasonal vaccine virus in humans as measured by shedding has been shown to be age dependent with more shedding at younger age. However, the incidence of reactogenicity does not seem to vary by age groups 5-8 years, 9-17 years or 18-49 years.

For the vaccine virus strains with a pandemic potential, the data are limited to a small number of subjects per strain. When shedding is compared to the rate of adverse events there is no obvious association. In conclusion, the data for the seasonal strains as well as the pandemic strains do not support an association between replication of the vaccine strains and the rate of adverse events.

Serious adverse event / deaths / other significant events

P/LAIV studies (pivotal and supportive)

SAEs and deaths during the 2 pivotal H5N1 P/LAIV studies were collected through Day 56 for subjects receiving a single dose of vaccine and through Day 28 following the second dose of vaccine for subjects receiving 2 doses. The two vaccine doses were administered at 4-8 week intervals. Deaths and SAEs in the supportive P/LAIV studies were collected until study end.

There were 4 SAEs in H5N1 study CIR 217, while there was none in H5N1 study CIR 239.

In subjects receiving H5N1 P/LAIV only one SAE considered to be possibly related to the vaccine by the Principal Investigator occurred in study CIR 217. The event was considered to be unrelated to the vaccine by the NIH Medical monitor. A 20 year old Black/African American received 2 doses of *ca* A/Vietnam/1203/2004 (H5N1) vaccine ($10^{6.7}$ $TCID_{50}$). At Day 7 following Dose 2 laboratory tests revealed a Grade 4 absolute neutrophil count (ANC) of 443 cells/ ml. The subject was asymptomatic. Subject`s baseline account was within a normal range (1526 cells/ml). The result was confirmed at Day 8 on the original blood sample. ANC performed at Day 9 was within a normal range (2036 cells/mm3).

Other SAEs occurring during the H5N1 P/LAIV studies were swelling/pain of right leg occurring 122 days post vaccination, traumatic brain damage occurring 129 days post-vaccination and pain at neck and shoulder, road traffic accident occurring 144 days post-vaccination. SAEs in the supportive pandemic P/LAIV studies were asthma exacerbation occurring at Day 102 following Dose 2 of H7N7 P/LAIV and gastroenteritis occurring at Day 12 following Dose 1 of H9N2 P/LAIV. All SAEs except the traumatic brain damage resolved.

There were no deaths reported from the H5N1 P/LAIV studies. One death was reported from one supportive P/LAIV study (study CIR 293). A 49-year old male died 16 days following the second dose of H7N9 P/LAIV (A/Anhui/1/2013 (H7N9)). As the final autopsy indicated the cause of death was an overdose of heroin and fentanyl; the death was assessed as not related to the study vaccine.

H1N1 P/LAIV studies

SAEs were formerly assessed by the US FDA for the approval of the H1N1pdm LAIV.

A total of 5 SAEs were reported by 5 adult subjects, 3 in the vaccination group (depression, cellulitis and premature delivery) and 2 in the placebo group (gall bladder disease and possible cervical cancer). None of the reported SAEs was considered to be vaccine related.

Two new onsets of chronic diseases (NOCDs) were reported during the study. One subject in the monovalent vaccine group was diagnosed with hypothyroidism on Day 15 post dose 1. One subject in the placebo group reported a diagnosis of possible cervical cancer approximately 4 months post dose 1 (this event was also considered an SAE). Both NOCDs were considered by the Investigator to be unrelated to investigational product.

There were no deaths reported during the adults H1N1 P/LAIV study.

Three subjects from the paediatric study, two in the vaccine group and one in the placebo group reported an SAE during the study (depression 27 days post dose1, osteomyelitis 132 days post dose 2 and staphylococcal cellulitis of the abdomen and face). None of the SAEs was considered to be vaccine related by the investigator.

One paediatric subject in the placebo group reported a NOCD (attention deficit hyperactivity disorder) on Day 53 of the study. This event was considered by the Investigator to be not related to investigational product.

Seasonal LAIV studies

SAEs were formerly assessed for the approval of Q/LAIV and T/LAIV.

Q/LAIV studies

Serious adverse events related to vaccination were only experienced by 2 subjects: 1 hypersensitivity event in a T/LAIV recipient and 1 spontaneous abortion in a Q/LAIV-BSF recipient. For the SAE spontaneous abortion, the temporal association led to the assessment of a possible relationship to Q/LAIV, but according to the Applicant there is no overall pattern to suggest a causal relationship between Q/LAIV and spontaneous abortion. It can be argued that the study population is too small for such a pattern to emerge. No definite conclusions on a putative causal relation between Q/LAIV and spontaneous abortion can therefore be drawn (see also EPAR Fluenz Tetra, 2013).

T/LAIV studies

Analysis of SAEs and deaths case-reports in any ages did not reveal any significant safety concern with the use of the product. No death (119 cases for >141,000 Fluenz recipients) was considered to be related to Fluenz (see also EPAR Fluenz, 2011).

Laboratory findings

In study CIR 217 8 events of ALT increase, 3 events of ANC decrease, one event of microcytic anaemia and one event of AST increase were recorded in the Cumulative List. All abnormal findings were of mild to moderate intensity and all resolved. Two of the cases of ALT increases (in the high dose group) were considered as possibly related to vaccine.

Three more cases of ALT elevation were assessed as unrelated to P/LAIV and were not listed. In study 239 one ALT elevation and one thrombocytopenia both on Day 7 following the first vaccination were recorded. Both abnormal findings were of mild intensity and resolved. The laboratory anomalies were considered to be possibly related to the study vaccine. The platelet count returned to within normal limits on Day 9 and the ALT had fully normalized by Day 15.

The biological plausibility of the ALT elevations in Study CIR 217 and the live attenuated vaccine is considered low because of the following aspects:

1) there does not appear to be a dose-dependent relationship (ALT were elevated in subjects with lower doses of vaccines);

2) ALT elevations at the time of hospital admission are not a prominent feature of the wild-type H5N1 influenza illness;

3) ALT elevations, transaminitis and AEs associated with abnormal liver function have been very rarely reported with the seasonal LAIV, which contains the same Master Donor Viruses as the pandemic vaccine;

4) potential alternative explanations exist for the mild to moderate asymptomatic increases in ALT levels that were seen.

The assessment of the safety of the seasonal LAIV at the time of marketing authorisation did not indicate that hepatobiliary disorders occur at higher levels in LAIV recipients. Liver abnormalities are not a known or potential risk for the seasonal LAIV. These findings are therefore considered relevant for the P/LAIV dossier, since P/LAIV contains the identical *ts*, *ca* and *att* genetic elements.

Safety in special populations

No special populations have been studied with P/LAIV.

Paediatric population

No paediatric studies have been performed with P/LAIV. A paediatric study included in the paediatric investigational plan is deferred until the next pandemic is declared and a pandemic virus identified. The data with the seasonal LAIVs and the H1N1 pandemic vaccine have been submitted and are considered supportive for this application. A large amount of confirmatory safety data in paediatrics is expected to be generated during the course of the next pandemic (see section on clinical safety discussion).

The most important group that is different in the indication for use for the P/LAIV compared to Fluenz/Fluenz Tetra is the age group 12-24 months. FluMist Quadrivalent in the US, Fluenz and Fluenz Tetra in Europe is indicated for children and adolescents from 24 months to less than 18 years of age. The reason for the lower limit of the age range of the seasonal LAIVs indication is the increased rate of medically significant wheezing and any wheezing observed with Fluenz compared to inactivated influenza vaccine (TIV) in the age group 6-23 months and 12-23 months. Below 12 months of age, Fluenz also was associated with higher rate of hospitalization for any cause than TIV.

Increased rates of medical significant wheezing and all cause hospitalisation were observed in the pivotal study MI-CP 111 for T/LAIV that was previously submitted to the EMA. No new data pertaining to the safety signal of wheezing or all cause hospitalisation were submitted in the dossier for H5N1 P/LAIV.

The increased rate of wheezing in infant between 1 and 2 years of age seen with T/LAIV is not considered a deterrent for the use of P/LAIV in a H5N1-like pandemic scenario. This is based on different benefit/risk considerations in a pandemic vs. a seasonal scenario (see also the sections on clinical safety discussion and on benefit/risk assessment).

Individuals with asthma, wheezing or respiratory disease

A few T/LAIV studies included subjects with a history of respiratory illness, asthma or wheezing. In summary, the safety profile of T/LAIV in subjects of 24 months of age and older was comparable to that of the comparator in studies prospectively designed to assess the safety of T/LAIV in subjects with asthma and wheezing.

Subjects with severe asthma and subjects < 5 years of age with recurrent wheezing were excluded from all Q/LAIV and P/LAIV studies and from the majority of T/LAIV studies.

Safety of T/LAIV in children with severe asthma has only been evaluated in one small placebo-controlled study (Study AV10) were children 9 to 17 years of age with moderate or severe asthma where included. The mean change in percent of predicted FEV1 (forced expiratory volume in 1 second) between baseline and visit 3 was similar in both study groups. Other spirometry measures, use of albuterol, and various measures of asthma symptoms were also similar in both study groups.

The Fluenz and Fluenz Tetra SmPC states that “Fluenz/Fluenz Tetra should not be administered to children and adolescents with severe asthma or active wheezing because these individuals have not been adequately studied in clinical studies”. The use of P/LAIV in these individuals should be considered according to the individual benefit/risk considerations during the pandemic.

Immunocompromised individuals

Overall, data from a small number of subjects indicated that the use of T/LAIV was safe in subjects with mildly to moderately non-HIV related compromised immune function, asymptomatic or mildly symptomatic HIV infection or cancer (solid tumours and haematological malignancies). The safety profile of LAIV in the clinical trials was comparable to that in healthy individuals. The currently available data regarding the use of T/LAIV in the special risk group of mildly to moderately immunosuppressed individuals do not indicate any untoward effect, hence the use in these individuals may be considered after weighing the anticipated benefits against the potential risks for the individual. No data are available for individuals with severe or symptomatic immunosuppression (i.e. clinically significant). The use in this latter group might only be considered following the HCP individual benefit/risk assessment at the time of a pandemic.

In 24 HIV-infected children and 25 HIV-negative children 1 through 7 years of age, and in 243 HIV-infected children and adolescents 5 through 17 years of age receiving stable anti-retroviral therapy, the frequency and duration of vaccine T/LAIV virus shedding were comparable to that seen in healthy individuals. No adverse effects on HIV viral load or CD4 counts were identified following seasonal T/LAIV administration.

Use during pregnancy

There are no clinical data available on the use of H5N1/PLAIV in pregnant or lactating women. Limited data are available from the use of the seasonal influenza vaccines (T/LAIV and Q/LAIV) in pregnant women. Data is available from pregnant females inadvertently dosed with LAIVs in clinical or post-marketing studies, from spontaneous reports and medical literature.

Study MI-MA 225 was a Phase 4, retrospective, descriptive, uncontrolled database study to evaluate maternal AEs in women exposed to T/LAIV during pregnancy. The study was conducted by analysis of an US electronic health insurance claims database of claims from over 50 million individuals. 138 pregnant women were identified. Overall, no safety signal was detected in maternal outcomes; however, the sample size was only sufficient (i.e. with 95% probability) to detect at least 1 event for outcomes occurring at a frequency of at least 2.2%.

Data from the US Vaccine Adverse Event Reporting System (VAERS) database from 1990 to 2009 were published by US CDC. 27 reports of LAIV administration to pregnant women were reported and no unusual patterns of pregnancy complications or foetal outcomes identified (Moro, 2011). A similar US CDC study utilizing data from VAERS reported 113 pregnant women who had received H1N1 2009 LAIV. No unusual patterns of pregnancy complications or foetal outcomes were observed.

From the spontaneous reports received, no congenital anomalies were identified. This is consistent as influenza infection is not considered to be teratogenic. Therefore it is unlikely that an influenza vaccine containing an attenuated influenza virus strain is teratogenic. This is further supported by the knowledge that additionally animal developmental toxicity studies conducted with T/LAIV and Q/LAIV do not indicate direct or indirect harmful effects with respect to reproductive toxicity.

The human data are limited to assure the safety of P/LAIV during pregnancy so that clear recommendations cannot be made, however the available data do not suggest any adverse effect of T/LAIV or Q/LAIV on pregnancy or maternal health, which can be reasonably extrapolated to P/LAIV if a vaccination is considered necessary based on the benefit/risk considerations by the HCP at the time of a pandemic.

Elderly

Elderly were excluded from the P/LAIV studies as they are not included in the age indication for the seasonal LAIVs in the US were the P/LAIV studies were conducted. The indication for P/LAIV does not include the elderly population.

Safety related to drug-drug interactions and other interactions

No studies of potential drug-drug or drug-food interactions were conducted with P/LAIV.

The concurrent use of P/LAIV with antiviral agents that are active against influenza A and/or B viruses has not been evaluated; however, based upon the potential for antiviral agents with activity against influenza virus to reduce the effectiveness of any LAIV, P/LAIV should not be administered until 48 hours after the cessation of such antiviral therapy, and these antiviral agents should not be administered until 2 weeks after administration of P/LAIV unless medically indicated. If antiviral agents and P/LAIV are administered concomitantly, revaccination should be considered when appropriate based on clinical judgement.

Although there are no data linking P/LAIV or Fluenz with Reye's syndrome, because of the association of Reye's syndrome with aspirin and wild-type influenza infection, P/LAIV co-administration should in principle be avoided. P/LAIV should be administered to children and adolescents (2 to 17 years of age) who are receiving aspirin, salicylates, or aspirin containing therapy only based on the HCP assessment of the risks of administering the vaccine vs. the benefits of receiving the vaccine in a pandemic situation.

The safety and immunogenicity of P/LAIV when administered concurrently with inactivated vaccines have not been determined. In the USA, where FluMist has been commercially available since 2003, the recommended practice as advised by the United States Advisory Committee on Immunization Practices (US ACIP) has been to consider that FluMist and inactivated vaccines "can be administered simultaneously or at any interval between doses" (CDC, 2006). This recommendation remains current in the US ACIP's 2011 recommendation: "Any inactivated vaccine can be administered either simultaneously or at any time before or after a… live vaccine" (CDC, 2011).

P/LAIV can be administered concurrently with the following live, attenuated vaccines: measles, mumps, and rubella vaccine (MMR), varicella vaccine, and orally-administered polio vaccine (OPV) based on data from Studies D153-P522, AV018, and D153-P511, conducted with T/LAIV. These studies

were previously submitted to the EMA. Concomitant administration of these vaccines did not change their safety profile.

Discontinuation due to adverse events

In the pivotal H5N1 studies no subject discontinued due to AEs. In study CIR 217 one subject did not receive a second vaccine dose due to persistent elevated ALT levels and another subject because of asymptomatic wheezing noted by auscultation at Day 2 and Day 7.

Post marketing experience

T/LAIV was licensed in the US under the trade name of FluMist and was approved for use in the EU in 2011 under the trade name of Fluenz. QLAIV was approved in the USA in February 2012 under the trade name of FluMist Quadrivalent and in the EU in December 2013 as Fluenz Tetra. Post marketing and safety information has been continually monitored and updated for T/LAIV and Q/LAIV. The reviewed post-marketing safety data of Fluenz and Fluenz Tetra generally support the safety of LAIVs. The benefit/risk for Fluenz and Fluenz Tetra is considered positive since marketing authorisation was granted.

Since LAIV was approved in 2003, a total of 7,864 AEs have been reported in 4,512 unique case reports received from all post marketing sources: regulatory authority reports, spontaneous reports, and post marketing studies. Of these AEs, 76.2% (5,993/7,864 [5,624 non serious; 369 serious]) were from 3,729 unique spontaneous case reports; 14.8% of the AEs (1,163/7,864 [657 non serious; 506 serious]) were from 361 unique case reports received through the regulatory authorities; and 8.8% of the AEs (693/7,864 [225 non serious; 468 serious]) were from 415 unique case reports involving subjects enrolled in post marketing studies. Seven unique case reports (15 AEs; 9 non serious; 6 serious) were obtained from literature sources.

The most commonly reported AEs (combined non serious AEs and SAEs as reported by MedDRA preferred term) from all post marketing sources through the period ending 16 December 2013 were expired drug administered (n =1,212), pyrexia (n = 399), drug administered to patient of inappropriate age (247), exposure during pregnancy (n = 204), headache (n = 202), drug administration error (n = 193), cough (n = 183) rhinorrhoea (n = 178), influenza (n = 169), nasal congestion (n = 169) and oropharyngeal pain (n = 161).

The most commonly reported SAEs from all post marketing sources (as coded by MedDRA preferred term) were pyrexia (n = 45), vomiting (n = 36), pneumonia (n = 30), convulsion (n = 22), dyspnoea (n = 22), influenza (n = 22), cough (n = 21) and injury (n = 21).

The SOCs from post marketing studies with the most commonly reported AEs were Injury, Poisoning and Procedural Complications (n = 139), Infections and Infestations (n = 112), Gastrointestinal Disorders (n = 58), and Pregnancy, Puerperium and Perinatal Conditions (n = 58). The SAEs by MedDRA preferred term that were most commonly reported from post marketing studies were injury (n = 21), mental disorder (n = 18), pneumonia (n = 15), appendicitis (n = 14), abortion spontaneous (n = 12), abdominal pain (n = 10), and dehydration (n=10).

Important identified risks for LAIVs include Wheezing in children under the age of 2 years and Hypersensitivity disorders including anaphylaxis.

Important potential risks for LAIVs include: Guillain-Barré Syndrome, Bell's palsy Secondary transmission to severely immunocompromised patients, Inadvertent administration to immunocompromised patients, Seizures and convulsions, Encephalitis, Neuritis, Vasculitis, Vaccination failure (lack of efficacy), Narcolepsy with or without Cataplexy.

2.6.1. Discussion on clinical safety

The proposed indication for MedImmune's pandemic influenza vaccine H5N1 in the EU is the prophylaxis of influenza in children and adolescents from 12 months to less than 18 years of age in an officially declared pandemic situation.

In the 3 pivotal studies investigating H5N1 P/LAIV candidate vaccine and the 10 supportive studies evaluating other influenza strains with pandemic potential (i.e. H2N2, H2N3, H6N1, H7N3, H7N7, H7N9, and H9N2) only adult subjects were enrolled. Safety and immunogenicity of a monovalent P/LAIV carrying the actual pandemic strain in children from 1 year to less than 18 years of age should be evaluated when a pandemic is declared. The latter will be reflected as an obligation to the terms of the marketing authorisation.

The extensive safety database that derives from the seasonal LAIVs Fluenz and Fluenz Tetra and from the monovalent 2009 H1N1 P/LAIV supports the claim of a comparable safety profile of the P/LAIV. The P/LAIV candidate vaccines administered in the clinical trials were generally well tolerated with a safety profile comparable to that of seasonal trivalent or quadrivalent LAIVs. No safety signal was observed with the P/LAIV. The most commonly reported AEs were headache (25.4%) and symptoms affecting the upper respiratory tract (nasal congestion, sore throat – 10.2%). The majority of events was of mild intensity and resolved within a few days. Occasionally, changes in safety laboratory parameters were noted, including mild to moderate ALT increase, and decrease in platelet count and absolute neutrophil count. The assessment of the safety of the seasonal LAIV at the time of marketing authorisation did not indicate that hepatobiliary disorders occur at higher levels in LAIV recipients. Liver abnormalities are not a known or potential risk for the seasonal LAIV. These findings are considered relevant for the PLAIV, since P/LAIV contains identical *ts*, *ca* and attenuation *att* genetic elements as the seasonal LAIVs.

The safety of P/LAIV has not been assessed in special populations such as subjects with asthma, wheezing, respiratory disease, or immunosuppression. However, supportive data are available from the seasonal LAIVs. Restrictions made for the seasonal LAIVs are deemed applicable for the P/LAIV as detailed in previous section and in the SmPC.

From clinical studies and post-marketing surveillance with T/LAIV and Fluenz Tetra in over 110,000 children and adolescents 2 to 17 years of age, the following adverse reactions are reported: Decreased appetite, Headache, Nasal congestion/rhinorrhoea, Malaise as very common, and Myalgia and Pyrexia as common.

FluMist Quadrivalent in the US, Fluenz and Fluenz Tetra in the European Union all have an age restriction in the indication from 2 years of age onwards, children below 2 years of age are excluded from the indication. The age restriction in the indication for seasonal LAIVs and the H1N1 P/LAIV is based on adverse drug reactions that were seen during the clinical development of seasonal T/LAIV. The pivotal study MI-CP 111 was conducted with more than 8000 infants 6 to 59 months of age to estimate the safety of T/LAIV compared to TIV. Medically significant wheezing was a predefined endpoint. In this study higher rates of medical significant wheezing in children below 2 years of age (5.9% versus 3.8%, p-value 0.002) and all cause hospitalisation in toddlers from 6 to 11 month of age (6.1% versus 2.6%, p-value 0.002) were observed in the LAIV vs. the TIV vaccine group. The difference was statistically significant. For age groups older than 24 months, no differences were seen. Study MI-CP111 was previously submitted to the EMA. Notably no deaths resulted from these events, and none of the hospitalized children required mechanical ventilation or admission to an intensive care unit. In the scenario of a pandemic, particularly with a highly pathogenic influenza virus subtype such as H5N1, it is likely that the risks from the use of P/LAIV in children from 12 to 24 months of age may be acceptable due to the highest risk of death or severe influenza in this young population. In the PIP,

a waiver to investigate the paediatric population less than 1 year of age was granted on the grounds that the specific medicinal product is likely to be unsafe, and a deferral was granted for conducting a clinical trial to evaluate safety and immunogenicity of a monovalent P/LAIV in children from 1 year to less than 18 years of age until a pandemic is declared (P/0313/2014).

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

Assessment of paediatric data on clinical safety

The safety data gathered in the paediatric population with the seasonal LAIVs and the pandemic H1N1 LAIV have been submitted as supportive for this application and have been assessed in the sections above to conclude that they support the claim of a comparable safety profile to P/LAIV.

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

Additional safety data to be provided in the context of a pandemic preparedness vaccine MA

Due to the limited knowledge gathered in children with a pandemic LAIV, the CHMP requests the Applicant to conduct an observational prospective cohort safety study in a large sample of children and adolescents from 12 months to less than 18 years of age during the next pandemic. The aim of this study is to further investigate the tolerability of P/LAIV and to estimate the incidence of adverse reactions of special interest in children and adolescents with the strain causing the next pandemic.

The CHMP noted that a clinical trial will be conducted in children with the actual pandemic strain as per the agreed PIP. This will be an open-label single arm interventional study to evaluate the safety (and immunogenicity as secondary endpoint) of P/LAIV in children and adolescents from 12 months to less than 18 years of age during the pandemic.

Both the studies above constitute specific obligations in the context of a MA for a pandemic preparedness vaccine.

2.6.2. Conclusions on clinical safety

The exposure of subjects to H5N1 P/LAIV and P/LAIV based on other strains with pandemic potential is limited with 59 subjects exposed to H5N1 and 288 subjects exposed to the other candidate vaccines and the study population does not reflect the target population as it is restricted to adults 18 to 49 years of age. In the 3 pivotal H5N1 clinical studies and in the supportive P/LAIV studies the safety profile of the P/LAIVs was comparable to that of the seasonal vaccines T/LAIV and Q/LAIV. The majority of reactogenicity events included headache and upper respiratory tract infection.

The monovalent pandemic H1N1 LAIV was demonstrated to be safe in children and adults during the 2009 pandemic. Extrapolation from data of the extensive safety database of seasonal LAIVs and the monovalent H1N1 P/LAIV to the monovalent H5N1 P/LAIV is justified.

From current knowledge it can be expected that the safety profile of P/LAIV is likely to be comparable to that of seasonal LAIVs, i.e. overall safe and well tolerated. However, no data with H5N1 P/LAIV for the target population are available; therefore plans have been put in place to confirm the current knowledge with data generated at the time of the next pandemic (see below), which will be reflected as obligation to the terms of the marketing authorisation.

FluMist Quadrivalent in the US, Fluenz and Fluenz Tetra in the European Union all have an age restriction in the indication from 2 years of age. The lower age limit of 2 years in the seasonal indication is based on data from clinical study MI-CP 111 with seasonal inactivated influenza vaccine as

comparator, where a higher rate of hospitalisation was observed for LAIV in the age group 6-11 months. Also, in the age groups 6-23 months and 12-23 months a higher rate of medical significant wheezing was observed for LAIV. For the age group from 24 months and higher, no differences were seen.

As per the agreed PIP, the Applicant will be conducting a clinical trial in children from 1 year to less than 18 years of age to evaluate safety and immunogenicity of a monovalent P/LAIV when a pandemic will be declared, i.e. the vaccine will contain the circulating pandemic strain. The clinical trial conducted in the paediatric population during the pandemic will provide further safety data for the benefit/risk assessment in respect to the use of the P/LAIV in this population in the pandemic situation. In addition the CHMP requests the Applicant to conduct during the pandemic a large observational prospective cohort safety study in the paediatric population in order to further investigate the tolerability of P/LAIV and to estimate the incidence of adverse reactions of special interest in children and adolescents.

The CHMP considers the following measures necessary to address the missing safety data in the context of a pandemic preparedness vaccine MA:

Description	Background
1. In order to further investigate the tolerability of P/LAIV and estimate the incidence of adverse reactions of special interest in children and adolescents, the MAH should conduct an observational prospective cohort safety study in a large sample of children and adolescents from 12 months to less than 18 years of age during the next declared pandemic. The MAH should submit the results of this study.	Limited data are available in children with a live attenuated vaccine strain with pandemic potential
2. In order to further investigate the safety and reactogenicity of the P/LAIV, the MAH should conduct an open-label single arm interventional study to evaluate the safety and immunogenicity of P/LAIV in children and adolescents from 12 months to less than 18 years of age during the next declared pandemic. The MAH should submit the results of this study.	Limited knowledge is available regarding the safety of P/LAIV in children before vaccine deployment

2.7. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

The PRAC considered that the risk management plan version 1.3 is acceptable. The PRAC advice is attached.

The CHMP endorsed the Risk Management Plan version 1.3 with the following content:

Safety concerns

Important identified risks	Medically significant wheezing in children 12 to <24 months of age Hypersensitivity (including anaphylaxis)
Important potential risks	Guillain-Barré Syndrome Bell's Palsy Secondary transmission to severely immunocompromised patients Inadvertent administration to immunocompromised patients Seizures and convulsions Encephalitis Neuritis Vasculitis Vaccination Failure (Lack of Efficacy)
Missing information	There is limited information regarding safety of P/LAIV in the following populations: Children 12 through 23 months Pregnant/lactating women Severe asthmatics Immunocompromised vaccine recipients Individuals with severe chronic illness

Pharmacovigilance plan

Study / Activity Type, Title and Category (1-3)	**Objectives**	**Safety Concerns Addressed**	**Status (Planned, Started)**	**Date for Submission of Interim or Final Reports (planned or actual)**
A Post-marketing Non-interventional Cohort Study of the Safety of Pandemic Live Attenuated Influenza Vaccine (pandemic LAIV) in Subjects 12 months through 17 Years of Age (Category 2)	To estimate the incidence of adverse events of interest (AEIs) through active surveillance of a large sample of children given P/LAIV	All cause Serious Adverse Events (SAEs), lower respiratory SAEs and other Medically Attended Events (MAEs) in patients receiving LAIV	The study will be initiated once a pandemic has been announced	The final report will be available 16 weeks after study completion
A Test Negative Case Control Study of the Effectiveness of Pandemic Live Attenuated Influenza Vaccine (pandemic LAIV) in Subjects 12 months through 17 Years of Age (Category 2)	To evaluate the effectiveness of a pandemic LAIV compared to no vaccine and/or inactivated influenza vaccine in community-dwelling subjects 12 months through 17 years of age against laboratory-confirmed pandemic influenza	Potential Risk: Vaccination Failure (Lack of Efficacy)	The study will be initiated once a pandemic has been announced	The final report will be available 16 weeks after study completion
Open-label, single arm trial to evaluate safety and reactogenicity of a monovalent live attenuated pandemic Influenza vaccine in children from 1 year to less than 18 years of age. (Category 2)	To evaluate safety including reactogenicity of a monovalent live attenuated pandemic Influenza vaccine in children from 1 year to less than 18 years of age Secondary, provision of immunogenicity data on actual pandemic vaccine in the target population	Reactogenicity; Overall safety profile,	The study will be initiated once a pandemic has been announced.	The final report will be available 16 weeks after study completion

Risk minimisation measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Medically significant wheezing in children 12 to <24 months of age	*Pandemic influenza vaccine H5N1 MedImmune* is not to be used in infants and toddlers below 12 months of age because of safety concerns regarding increased rates of hospitalisation and wheezing in this population. The risk to children 12 through 23 months is considered under missing information. Information on this risk is included in sections 4.2, 4.4 and 4.8 of the SmPC: "Posology and method of administration", "Special warnings and precautions for use" and "Undesirable effects". A clinical study will be conducted at the time that a pandemic is declared or is considered imminent that includes children 12 through 23 months of age to gather further information on the safety of P/LAIV in this age group.	None applicable, routine risk minimisation activities are sufficient.
Hypersensitivity (including anaphylaxis)	The vaccine is contraindicated in people with history of an anaphylactic (i.e. life-threatening) reaction to any of the active substances, or to any of the inactive substances listed in section 6.1 (e.g. gelatin), or to gentamicin (a possible trace residue), to eggs or to egg proteins (e.g. ovalbumin). Information on this risk is included in the SmPC, section 4.3 "Contraindications." However, in a pandemic situation, it may be appropriate to give the vaccine, provided that facilities for resuscitation are immediately available in case of need. Caution is needed when administering this vaccine to persons with a known hypersensitivity (other than anaphylactic reaction) to the active substance(s), to any of the excipients, and to eggs or to egg proteins (e.g. ovalbumin). Information on this risk is included in the SmPC, section 4.4, "Special warnings and precautions." As with most vaccines, appropriate medical treatment and supervision should always be readily available to manage an anaphylactic event or serious hypersensitivity event after giving Pandemic influenza vaccine H5N1 MedImmune.	None applicable, routine risk minimisation activities are sufficient.
Guillain-Barré syndrome	Very rare reports of Guillain-Barré syndrome have been observed in the post-marketing setting with seasonal LAIV. This event is listed in the SmPC, section 4.8, "Undesirable Effects".	None applicable, routine risk minimisation activities are sufficient.
Bell's palsy	Bell's palsy is included in this RMP as a potential risk, not yet identified in people using LAIV products. Therefore, no risk minimisation activities are deemed necessary until such time it may be confirmed as an identified risk.	None applicable
Secondary transmission to severely immunocompromised patients	Those receiving the vaccine are to be warned against contact with people whose immune system is compromised. The SmPC states in section 4.4, "Special warnings and precautions for use": *Pandemic influenza vaccine H5N1 MedImmune is an attenuated live virus vaccine and has the potential for transmission to immunocompromised contacts. Immune response in patients with endogenous or iatrogenic immunosuppression may be insufficient.* *Vaccine recipients should be informed that Pandemic influenza vaccine H5N1 MedImmune is an attenuated live virus vaccine and has the potential for transmission to immunocompromised contacts. Vaccine recipients should attempt to avoid, whenever possible, close association with severely immunocompromised individuals (e.g. bone marrow transplant recipients requiring isolation) for 1-2 weeks following vaccination. Shedding of the H5N1 vaccine virus in adults was extremely limited. Peak incidence of vaccine virus recovery occurred 1-2 days post-vaccination in clinical studies with Pandemic influenza vaccine H5N1 MedImmune. In circumstances where contact with severely immunocompromised individuals is unavoidable, the potential risk of transmission of the influenza vaccine virus should be weighed against the risk of acquiring and transmitting wild-type influenza virus.*	None applicable, routine risk minimisation activities are sufficient.

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Inadvertent administration to immunocompromised patients	Immune response in patients with endogenous or iatrogenic immunosuppression may be insufficient. The SmPC states in section 5.1, "Pharmacodynamic properties", that the effectiveness of Pandemic influenza vaccine H5N1 MedImmune and of the seasonal vaccines T/LAIV and Q/LAIV (Fluenz Tetra) LAIV products in preventing influenza illness in immunocompromised individuals has not been evaluated.	None applicable, routine risk minimisation activities are sufficient.
Seizures and convulsions	Seizures and convulsions are included in this RMP as a potential risk, therefore, no risk minimisation activities are deemed necessary until such time it may be confirmed as an identified risk.	None applicable
Encephalitis	Encephalitis is included in this RMP as a potential risk, therefore, no risk minimisation activities are deemed necessary until such time it may be confirmed as an identified risk.	None applicable
Neuritis	Neuritis is included in this RMP as a potential risk, therefore, no risk minimisation activities are deemed necessary until such time it may be confirmed as an identified risk.	None applicable
Vasculitis	Vasculitis is included in this RMP as a potential risk, therefore, no risk minimisation activities are deemed necessary until such time it may be confirmed as an identified risk.	None applicable
Vaccination failure (lack of efficacy)	There is no data on the demonstrated efficacy of Pandemic influenza vaccine H5N1 MedImmune from controlled clinical studies. The assumption of efficacy is based on the seasonal LAIV products. Efficacy of seasonal LAIV was shown to be below 100%, therefore it should be understood that the pandemic vaccine may not be effective in all recipients.	None applicable, routine risk minimisation activities are sufficient.
Children under the age of 12 months	Pandemic influenza vaccine H5N1 MedImmune is not to be used in children younger than 12 months. The SmPC states in section 4.2, "Posology and method of administration": *MedImmune pandemic influenza vaccine should not be used in infants below 12 months of age because of safety concerns regarding increased rates of hospitalisation and wheezing in this population (see section 4.8).*	None applicable, routine risk minimisation activities are sufficient
Pregnant and breast-feeding women	Pandemic influenza vaccine H5N1 MedImmune needs to be given special consideration in pregnancy. It is not to be used in women who are breast-feeding. The SmPC states in section 4.6, "Fertility, pregnancy and lactation": *There are no data on the use of Pandemic influenza vaccine H5N1 MedImmune in pregnant women. Healthcare providers need to assess the benefit and potential risks of administering Pandemic influenza vaccine H5N1 MedImmune to pregnant women.* *It is not known whether Pandemic influenza vaccine H5N1 MedImmune is excreted in human milk. Therefore, as some viruses are excreted in human milk, the vaccine should not be used during breast-feeding.*	None applicable, routine risk minimisation activities are sufficient
Severe asthmatics	The SmPC states in section 4.4 "Special warnings and precautions for use": *The safety of seasonal LAIV in children with severe asthma and active wheezing has not been adequately studied. Healthcare providers need to assess the benefits and potential risks of administering Pandemic influenza vaccine H5N1 MedImmune to these individuals.*	None applicable, routine risk minimisation activities are sufficient
Immunocompromised vaccine recipients	The following text is included in the SmPC, section 4.4 "Special warnings and precautions for use": *"Immune response in patients with endogenous or iatrogenic immunosuppression may be insufficient.* *No data are available for individuals with significant clinical immunodeficiency. In a pandemic situation, healthcare providers need to assess the potential benefits, alternatives, and risks of administering the vaccine to children and adolescents with significant clinical immunodeficiency due to conditions or immunosuppressive therapy such as: acute and chronic leukaemias; lymphoma; symptomatic HIV infection; cellular immune deficiencies; and high-dose corticosteroids.*	None applicable, routine risk minimisation activities are sufficient
Serious chronic disease	There is currently no evidence to suggest that vaccine recipients with serious chronic diseases are at increased risk compared to the general population. Therefore, no action is deemed necessary. The exception is those who are clinically immunodeficient: see "Immuno-compromised vaccine recipients", above.	None applicable

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the Applicant fulfils the requirements of Article 8(3)(ia) of Directive 2001/83/EC.

2.9. Significance / Non-Conformity of paediatric studies

Not applicable.

2.10. Product information

2.10.1. 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.*

No full user consultation with target patient groups on the package leaflet was performed. Instead, a bridging report making reference to Fluenz was submitted. As the Fluenz PL covers all format aspects of the Pandemic Influenza Vaccine H5N1 MedImmune PL and their overall formats are sufficiently similar, the bridging report submitted by the Applicant was considered acceptable.

2.10.2. Labelling exemptions

A request to omit certain particulars from the labelling as per Article 63(3) of Directive 2001/83/EC has been submitted by the Applicant and has been found acceptable by the QRD Group for the following reasons:

The Applicant requested an exemption to display the common name “Pandemic influenza vaccine” on the sprayer label based on the limited available space. The proposed approach would ensure improved readability by accommodating the minimum font size of 7pt. The outer carton and intermediate packaging would display the common name in the national languages.

No objections were raised by the QRD Group to omit the common name from the sprayer label.

The particulars to be omitted described above will however be included in the Annexes published with the EPAR on EMA website, and translated in all languages but will appear in grey-shaded to show that they will not be included on the printed materials.

2.10.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EC) No 726/2004, Pandemic influenza vaccine H5N1 MedImmune (pandemic influenza vaccine H5N1 (live attenuated, nasal)) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore the summary of product characteristics and the package leaflet shall include a statement ”This medicinal product is subject to additional monitoring” and this will allow for quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

2.10.4. Conditional Marketing Authorisation

The CHMP has reviewed the claims from the Applicant and considers that this application is eligible to a Conditional Marketing Authorisation in accordance with Article 14(7) of Regulation (EC) No 726/2004 as it is a medicinal product to be used in emergency situations, in response to public health threats duly recognised either by the World Health Organisation or by the EU (Article 2(2) of Commission Regulation (EC) No 507/2006).

The CHMP concludes that:

- The risk-benefit balance of the medicinal product is positive (see section 3);
- it is likely that the Applicant will be able to provide comprehensive data when a pandemic occurs;
- there is a an unmet medical need fulfilled by providing a live attenuated pandemic influenza preparedness vaccine in children and adolescents;
- the benefit to public health of authorising this pandemic preparedness vaccine in the recommended indication outweighs the risk inherent in the fact that additional data are still required on the actual pandemic strain.

3. Benefit-Risk Balance

Benefits

Beneficial effects

P/LAIV H5N1 VN04 is able to effectively prime naïve adults when administered with a 2-dose regime, resulting in an immune memory response that could last for at least 4-5 years. This is based on the fact that in response to re-vaccination with an inactivated vaccine given 5 years after priming, an immune memory response could still be detected in 73% of the re-vaccinated subjects by two functional antibody assays, HAI and MN, measured against the wild-type virus. The antibodies detected in this response were broadly cross-neutralising up to 4 heterologous strains, including clade 2.1.3, clade 2.3.4, clade 2.2.1, and clade 2.2.1, and persisted for about 6 months before becoming undetectable. The measured functional immune response is considered as an acceptable surrogate endpoint for clinical protection in the context of the current application, but is not a validated correlate of protection for LAIVs at this point in time. In addition the posology of the P/LAIV will consist of 2 doses of pandemic LAIV only as per current SmPC.

Produced by the same platform technology as used for P/LAIV H5N1 VN04, P/LAIV H7N7 and P/LAIV H7N9 candidates demonstrated a similar priming capability in naïve adults. The available data generated in prime-boost setting with both candidates support the conclusion that detection of greater antibody responses to a boost with an inactivated vaccine is possible even when re-vaccination with the inactivated pandemic vaccine is performed at earlier intervals, i.e. as early as 4 weeks from P/LAIV vaccination (~90% responders in P/LAIV vaccinees vs. 30% responders in P/LAIV naïve at 14 days post IIV vaccination - with H7N9 antigens).

In ferrets challenged with the homologous and the heterologous wild-type H5N1 viruses, the 2-doses of P/LAIV H5N1 VN04 demonstrated to be highly efficacious in preventing challenge virus replication both in lungs and in nasal turbinates. In African Green Monkeys, one fifth clinical dose of the vaccine given intranasally and intratracheally, at Day 0 and 28, provided effective protection against homologous wild-type virus challenge. In the latter animal model, induction of serological functional antibodies by vaccination was evidenced, suggestive of correlation with protection.

Together human adult immunogenicity data and animal challenge data provide cumulative proof of concept for inferring priming capacity of P/LAIV H5N1 VN04 in naïve paediatric subjects 1 to 17 years of age.

The potential efficacy of P/LAIV H5N1 VN04 in this population may further be predicted based on other existing data, including the large body of clinical efficacy data generated with the seasonal T/LAIV in subjects aged 6 months and above, and effectiveness data of pandemic monovalent H1N1pdm09 LAIV reported for children aged 2 years and above. The latter data showed an estimated effectiveness of up to 82% in the 2-9 year age strata, which is quite favourable for a pandemic vaccine.

Uncertainty in the knowledge about the beneficial effects

No validated HAI and MN assays were used for the clinical testing programme, including the CIR277 study, although fully qualified HAI and MN assays were used for the key studies CIR277 and CIR293. Lack of formal assay validation was accepted, given the reassurance on assay performance provided by the extensive qualification.

Studies conducted in prime-boost setting did not enrol a large number of subjects, however the limitations in conducting studies with a live virus with pandemic potential in the interpandemic period are acknowledged. Also small size is not considered as a major issue, in view of the fact that adult data serve as proof of concept and are not intended for supporting an adult indication. Similarly, lack of P/LAIV paediatric data is acceptable in the context of this application for a pandemic preparedness vaccine in the interpandemic period, i.e. lack of immediate threat from avian influenza and lack of circulating virus with sustained human-to-human transmission may raise serious feasibility and ethical concerns for investigating a paediatric population. Indeed, based on adult data and extensive experience gained in paediatrics with seasonal and pandemic H1N1pdm09 LAIV vaccines, it can be reasonably assumed that P/LAIV H5N1 VN04 is able to prime the naïve paediatric population too.

To further support the conclusions of this application, a safety and descriptive immunogenicity study in subjects 1 to 17 years of age will be conducted upon pandemic declaration. It is also required that surveillance studies for effectiveness and safety of P/LAIV H5N1 VN04 vaccine will be conducted upon massive use during the pandemic. These studies are required as obligations to the terms of the marketing authorisation for this pandemic preparedness vaccine and are reflected in Annex II.

Whether a single dose of P/LAIV H5N1 VN04 vaccine suffices to prime naïve subjects remains to be fully elucidated. The potential advantage of 1-dose regime in a pandemic situation, with respect to schedule compliance and vaccination coverage, is acknowledged. However, seasonal LAIV efficacy data obtained from very young children showed that the second dose had additional clinical benefit. In the monkey challenge model, a single $2x10^6$ $TCID_{50}$ dose was ineffective, in contrast to complete protection offered by 2 doses of vaccine. In addition existing evidence in the prime-boost studies CIR 277 (H5N1) and CIR 293 (H7N9) indicate the need for 2 doses to achieve better immune responses to booster. Collectively the gap of knowledge and the existing data hampers the possibility at present to recommend a 1-dose regime for P/LAIV H5N1 VN04. There is also lack of knowledge regarding the minimum time needed from immunisation until protective immune responses are elicited, although based on the evidence from study CIR 293 booster responses were detected as early as 4 weeks post priming.

The supportive data from study CIR293 are still incomplete at the current time; the final results will be submitted as soon as available and are expected to provide significant information.

The Applicant did not include stability data for the finished product using the H5N1 strain in this application. The claimed finished product shelf life of 20 weeks at -25°C ± 5°C (prior to distribution) and subsequent storage at 2°C - 8°C for not more than 18 weeks is based on the H1N1 pandemic formulation (2009). These data were also not generated with the requested and newly implemented

thermostability assay that allows detection of “thermolabile” HA molecules. The CHMP has thus requested the Applicant to generate and provide strain-specific stability data for the actual pandemic vaccine strain in order to define the shelf life on an evidence-based, strain-specific basis. This request is reflected in Annex II as obligation to the terms of the marketing authorisation.

Risks

Unfavourable effects

In the adults P/LAIV studies headache was most frequently reported, followed by events of the upper respiratory tract including nasal congestion and rhinorrhoea. These adverse reactions were of mild to moderate intensity.

Overall, based on the very limited number of adult subjects tested, the safety profile of P/LAIV H5N1 VN04 and other P/LAIV candidates can be described as comparable to that of seasonal LAIVs.

Seasonal LAIVs are proved to be safe and well tolerated across the paediatric population. From clinical studies and post-marketing surveillance with Fluenz and Fluenz Tetra in over 110,000 children and adolescents 2 to 17 years of age, the following adverse reactions are reported: Decreased appetite, Headache, Nasal congestion/rhinorrhoea and Malaise as very common, and Myalgia and Pyrexia as common.

One of the main concerns for the seasonal LAIVs was the increased incidence or wheezing in younger children below the age of 2 years, and the increased risk of hospitalisation in children below the age of 1 year (see the following sections for further details on this topic). These risks are not found in children from 2 years of age onward.

The monovalent pandemic H1N1 LAIV was demonstrated to be safe in children and adults during the 2009 pandemic. Extrapolation from data of the extensive safety database of seasonal LAIVs and the monovalent H1N1 P/LAIV to the monovalent H5N1 P/LAIV is justified.

Uncertainty in the knowledge about the unfavourable effects

The safety dataset from the P/LAIV programme in adults is very limited.

The tolerability and safety of P/LAIV H5N1 VN04 has not been assessed in paediatric subjects aged 1 to 17 years. This will be subject to post-marketing evaluation once a pandemic is declared. Collecting safety data from this population, especially from 1-2 years of age, in the inter-pandemic period raise significant concerns and appears unfeasible and not ethically acceptable, due to the lack of risk of contracting the disease in the absence of pandemic virus circulation from human to human.

The available data gained with seasonal T/LAIV showed increased risks of wheezing through Day 42 in subjects below 24 months of age, compared to inactivated trivalent vaccines. Whether or not such potential risks can be detected for the monovalent P/LAIV H5N1 VN04 too, it could only be appropriately addressed during use in a pandemic situation.

It is required that surveillance studies for safety of the vaccine be conducted upon use during the next pandemic. This will be reflected as specific obligations to the terms of the marketing authorisation.

Effects table

Table 20. Effects Table for H5N1 P/LAIV

Effect	Short Description	Uncertainties/ Strength of evidence	References
Favourable Effects			

Effect	Short Description	Uncertainties/ Strength of evidence	References
Efficacy (H5N1 VN/04) (Immunogenicity)	Efficient priming of naïve adults as measured indirectly up to 5-y post vaccination (i.e. measuring % of seroconverts post IIV booster vaccination)	Boosted immune responses were characteristic of a memory response induced by P/LAIV in naïve subjects, and lasted up to 6 months The establishment of efficacy is based on indirect measurement on surrogate endpoints Immune responses were broader with P/LAIV than control (cross-clade H5N1 neutralisation) Similar results were shown for other P/LAIV candidates (e.g. H7N7, H7N9)	CIR277
Efficacy in animal models	Studies in ferrets and monkeys showed complete protection by P/LAIV against homologous and heterologous challenge (proof of concept)		Suguitan et al, 2006; Matsuoka et al, 2014
Efficacy with other LAIV constructs	Clinical efficacy was demonstrated in the target population for seasonal LAIV. Effectiveness was demonstrated for the pandemic H1N1v vaccine during the 2009/2010 swine flu pandemic in the US (61% protection in subjects aged 2-49 years, and 82% in subjects aged 2-9 years, 7 days after 1-dose of vaccine)		AV006, D153-P501, -P502, -504, -P513, and -P522 Griffin, et al, 2011
Unfavourable Effects			
Wheezing	Observed through day 42 in subjects below 24 months of age following seasonal LAIV vaccination (5.9%) compared to seasonal TIV vaccination (3.8%)		Study MI-CP 111
Headache, nasal congestion, rhinorrhoea, malaise	Observed as very common adverse reactions in the paediatric population with the seasonal LAIV and in adults with P/LAIV H5N1		Study MI-CP 111, CIR277, CIR217, CIR239

Abbreviations: IIV (inactivated influenza vaccine); TIV (trivalent inactivated influenza vaccines); LAIV (live attenuated influenza vaccines)

Benefit-risk balance

Importance of favourable and unfavourable effects

A pandemic caused by H5N1 can result in high morbidity and mortality in the entire age range of the general population. Vaccination is a viable effective measure to prevent disease and disease spread.

The most important benefit of P/LAIV H5N1 VN04 is to provide protection against H5N1 in naïve paediatric subjects. Such protection is inferred by proving that the vaccine can prime, i.e. by looking at the immune memory response following a booster with an inactivated vaccine containing the same strain. In fact, when administered with 2 doses separated by 4-8 weeks, P/LAIV H5N1 VN04 can prime naïve young adults and elicit long-lasting memory immune responses that could be recalled by re-exposure to H5N1 antigen. This has been evidenced by means of two different functional antibody assays, HAI and MN, against the wild-type H5N1 VN04 virus.

Another notable aspect of antibody responses to re-vaccination is the broadly cross-neutralising activity against up to 4 different H5N1 clades.

P/LAIV H5N1 VN04 priming capacity has been supported by data generated for other P/LAIV candidates, with a recall/boost antigen given between 24 and 3 months after priming. Preliminary results from a prime-boost study with H7N9 additionally showed that robust booster responses can be achieved as early as 4 weeks post priming. Data of from this study are of highly supportive value because of the relevance of prime-boost interval to re-exposure in a pandemic situation.

These naïve adult data provide important proof-of concept for inferring the capacity of P/LAIV H5N1 VN04 to prime subjects 1 to 17 years of age.

The protective efficacy of P/LAIV H5N1 VN04 in ferret and monkey challenge studies, the efficacy of the licensed seasonal LAIVs in very young children, and the effectiveness of H1N1pdm09 monovalent

LAIV vaccine, provide additional supportive evidence for the efficacy of P/LAIV H5N1 VN04 in subjects aged 1-17 years.

No paediatric safety data have been generated for P/LAIV H5N1 VN04 and other P/LAIV candidates. Based on a small number of naïve adults tested thus far, these candidate vaccines can be described as low reactogenic, with similar safety profiles to the seasonal LAIVs.

Seasonal T/LAIV show increased risks of wheezing through Day 42 in subjects below 24 months of age. With a highly pathogenic avian influenza virus such as the H5N1 subtype, which might be associated with high rate of mortality in a naive population, a safety signal such as wheezing is considered acceptable compared to the potential benefit of an effective life-saving vaccine in young children.

Discussion on the benefit-risk balance

Several lines of proof-of-concept have been provided in support of the predicted priming capacity and protective efficacy of the 2-dose regime of P/LAIV H5N1 VN04 in subjects aged 1-17 years. These include non-clinical and clinical data generated with several other P/LAIV candidate containing pandemic virus strains such as H7N7 and H7N9.

The limited adult data available from the entire P/LAIV programme show a well-tolerated and safe profile comparable to that of seasonal LAIVs. Although no major safety concern is raised at present, the potential risk of increased wheezing upon vaccination with P/LAIV H5N1 VN04, in the infants and toddlers aged 1-2 years, has been suggested by previous experience with seasonal T/LAIV. However, the predicted efficacy of P/LAIV H5N1 VN04 in 1-2 year of age children, together with the potential severity of a H5N1 pandemic that might be associated with high rate of mortality in a naive population, outweighs the potential risk of increased wheezing in this age group, therefore supporting the age indication with a lower age cut-off of 1 year. It will be imperative to monitor the safety of the vaccine after declaration of a pandemic as per defined specific obligations.

Conclusions

The overall benefit/risk balance for P/LAIV H5N1 VN04 is positive for the prophylaxis of influenza in an officially declared pandemic situation in children and adolescents from 12 months to less than 18 years.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Pandemic influenza vaccine H5N1 MedImmune in the prophylaxis of influenza in an officially declared pandemic situation in children and adolescents from 12 months to less than 18 years of age is favourable and 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 of Directive 2001/83/EC, the official batch release will be undertaken by a state laboratory or a laboratory designated for that purpose.

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports 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.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The 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.

- **Additional risk minimisation measures**

Not applicable

- **Obligation to complete post-authorisation measures**

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14(7) of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
Non-interventional post-authorisation safety study (PASS) in order to further investigate the tolerability of Pandemic influenza vaccine H5N1 MedImmune and estimate the incidence of adverse reactions of special interest in children and adolescents. The MAH should conduct an observational prospective cohort safety study in a large sample of children and adolescents from 12 months to less than 18 years of age during the next declared pandemic. The MAH should submit the final results of this study.	After declaration in the EU of a pandemic and after implementation of the pandemic vaccine
In order to further corroborate the efficacy of Pandemic influenza vaccine H5N1 MedImmune, the MAH should conduct an observational effectiveness study in community dwelling children and adolescents from 12 months to less than 18 years of age against laboratory confirmed influenza during the next declared pandemic. The MAH should submit the final results of this study.	After declaration in the EU of a pandemic and after implementation of the pandemic vaccine
In order to further investigate the safety and reactogenicity of Pandemic influenza vaccine H5N1 MedImmune, the MAH should conduct an open-label single arm	After declaration in the EU of a pandemic and

Description	Due date
interventional study to evaluate the safety and immunogenicity of P/LAIV in children and adolescents from 12 months to less than 18 years of age during the next declared pandemic. The MAH should submit the final results of this study.	after implementation of the pandemic vaccine
In order to define the shelf life of Pandemic influenza vaccine H5N1 MedImmune on a strain-specific basis, the MAH should generate strain-specific stability data for the actual pandemic strain. The MAH should submit the final results of this study.	At the time of approval of the next pandemic variation

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

New Active Substance Status

Based on the CHMP review of data on the quality properties of the active substance, the CHMP considers that the active substance is qualified as a new active substance. The active substance is:

Reassortant influenza virus* (live attenuated) of the following strain**:

A/Vietnam/1203/2004 (H5N1) strain

(A/Vietnam/1203/2004, MEDI 0141000136)

* propagated in fertilised hens' eggs from healthy chicken flocks.

** produced in VERO cells by reverse genetic technology. This product contains a genetically modified organism (GMO).