EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Assessment report

FLUENZ

Common name: influenza vaccine (live attenuated, nasal)

Procedure No. EMEA/H/C/001101

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

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1. Background information on the procedure

1.1. Submission of the dossier

The applicant Medimmune, LLC submitted on 26 November 2008 an application for Marketing Authorisation to the European Medicines Agency (EMA) for FLUENZ, 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 September 2007.

The applicant applied for the following indication: Prophylaxis of influenza in individuals 12 months of age and older.

The legal basis for this application refers to:

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

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and of 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/101/2008 for the following condition:

Prophylaxis of influenza.

Information relating to Orphan Market Exclusivity

Similarity

Not applicable.

Market Exclusivity

Not applicable.

Scientific Advice:

The applicant received Scientific Advice from the CHMP on 21 June 2007. The Scientific Advice Assistance pertained to quality, non-clinical and clinical aspects of the dossier.

Licensing status

FLUENZ has been given a Marketing Authorisation in the USA on 17 June 2003 and in Canada on 22 June 2010.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Pierre Demolis ; Co-Rapporteur: Pieter Neels

- The application was received by the EMA on 26 November 2008.
- The procedure started on 17 December 2008.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 25 March 2009 . The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 18 March 2009.
- During the meeting of 13 to 15 April 2009, the BWP discussed the quality aspects of the application and prepared a report to CHMP.
- During the meeting on 23 April 2009, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 23 April 2009.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 24 September 2009.
- The report of the GMP inspection carried out at the following site Medimmune LLC, 3001 Red Lion Road, Philadelphia, USA, between 19 – 20 April 2010 was issued on 6 June 2010.
- The summary report of the GCP inspection carried out at a site Medimmune in Gaitherburg, MA, USA, from 08-11 September 2009 was issued on 16 October 2009.
- At the CHMP meeting of 22 October 2009 the CHMP adopted a 2nd List of Questions pertaining to GCP-related concerns and extended the clock-stop until February 2010.
- On 3 November 2009 the Applicant asked for an extension to the timetable for providing his responses to the 2nd List of Questions.
- The Applicant submitted the responses to the CHMP 2nd consolidated List of Questions on 23 March 2010.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 4 June 2010.
- During the meeting of 14 to 16 June 2010 and of 13 to 15 September 2010, the BWP discussed the quality aspects of the application and prepared a report to CHMP.
- During the CHMP meeting on 24 June 2010, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- During the CHMP meeting on 23 September 2010, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 19 to 21 October 2010, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to FLUENZ on 21 October 2010. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled post-authorisation on 20 October 2010.

2. Scientific discussion

2.1. Introduction

Influenza disease

Influenza is a highly contagious, acute febrile respiratory disease caused by one of two types of influenza viruses: influenza A and influenza B. As the circulating influenza strains may vary annually, epidemics occur on a yearly basis. The primary transmission of the disease is respiratory by means of large particle droplets. The incubation period generally ranges from 1-4 days, and viral shedding usually peaks around the second day of influenza symptoms. Children shed the greatest amount of virus and pose the greatest risk for further transmission of influenza into the community. Young children may shed virus for several days before the onset of symptoms and can be infectious for more than 10 additional days. Thus, infectivity is higher among preschool and school-aged children compared to other children and adults.

Type A influenza strains have been responsible for large epidemics. Since 1977, influenza A/H1N1, A/H3N2 and B viruses have circulated globally and have been included in all licensed influenza vaccines, as recommended by the World Health Organization (WHO). Influenza epidemics of variable severity occur annually worldwide in all age groups, typically during the winter months in temperate climates. These annual epidemics are thought to result in 3 million to 5 million cases of severe illness and approximately 250,000 to 500,000 deaths every year around the world (WHO, 2005).

Influenza attack rates vary from year to year as do the circulating virus strains. The collaborative project European Influenza Surveillance Scheme (EISS) age-specific incidence rates reported have routinely been highest among those 0-4 and 5-14 years of age, though large variation was observed by countries (ECDC, 2007).

Influenza causes disease in all age groups. The clinical presentation of influenza in school-age children and adolescents is similar to that in adults and includes fever, cough, myalgia, headache, sore throat, chills tiredness, and general malaise.

Uncomplicated influenza illness in healthy individuals is generally a self-limited febrile respiratory disease of 3-7 days' duration, sometimes with persistence of cough and malaise for several weeks. Influenza illness is characterized by the abrupt onset of signs and symptoms such as fever, myalgia, headache, malaise, chills, nonproductive cough, anorexia, sore throat, and rhinitis. Children may also have otitis media, croup, nausea, and vomiting. Severe cases can occur in children with underlying chronic diseases.

Severe morbidity and mortality occur mainly in the elderly (> 65 years of age) and the very young (< 24 months of age) and in other populations with specific "high-risk" conditions, such as chronic lung, heart, renal diseases or metabolic diseases, persons with conditions or medical treatments resulting in suppressed immune function, and persons living in institutional settings are at increased risk for influenza illness, development of serious influenza-associated complications (such as pneumonia and respiratory failure), and death.

Although influenza-associated deaths are uncommon among children (usually less than 100 per year in the United States but can be higher during years of vaccine mismatch), they represent a substantial proportion of vaccine-preventable deaths exceeding the annual child mortality from invasive pneumococcal disease, varicella, pertussis, or measles; 47% of the influenza-related deaths were in previously healthy children with no known risk factors or underlying chronic diseases.

The risk of influenza-associated hospitalization is greatest among the elderly (> 65 years old) and the very young (< 2 years of age). Infected children also appear to play a pivotal role in secondary transmission of influenza to household members and to other members of the community, leading to further increases in medical utilization and medication use.

Influenza virus

Two types are responsible for the disease: influenza A, which is categorized into subtypes on the basis of its hemagglutinin (H) and neuraminidase (N) surface antigens, and influenza B, which is separated into two genetic lineages.

Within each influenza subtype, the viruses undergo frequent changes in their surface antigens (antigenic drift), leading to the perpetuation of different viral strains (CDC, 2007).

A/H3N2 and A/H1N1 are the 2 influenza A subtypes that have circulated and caused human disease since 1977 (Kilbourne, 2006). Seasonal outbreaks in the last 2 decades have most commonly been associated with A/H3N2 strains. Influenza A/H3N2 strains have been associated with more severe illness and with higher mortality compared to seasons when A/H1N1 and B strains predominated (Simonsen et al, 1997; Thompson et al, 2003; Meijer et al, 2007).

Influenza seasonal vaccines

MedImmune has developed a cold-adapted live attenuated influenza vaccine, FLUENZ, which is trivalent (A/H1N1, A/H3N2 and B), and indicated for prophylaxis against influenza. The vaccine is to be administered intranasally, at the posology of 0.2 ml dose (0.1 ml per nostril).

Vaccination is the most effective method for prevention of influenza (CDC, 2007). All current vaccines include antigens that can provide protection against influenza A and influenza B. Annual vaccination (1 or 2 doses depending on age and prior influenza vaccination history) provides protection through an entire influenza season.

Each year, one or more strains contained in influenza vaccines might be changed to reflect the strains expected to circulate around the world. Since 1972, WHO has recommended 39 changes in the influenza vaccine formulation (WHO, 2005). Influenza vaccines must be administered annually to assure that populations are vaccinated with antigens that are relevant to circulating strains and provide optimal protection.

Fluenz contains a genetically modified organism (GMO) and has been evaluated for the potential risk to the environment.

2.2. Quality aspects

2.2.1. Introduction

The drug product FLUENZ is a trivalent (A/H1N1, A/H3N2 and B); cold-adapted live attenuated influenza vaccine. It is presented in a refrigerated liquid formulation. It is to be administered intranasally, at the posology of 0.2 ml dose (0.1 ml per nostril). The drug product is produced by MedImmune, LLC (Philadelphia, Pennsylvania, USA), and imported and released in the European Union by MedImmune UK Limited (Speke, Liverpool, UK).

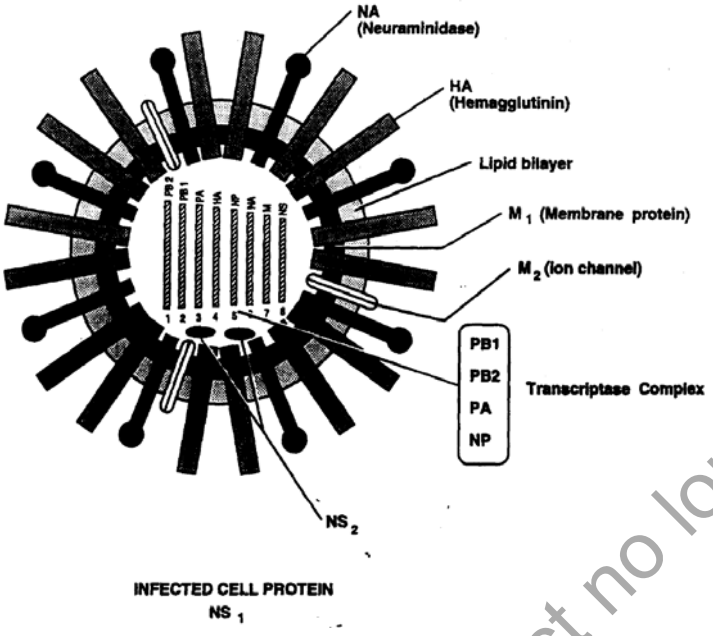
The drug substance consists of 3 different monovalent bulks of live attenuated influenza viruses (cold-adapted, temperature sensitive and attenuated), produced by MedImmune UK Limited (Speke, Liverpool UK). Monovalent bulks are prepared from purified harvests derived from the inoculation of the master virus seed (MVS) in embryonated Specific Pathogen Free (SPF) eggs. The master virus seeds (MVS) are prepared by a plasmid rescue process and contain a specific constellation of viral gene segments from an attenuated Master Donor Virus (MDV) and a wild-type (wt) influenza virus.

2.2.2. Active Substance

Influenza viruses Type A and Type B belong to the family of Orthomyxoviruses, and are morphologically indistinguishable from each other. Influenza viruses are enveloped and do not have a rigid capsule structure. 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. NP and M1 proteins contain epitopes that provide the basis for antigenic distinction between Type A and Type B influenza viruses. The external surface of the lipid bilayer of influenza viruses is decorated with two major viral transmembrane protein spikes. Approximately 80% of these transmembrane protein spikes 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)



FLUENZ contains three active components: two attenuated influenza A strains, H1N1 and H3N2, based on the influenza A master donor virus and one attenuated influenza B strain based on the influenza B master donor virus. The cold-adapted reassortant vaccine strains in FLUENZ are produced by genetic reassortment (reverse genetic techniques) between a wild-type influenza virus and a cold-adapted master strain. Such reassortant viruses contain gene segments encoding haemagglutinin (HA) and neuraminidase (NA) antigens that have been contributed by the wild-type virus, and gene segments encoding other proteins that have been contributed by the cold-adapted master donor virus. These vaccine strains are called 6:2 reassortants. Thus, cold-adapted reassortant vaccine strains derive their antigenic phenotypes from the wild-type strain and their cold-adapted (ca), temperature-sensitive (ts), and attenuated (att) phenotypes from the cold-adapted master donor virus.

The ca phenotype refers to the ability of the Type A or Type B cold-adapted master strain viruses to replicate to similar infectious titer in cell culture at either 33°C or 25°C. The ts phenotype of the Type A master strain viruses refers to the 39°C shut-off temperature of replication and the 100-fold or greater reduction in the number of plaques when compared to the permissive replication temperature of 33°C. The ts phenotype of the Type B ca master strain virus refers to the 37°C shut-off temperature of replication and the 100-fold or greater reduction in the number of plaques when compared to the permissive replication temperature of 33°C.

Manufacture

Development

The drug substance process development was initiated by the National Institutes of Health (NIH) in collaboration with Wyeth and Aviron, in the US. The clinical trial materials were manufactured by Wyeth in five different manufacturing campaigns (CTM1 through 5) between 2000 and 2004. In 2004, MedImmune obtained the rights from Wyeth to further develop and commercialize refrigerated FLUENZ, and in 2004, the process was transferred to MedImmune, UK.

Several changes were introduced at MedImmune, including scale up, use of a closed system and use of reverse genetics to establish the MVS.

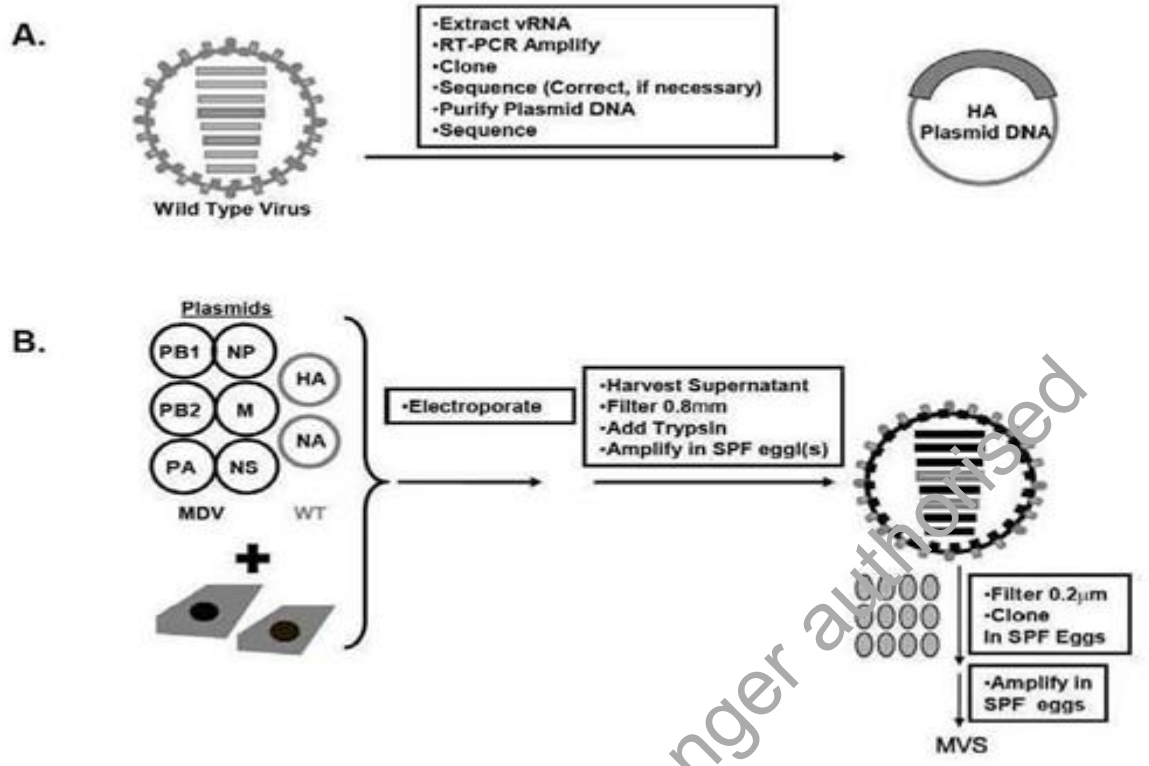
Comparability exercises were performed at the Quality level between Wyeth (US) refrigerated formulation (produced in 2004 or before) vs. MedImmune (UK) refrigerated formulation (produced in 2004). During the review, the CHMP raised concern on the comparability of batches produced with the final commercial process at MedImmune (after 2008) and the batches used in clinical trials (2004). In response to these concerns, MedImmune provided comparison of the results of batches used in the pivotal clinical trials at Wyeth 2004 with one batch of each virus strain from the 2008 manufacturing campaign. The Applicant also provided a summary of the clinical experience obtained with monovalent bulks produced with the final process and site, including link to clinical trials and their US marketing experience. The CHMP concluded that the submitted data satisfactorily resolved the concerns raised on comparability.

Manufacturing process

The master virus seeds (MVS) used in production consists of 6:2 genotype corresponding to: i) 6 gene segments (PB2, PB1, PA, NP, M, and NS) that confer the characteristics of the cold-adapted (ca), temperature sensitive (ts), and attenuated (att) phenotypes derived from the MDV, and ii) 2 gene segments, encoding the haemagglutinin (HA) and neuraminidase (NA) surface antigens derived from the wild-type(wt) influenza strains recommended by the World Health Organization (WHO). MVS are prepared by a plasmid rescue process / reverse genetics.

Figure 2 schematically depicts the procedure used to create the MVS using plasmids containing the expanded MDV (EMDV) and the expanded WT (EWT) gene segments. A new MVS is manufactured for each new wt strain recommended by the World Health Organization (WHO).

Figure 2: Diagram of Plasmid Rescue Process



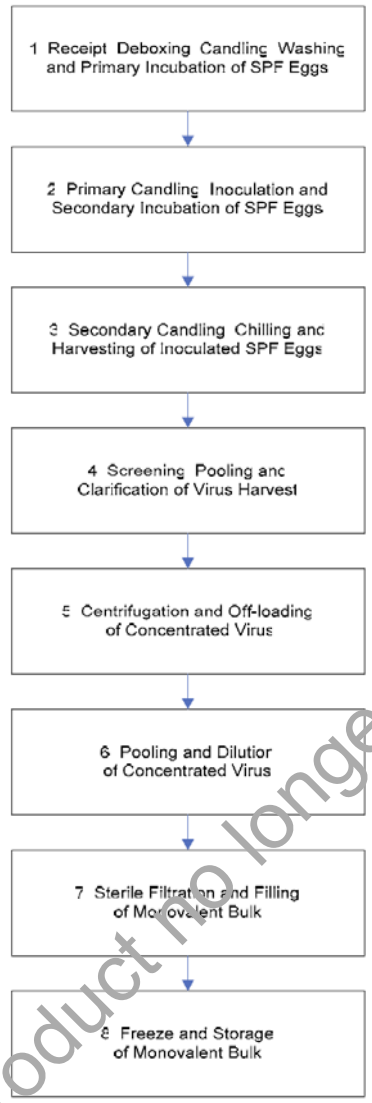
The plasmid rescue process is initiated by extracting viral RNA from the MDV and the WT, and converting six viral gene segments (PB1, PB2, PA, NP, M, NS) from the MDV, and the HA and NA gene segments from the wt strain, into cDNA by Reverse Transcriptase and Polymerase Chain Reaction (RT-PCR). These amplified cDNAs are inserted into plasmids and transformed into E. coli cells which are grown in animal free medium. The transformed E.coli cells are grown and plasmid DNA purified for testing and further processing. The cDNA containing the plasmids are characterized and their sequences analyzed to ensure that the representative viral genetic sequences have been obtained. The cDNA containing plasmids corresponding to the MDV PB1, PB2, PA, NP, M and NS gene segment, as well as the cDNA containing the plasmids corresponding to the wt HA and NA gene segments are combined for electroporation into serum-free Vero (African green monkey kidney) cells that are derived from an extensively tested and characterized cell bank produced in serum-free medium. The electroporated Vero cells are plated on tissue culture dishes, or, onto sub-confluent monolayers of CEK cells already seeded on tissue culture dishes in order to amplify the rescued 6:2 reassortant virus. The 6:2 reassortant is then propagated in SPF embryonated chicken eggs to produce sufficient amount of material, this material is designated the accession virus seed. An accession seed is made for each vaccine strain in the Research and Development Departments at the MedImmune CA facility. The accession seed is then transported to the MedImmune-UK facility for further production of the MVS. The accession seed is biologically purified and amplified in SPF eggs to produce the MVS batch.

All MVS and drug substance lots are tested for their 6:2 genotype by Restriction Fragment Length Polymorphism (RFLP), as part of their respective lot release. Initially the proposed test was not considered sufficient to demonstrate genetic stability. The Applicant was requested to demonstrate genetic stability on a number of passages beyond production level. During the procedure the Applicant submitted data in support of the genetic stability. The CHMP concluded that the genetic stability of the

MVS has been sufficiently documented. In addition the Applicant committed to repeat this study on each new MVS until sufficient experience will be acquired.

The drug substance is produced by MedImmune UK Limited (Speke, Liverpool UK). Monovalent bulks are prepared by the inoculation and growth of the master virus seed (MVS) in embryonated Specific Pathogen Free (SPF) eggs. Following secondary incubation, allantoic fluid is harvested and harvests that meet the acceptance criteria are pooled and mixed. The pooled harvest fluid is filtered and centrifuged to concentrate the virus particles and to reduce the quantity of egg-derived proteins, nucleic acids and other components. The concentrated virus is diluted prior to sterile filtration. The resulting monovalent bulk is mixed and then dispensed into bottles using a closed system and stored at ≤ -60°C. The drug substance manufacturing process flow is shown **in Figure 3.**

Figure 3: Overview of the manufacturing process



Receipt, preparation, incubation and inoculation of Specific Pathogen Free (SPF) Eggs

Upon shipments SPF eggs are transferred to refrigerated storage and checked for compliance with predefined specifications and integrity. Eggs are washed, rinsed, dried and transferred into the Primary Incubation suite.

Following the primary incubation period, the trolleys of eggs are removed from the primary incubators and transferred to the Primary Candling Area where they are candled. The trays of acceptable candled eggs transferred and held under controlled temperature conditions until inoculation. The eggs are inoculated with diluted Master Virus Seed (MVS) and incubated. After incubation, allantoic fluid is removed using a sterile graduated pipette. Clear allantoic fluid is dispensed into bottles and transferred to the Harvest Cold Room until released for further processing.

Screening, Pooling and centrifugation of Virus Harvest

Harvest bottles are screened for lack of bioburden using a rapid bioburden screening assay and pooled. The pooled and mixed fluid is then sampled to provide material for in-process and lot release

bioburden testing. The pooled material is filtered to obtain Clarified Harvest Fluid (CHF). The CHF is mixed and samples are removed for bioburden in-process testing. Pooled virus harvest is concentrated using continuous flow ultracentrifugation in a sucrose gradient in order to increase the density of virus particles present in the CHF and to reduce the quantity of egg-derived proteins, nucleic acids and other components. Concentrated virus harvest is pooled and pooled fractions are then diluted using phosphate buffer/EDTA prior to sterile filtration.

Sterile Filtration and Filling

The diluted virus harvest pool is sterile filtered to obtain the monovalent bulk. Following flushing and equilibration, the sterilizing grade filter is subjected to a filter integrity test.

Batch size is defined by the volume of material pooled and processed through the centrifuge.

Sterility and mycoplasma testing is performed on the MVS and provides assurance that no extraneous adventitious agents that may be present in the expanded wild type virus are carried over into the MVS. The bulk expanded wild type virus is filtered through a 0.2 µm filter, and an integrity test is performed on the filter after the filtration step has been completed. The MVS that is generated by plasmid rescue is screened in the mycoplasma test (microbiological culture and cell culture) as well as the sterility test before it is released for further manufacturing.

Critical Process Parameters are identified for each step of the drug substance manufacturing process, and are based on their ability to impact on Quality Attributes (QA) such as embryo viability, harvest volume yields, virus titre, virus concentration and egg-related impurities, pooling and dilution, potency. In-process control (IPC) limits have been established for key process parameters (KPP) that are used to monitor ongoing production of the monovalent bulk. The Applicant proposed a specification for the pooled harvest fluid, which consists of microbiological tests and viral tests.

Characterisation

The characterization studies of the drug substance have been presented as a summary of the different comparability exercises and included analysis of attenuation and phenotype (ca/ts), maintenance of RNA or cDNA consensus sequence from the respective MVS, virus particle morphology, percent infectious particles, HAI, HA and Viral RNA content. Replication and immunogenicity studies and protection following wild type challenge were performed in ferrets.

The impurities identified by the Applicant are egg related components in the allantoic fluid (ovalbumin, protein and chicken DNA) and other process related impurities (Disodium EDTA and Gentamicin Sulfate). Based on the low theoretical maximum possible concentration of disodium EDTA in the final product the monovalent bulk and final product are not routinely assayed for EDTA. The theoretical concentration of gentamicin sulfate in the final product is below levels of detection using current assay methods.

The monovalent bulk specification is the same for all serotypes, and consists of sterility, endotoxin, appearance, potency (Fluorescent Focus Assay), genotype (6:2 reassortant), phenotype (ca & ts), and attenuation (in Ferrets).

Initially, no HA or NA inhibition tests have been proposed in the drug substance specification. MedImmune was requested to develop and include comparable tests for HA and NA inhibition in the drug substance and/or MVS specifications. In response to these concerns, tests for physical appearance, infectivity and identity by HA inhibition were introduced at the level of the Expanded Wild Type (EWT) virus used to generate the Master Virus Seed (MVS) using the plasmid rescue process.

The nucleotide sequence of the RNA segments coding for the HA and NA proteins is determined for each WT or EWT virus at MedImmune. MedImmune committed to the development of an NA identity test to demonstrate the functional expression (enzymatic activity) of NA in the MVS as a release assay (i.e., neuraminidase inhibition). The type/subtype specificity will be demonstrated using NA-specific (type/subtype) antibodies that inhibit enzymatic activity. Initial specifications will be determined using data collected from samples from lots manufactured for the 2010-11 season, and details regarding this assay will be provided as a variation to the authorized Marketing Authorisation Application.

For annual strain update, MedImmune further committed to conduct a neurovirulence assay on the master virus strains of any novel strain subtype(s) introduced into the vaccine that have not been previously tested. This neurovirulence assay will be conducted as a GLP characterization assay and a summary of the assay has been presented by the Company. In addition, MedImmune also committed to develop a control strategy for monitoring neurovirulence of any novel strain subtypes taking advantage of several surveillance and reporting methods already in place including post-marketing safety data, periodic safety update report, risk management plan.

Stability

Stability studies have been performed on drug substance lots throughout product development, and include products manufactured from influenza viruses created by both classical re-assortment and plasmid rescue. Final stability data are presented and no deviations or atypical results were reported. As per MedImmune SOP, the first commercial lot of each strain of monovalent bulk drug substance manufactured in each season's manufacturing campaign is placed into the stability testing program.

Based on the stability studies performed, a shelf-life of 24 months at ≤-60°C is considered acceptable. In accordance with EU GMP guidelines[1], any confirmed out of specification result, or significant negative trend, should be reported to the CHMP.

2.2.3. Finished Medicinal Product

The finished product is a sterile colourless to pale yellow liquid composed of three serotypes (A/H3N2, A/H1N1 and B), and formulated with monosodium glutamate, gelatin, arginine, sucrose, and phosphate buffer. The finished product is presented as a 0.2 mL nasal sprayer capable of delivering a dose of 7.0 ± 0.5 log10 FFU of each strain, and 0.1mL is sprayed in each nostril. The composition of FLUENZ drug product is included in Table 5.

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

[1] 6.32 of Vol. 4 Part I of the Rules Governing Medicinal Products in the European Union

Table 5. Composition of FLUENZ

Name of Ingredients	Content (Per Dose)	Function	Monograph
Active Ingredient			
Influenza Virus, Type A, H1N1	7.0 ± 0.5 $\log_{10}$ FFU [a]	Immunogen	MedImmune specification
Influenza Virus, Type A, H3N2	7.0 ± 0.5 $\log_{10}$ FFU [b]	Immunogen	
Influenza Virus, Type B	7.0 ± 0.5 $\log_{10}$ FFU [c]	Immunogen	
Inactive			
Sucrose		Stabilizer	Ph. Eur./NF
Dipotassium Phosphate		Buffer	Ph. Eur./USP
Potassium Dihydrogen Phosphate		Buffer	Ph. Eur./NF
Gelatin Hydrolysate, Porcine Type A		Stabilizer	Ph. Eur./NF
Arginine Hydrochloride		Stabilizer	Ph. Eur./USP
Monosodium Glutamate		Stabilizer	Ph. Eur./NF
Water for Injection		Solvent	Ph. Eur./USP

[a] FFU – Fluorescent Focus Units as measured by Fluorescent Focus Assay

Pharmaceutical Development

The initial manufacturing process was transferred from Aviron to Wyeth. Between the years 1999 and 2004, Wyeth produced vaccine in five production campaigns to support preclinical and clinical development. In 2004, the drug product manufacturing site was transferred to MedImmune. In 2004, this site produced drug product for clinical pivotal studies, but using drug substance produced by Wyeth. All batches produced at MedImmune appear to use the final formulation. In response to the D180 CHMP list of questions, MedImmune provided comparison of the results batches used in the pivotal clinical trials at Wyeth 2004 with one batch of each virus strain from the 2008 manufacturing campaign. The Applicant also provided a summary of the clinical experience obtained with monovalent bulks produced with the final process and site, including link to clinical trials and their US marketing experience.

During the review change to the fill line was implemented, which was based on conclusions of a GMP inspection (Red Lion Site, Philadelphia, USA). Fill Line 2 has been implemented as the primary fill line for Thaw/Blend/Fill operation at the Red Lion Site, Philadelphia, USA since 2010 and has been validated.

Adventitious agents

The master virus seeds (MVS) are prepared by a plasmid rescue process and contain a specific constellation of viral gene segments from an attenuated Master Donor Virus (MDV) and a wild-type (wt) influenza virus. Vero cells are electroporated with plasmids containing cDNA clones of the viral gene segment and then co-cultured with SPF CEK cells to produce pre-MVS/MVS. Raw materials of animal origin (FBS, NCS, porcine trypsin) were used for Vero cell banks establishment and during cultures of CEK cells.

The MVS are used to inoculate Specific Pathogen Free (SPF) embryonated eggs to produce individual monovalent bulks for each of the three virus strains.

The viral safety of FLUENZ relies on 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 production process at MVS level and pooled harvest fluid level.

Regarding virological controls, the Company has previously committed to perform specific tests for bovine and porcine viruses on the MVS as long as non irradiated NCS and non irradiated trypsin in CEK cells are used. However, since non irradiated porcine trypsin was also used for the establishment of Vero WCB (2003), the Company commits to performing specific tests for porcine viruses as long as MVS generated using the 2003 WCB remains in the vaccine formulation when the product is launched in Europe. The WCB produced in 2009 was prepared without the use of any animal derived components (i.e., no animal derived components in the medium, animal-component free recombinant trypsin). The new 2009 WCB will be used to generate all new MVS. But, in the case where non-recombinant trypsin would be used in future WCB establishment, MedImmune commits to performing the research for porcine viruses on either the cell bank or the MVS. The commitments by the Company were noted and considered acceptable by the CHMP.

Raw materials of animal origin used in the production of FLUENZ are fetal bovine serum, new born calf serum and porcine trypsin, which were used for Vero cell banks establishment and during cultures of CEK. Certificates of suitability for TSE safety have been provided for all bovine materials.

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

Manufacture of the product

The manufacturing process mainly consists of the thawing of the monovalent bulks, followed by a blending of the 3 strains with buffer and dilution to final volume with buffer. The blended trivalent formulated bulk is then 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).

Critical steps are controlled at several steps in both the blending and filling processes to ensure that the process performs as intended. As for the drug substance, the strategy employed by the Applicant with regards to the identification and selection of Critical Process Parameters and Quality Attributes was not documented. Nevertheless, the rationale provided for the selection of each CPP are generally considered reasonable. Most of the CPP identified appear to be based on their impact on potency. Furthermore, no test are performed to control the HA and NA titres through the process, or to control the proper gelatine content.

The drug product process validation at the MedImmune Pennsylvania (PA) facility was performed on 3 batches with drug substance that was manufactured in 2004 by the MedImmune UK-1 facility at pilot DS manufacturing scale). The results on previous batches have been provided and support the consistency of the process.

Product Specification

The drug product specification consists in identity, potency, ovalbumin, total protein, appearance, pH, endotoxin and sterility testing. The identity is based on the Fluorescent Focus Assay (FFA assay). No HA or NA inhibition tests have been proposed in the drug product specification.

During the procedure, concerns were raised regarding the lack of an appropriate test to study thermal stability in order to select incubation time and to study loss in potency in comparison to the unheated vaccine. MedImmune was requested to perform a test on an appropriate number of batches (e.g. annual strain change, process modification). MedImmune committed to establish a thermal stability assay before marketing of the product and will conduct studies to select the incubation temperature, incubation time, and sample treatment to establish an acceptance criterion for potency loss in comparison to that of an unheated vaccine. The test is not intended for routine lot testing and MedImmune will determine the appropriate test frequency.

Stability of the product

The drug product stability data support a shelf life at -25°C ± 5°C for up to 20 weeks prior to distribution and subsequent storage at 2°C to 8°C not to exceed 18 weeks.

In accordance with EU GMP guidelines2, any confirmed out of specification result, or significant negative trend, should be reported to the CHMP.

GMO

FLUENZ is a live attenuated influenza vaccine, whereby its virus strains have been generated through reverse genetic technology. For this reason, the vaccine has been classified a genetically modified organism (GMO) as defined in Directive 2001/18/EC3. The environmental risk assessment (ERA) is mostly based on information present in module 1.6.2. of the application for marketing authorisation. The scope of this ERA is the environment at large, excluding the patient but including people in the patient's environment. In general the current ERA follows the methodology described in the EU deliberate release Directive 2001/18/EC. Environmental national competent authorities have been consulted for this procedure. Following their expression of interest, the following national authorities have been consulted: Belgium, Finland, Sweden, Norway, Spain, Ireland, Bulgaria and Czech Republic.

FLUENZ contains two types A (ie, A/H1N1 and H3N2) and one type B attenuated (att), cold-adapted (ca) and temperature sensitive (ts) reassortant strains. Each dose is formulated to contain 107±0.5 fluorescence focus units (FFU) of each of the three reassortant influenza virus strains.

The plasmid rescue process utilizes recombinant DNA techniques to produce genetic reassortants. Each of the three vaccine strains in FLUENZ are 6:2 genetic reassortants. These vaccine strains have 6 gene segments (PB1, PB2, PA, NP, M and NS) from one master donor viruses (MDV, type A or B) and 2 gene segments, hemagglutinin (HA) and neuraminidase (NA) from the WHO recommended contemporary wt influenza virus.

The specific genotype of MDVs is designated 6:2 which indicates that 6 internal gene segments that confer the characteristics of the ca, ts and att phenotypes are derived from the MDV and that the 2 gene segments encoding HA and NA surface antigens are derived from the wt influenza strains.

The overall risk of FLUENZ to human health and the environment is concluded to be negligible. Therefore, the overall risk posed by the GMO to human health and the environment is considered low or negligible (in the scenario of the worst case assessment).

FLUENZ does not replicate in the environment, does not carry a toxic transgene, is specific to humans, does not integrate and therefore is very unlikely to transfer genes to any other species, and is well tolerated in vaccinated individuals at recommended administration doses.

The CHMP concludes that the overall risk to the environment from FLUENZ is low.

In addition, the CHMP is of the opinion that the assessment confirms the relevance of the initial ERA for future seasonal strain updates. There will be no need to submit an ERA at each seasonal strain update procedure, with all proper reserves of new scientific information publication on the ERA for this kind of vaccine.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The production process for FLUENZ has been adequately described and is considered controlled and sufficiently validated.

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

[3] • Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.

Starting materials used in the production of the drug substance of biological origin are Specific Pathogen Free (SPF) Eggs, Vero cells (which may use CEK cells as feeder cells) used in the production of the MVS and the master virus seed generated from plasmids containing the expanded MDV (EMDV) and the expanded WT (EWT) gene segments. Raw materials of animal origin used in the production of FLUENZ are fetal bovine serum, new born calf serum and porcine trypsin, which were used for Vero cell banks establishment and during cultures of CEK. Certificates of suitability for TSE safety have been provided for all bovine materials.

MVS have been established using plasmids containing the expanded MDV (EMDV) and the expanded WT (EWT) gene segments. A new MVS is manufactured annually for each new wt strain recommended by the World Health Organization (WHO).

All MVS and drug substance lots are tested for their 6:2 genotype by RFLP, as part of their respective lot release. Initially the proposed test was not considered sufficient to demonstrate genetic stability. The Applicant was requested to demonstrate genetic stability on a number of passages beyond production level as further replication. During the procedure the Applicant submitted data in support of the genetic stability. In addition the Applicant committed to repeat this study on each new MVS until sufficient experience will be acquired. The CHMP concluded that the genetic stability of the MVS has been sufficiently documented.

During the review, the CHMP raised concern on the comparability of batches produced with the final commercial process at MedImmune (after 2008) and the batches used in clinical trials (2004). In response to these concerns, MedImmune provided comparison of the results batches used in the pivotal clinical trials at Wyeth 2004 with one batch of each virus strain from the 2008 manufacturing campaign. The Applicant also provided a summary of the clinical experience obtained with monovalent bulks produced with the final process and site including link to clinical trials and their US marketing experience. The CHMP concluded that the submitted data satisfactorily resolved the concerns on comparability.

Critical Process Parameters are identified for each step of the drug substance manufacturing process, and are based on their impact on Quality Attributes such as embryo viability, harvest volume yields, virus titre, virus concentration and egg-related impurities, pooling and dilution, potency. In-process control limits have been established for key process parameters that are used to monitor ongoing production of the monovalent bulk. The Applicant proposed a specification for the pooled harvest fluid, which consists of microbiological tests and viral tests.

Based on stability studies performed with batches obtained during process development, a shelf-life of 24 months at ≤-60°C has been assigned, which is considered acceptable. The Applicant has committed to provide any confirmed out of specification result or significant negative trend to the CHMP, in accordance with EU GMP guidelines.

Drug Product

The finished product is a sterile colourless to pale yellow liquid composed of three serotypes (A/H3N2, A/H1N1 and B), and formulated with monosodium glutamate, gelatin, arginine, sucrose, and phosphate buffer. The finished product is presented as a 0.2 mL nasal sprayer capable of delivering a dose of 7.0 ± 0.5 log10 FFU of each strain, and 0.1mL is sprayed in each nostril.

The manufacturing process complies with standard procedures used for the formulation and filling of live attenuated viral vaccines. The manufacturing process including the process controls have been sufficiently described and the critical steps in the manufacture of the drug product have been identified and are adequately controlled. Sufficient information has been provided on the validation strategy.

The drug product process validation at the MedImmune Pennsylvania (PA) facility was performed on 3 batches produced in 2005 with drug substance that was apparently manufactured in 2004 by the MedImmune UK-1 facility at pilot DS manufacturing scale. The results on previous batches have been provided and support the consistency of the process.

Based on the currently available drug product stability data the Applicant proposed a shelf life at -25°C ± 5°C for up to 20 weeks prior to distribution and subsequent storage at 2°C to 8°C not to exceed 18 weeks. This was accepted by the CHMP.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The production process of FLUENZ drug substance and drug product is well defined and sufficiently validated. All manufacturing sites are in compliance with current GMP requirements. Several concerns on establishment, characterisation, control and stability of the master virus seed, comparability, drug substance specifications, identity testing and drug substance specifications, which were initially raised as major concerns, have been addressed by the Applicant. The Applicant has committed to further address some outstanding issues as follow-up measures.

2.3. Non-clinical aspects

2.3.1. Introduction

The pharmacological activity of FLUENZ 6:2 reassortant influenza viruses is related to its ability to replicate in the nasopharynx, thereby initiating immune responses (via mucosal and serum antibodies, and possibly cytotoxic T-cells), and to its inability to replicate efficiently in the lower airways and the lung due to the warmer temperatures. These properties enable FLUENZ to elicit a protective immune response without causing clinical disease.

Traditional non-clinical pharmacology studies were not initially performed, partly because of the extensive human database as well as the fact that several, specific nonclinical tests have been incorporated into the release testing scheme for each monovalent lot of the vaccine. Release testing includes an attenuation assay utilizing a ferret model which evaluates replication of influenza vaccine strains in the nasal turbinates and lungs of ferrets. This assay verifies that vaccine strains exhibit the attenuated (att) phenotype characterized by replication of vaccine strains in the upper airways of these animals but no, or highly restricted replication in lung tissues and no signs of influenza illness. Any evidence of clinical signs of influenza-like illness elicited in ferrets would be noted during release testing of the monovalent 6:2 vaccine strains and would prevent release of the strains for further manufacturing. Other routine cell culture tests characterise the phenotype of these attenuated strains including cold adaptation and temperature sensitivity.

Since the initial regulatory filing of FLUENZ in the USA (where it is marketed as FluMist), the non-clinical program has evolved due to the change from a frozen formulation to a refrigerated formulation. The active agents of the two formulations are identical from a clinical or non-clinical point of view. In addition to the routine attenuation testing in ferrets performed on each reassortant vaccine strain,

several non-clinical pharmacology and toxicology studies have been performed on selected lots of both the frozen and refrigerated formulations of the vaccine to demonstrate comparability.

Table 6. Pharmacodynamic studies

Type of Study	Species	Test Article	Method of Administration	Study Number
Primary Pharmacodynamics	Ferret	FluMist refrigerated and frozen formulations	Intranasal	MedImmune Research Report
Safety Pharmacology	Mouse	FluMist Master Virus Seeds	Intranasal	ACF-07-001
Pharmacodynamic Drug Interactions	Not Applicable [a]	Not Applicable [a]	Not Applicable [a]	Not Applicable [a]

[a] Pharmacodynamic drug interaction studies have not been conducted (see text)

The pharmacology program for FLUENZ included ferret immunogenicity and challenge studies with wt virus that was conducted to compare the frozen and refrigerated formulations, as well as a safety pharmacology study in mice to evaluate the potential for neurovirulence of monovalent vaccine strains and the trivalent vaccine. Moreover, toxicology studies included a repeat dose toxicology study in ferrets performed using the refrigerated formulation, two reproductive toxicology studies in rats (frozen formulation) and ferrets (refrigerated formulation), and two ocular toxicology studies (Draize tests) in a rabbit model.

2.3.2. Pharmacology

Primary pharmacodynamics

Mechanism of action

Naturally acquired immunity to wild-type influenza has not been completely elucidated. Likewise immune mechanisms conferring protection against influenza following receipt of FLUENZ are not fully understood. Serum antibodies, mucosal antibodies and influenza-specific T cells may play a role in prevention and recovery from infection.

Shedding and immunogenicity study of different FLUENZ formulations in ferrets

This immunogenicity and challenge study in ferrets showed that the intranasal administration of either the refrigerated or frozen formulation of FLUENZ prevented replication of a wt virus in the lung tissues of animals and significantly decreased the level of replication of the challenge virus in the upper airways. Nasal wash samples collected from vaccinated animals at several time points post inoculation indicated that the pattern of shedding was indistinguishable between animals receiving the refrigerated formulation and those that received the frozen formulation. Titres of vaccine virus in nasal wash specimens increased between 8 hours and 1 day post vaccination, remained elevated through Day 5, and returned to low levels by 7 days after vaccination, and measurements of immunity assessed by hemagglutination inhibition and neutralization titers present in the sera were highly similar for both vaccine formulations. This study showed that the performance of the tested refrigerated and frozen formulations were similar with respect to vaccine take, replication, immune response induction, and protection of animals from a challenge infection with wt virus.

Immunogenicity in immunologically immature animals

This vaccine is intended for infants above 24 months of age. The immunogenicity in ferrets is determined in castrated males or females 6 to 8 weeks old. These animals are prepubertal (ferret puberty occurs at 6 to 8 months - Life span of 5 to 11 years). It is not known whether ferrets are immunologically immature at this age. However a sufficient amount of clinical data is available for all intended age groups.

Protective efficacy in animals: animal challenge model with wild type influenza viruses

To further evaluate protective immunity following vaccination, ferrets immunized with either frozen or refrigerated FLUENZ or placebo were challenged with matched wt H1N1, H3N2, or B virus on Day 36 (2 weeks post dose 2). Placebo or vaccine immunized animals (n = 4 per wt virus) were challenged by intranasal inoculation with either wt A/New Caledonia/20/99 (H1N1), wt A/Texas/40/2003 (H3N2) (antigenically similar to A/Wyoming/03/2003), or wt B/Jilin/20/03 virus. Nasal washes were collected at 8 hours (challenge Day 0), Day 1 and Day 3 post-challenge. Finally, on Day 3 post-challenge the animals were euthanized and the nasal turbinate and lung tissues were harvested. Upon wt virus challenge, each of the three H1N1, H3N2 and B wt viruses were efficiently recovered from both the upper and lower respiratory tracts and the nasal washes of animals in the placebo group, demonstrating the susceptibility of the animals and the virulence of the challenge strains. In contrast, virtually no virus was recovered in the nasal washes of animals vaccinated with either the frozen or refrigerated FLUENZ formulations for any of the three wt challenge strains. On Day 3 of the challenge infection, whilst wt virus was recovered from extracts of the nasal turbinate tissues in the placebo group, it was infrequently recovered from vaccinated animals, and when recovered, only at very low quantities (see table 7). Nearly all animals in the placebo group had detectable virus in their lung tissues, whereas no virus was recovered from the lung tissues of animals vaccinated with either FLUENZ formulation, indicating complete protection of the lower respiratory tract by both formulations (see table 7). These data clearly demonstrated comparable and highly protective immunity elicited by FLUENZ in this good animal model of influenza disease.

Table 7. Frozen and Refrigerated FLUENZ Vaccinated Ferrets are Protected from Challenge with Wild-Type H1, H3 and B Viruses.

Group	Nasal Turbinate Tissues		Lung Tissues	
	Titer (Log_{10} $TCID_{50}$/ml)	% Animals with Virus Recovered	Titer (Log_{10} EID_{50}/ml)	% Animals with Virus Recovered
Placebo				
H1 (n = 4)	5.2 ± 0.4	100%	3.2 ± 1.2	75%
H3 (n = 4)	4.1 ± 0.2	100%	3.0 ± 0.2	100%
B (n = 4)	6.3 ± 0.2	100%	3.2 ± 0.2	100%
Frozen FluMist				
H1 (n = 4)	3.0 ± 0.0	25%	< 1.5 [a]	0%
H3 (n = 4)	< 3.0 [a]	0%	< 1.5 [a]	0%
B (n = 4)	3.1 ± 0.2	50%	< 1.5 [a]	0%
Refrigerated FluMist				
H1 (n = 4)	< 3.0 [a]	0%	< 1.5 [a]	0%
H3 (n = 4)	< 3.0 [a]	0%	< 1.5 [a]	0%
B (n = 4)	< 3.0 [a]	0%	< 1.5 [a]	0%

[a] No Virus detected. Number indicates lowest possible determinable titer.

Secondary pharmacodynamic studies

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

Safety pharmacology programme

The mouse is an established model to study neurovirulence and is valuable to investigate potential neurovirulence of laboratory adapted human influenza viruses. Non-adapted human strains of influenza generally undergo a single abortive cycle when injected into mouse brain tissue and do not yield productive infection. The A/WS/33 (WS) (H1N1) strain of influenza has been adapted to grow in the mouse, yielding variants A/NWS/33 and A/WSN/33.

Neurovirulence testing of influenza strains in mice (Study ACF061-07-001)

The mouse animal model was used to evaluate potential neurovirulence of FLUENZ strains using as controls murine neuroadapted strains that have been well characterized (A/NWS-33), since a mouse-adapted, neurovirulent type B influenza virus was unavailable. The objective of the study was to quantify the viral levels in the brain tissue of mice at 7 days following intranasal inoculation using real time qPCR as a measure of viral replication and neurovirulence. The established dose of the comparator virus used for the purposes of this study was $3\log_{10}$ $TCID_{50}$. By contrast, the dose of the vaccine strains used in this evaluation was $5\log_{10}TCID_{50}$. A range of 7.68×10^2 to 1.05×10^5 copies/mg of viral RNA were detected in the mouse brain tissue at Day 7 when a dose of 3 $\log_{10}$ $TCID_{50}$ of A/NWS/33 was delivered intranasally. In contrast, no viral RNA was detected when any of the type A, monovalent FLUENZ vaccine strains were administered. A β-actin assay was performed in parallel to show that comparable levels of RNA were analysed. Likewise, no viral RNA was detected for type B influenza vaccine viruses. No viral RNA was detected in the mouse brain tissue when trivalent FLUENZ vaccine viruses were included in the study.

It was concluded that the FLUENZ viruses, either in monovalent master seeds or trivalent formulation, did not exhibit any neurotropism or neurovirulence.

Pharmacodynamic drug interactions

Pharmacodynamic drug interaction studies have not been conducted with FLUENZ, 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). However, clinical studies were performed to assess safety, tolerability and immunogenicity of FLUENZ administered concurrently with measles, mumps, rubella (MMR), varicella and oral polio vaccines in young children (see section Clinical Pharmacology – Interaction studies).

2.3.3. Pharmacokinetics

While many of the typical pharmacokinetic studies, including absorption, metabolism and excretion, do not pertain to live vaccines, local deposition and distribution studies have been performed in humans. 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. Moreover the vaccine does not contain an adjuvant or new excipients which would require other pharmacokinetics studies.

2.3.4. Toxicology

Study Type (Duration)	Route	Species	FluMist Formulation	Study Number
Repeat dose Toxicity (14 weeks) Dose 1: 0 weeks Dose 2: 4 weeks Dose 3: 14 weeks	Intranasal	Ferret	Refrigerated	SVT01-18
Reproductive Toxicity (22 days)	Intranasal	Ferret	Refrigerated	SVT01-19
Reproductive Toxicity (21 days)	Intranasal	Rat	Frozen	3113-001
Ocular Toxicity (72 hours)	Intraocular	Rabbit	Refrigerated	SVT02-10
Ocular Toxicity (72 hours)	Intraocular	Rabbit	Frozen	8012730
Reassortant risk	Intranasal	Ferret	N/A [a]	N/A [b]
Environmental Safety Studies: Tropism for reassortant vaccine strains in multiple species	Intranasal or oral [c]	Multiple mammalian and avian species	Frozen	51123; 51124; 51125; 51126; 51157; 51158; 51159; 51191; 51192; 51193; 51194; 51195; 51196; 51197; 51198; 51199; 51200; 51201; 51202; 51203; 51204

[a] Not applicable; reassortants were experimentally created between vaccine and wild-type strains

[b] Data reported in: Parks, C. L., Latham, T., Cahill, A., O'Neill, R. E., et al. (2007) Phenotypic properties resulting from directed gene segment reassortment between wild type A/Sydney/5/97 influenza virus and the live attenuated vaccine strain. Vaccine 367: 275-287.

c Mammalian species were inoculated intranasally; avian species were inoculated orally

Single dose toxicity

A single dose toxicity study was incorporated into the repeat dose toxicity study.

Repeat dose toxicity

A repeat dose toxicology study was conducted in ferrets to investigate the potential adverse effects of refrigerated FLUENZ given one or three times to ferrets over a 15-week period. The regimen consisted of up to 3 human doses of FLUENZ administered intranasally at weeks 0, 4 and 14. No clinical indications of toxicity were manifest during the course of the study from any of the parameters evaluated. No test material-related toxicity was identified in the major organs by histopathological analysis at either the interim or terminal necropsies except in the nasal turbinates and cervical lymphoid tissues at interim necropsy. An acute multifocal suppurative inflammation of the nasal turbinates was present in vehicle (1/4 animals) and vaccinated (3/8 animals) groups at interim necropsy. This was not observed at terminal necropsy. These findings could be due to the inoculation 3 days prior to necropsy and the antigenic responses of the animals to the inoculums.

Genotoxicity

No Genotoxicity study was submitted for FLUENZ in accordance with the CHMP Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines (CPMP/SWP/465/95).

Carcinogenicity

No Carcinogenicity study was submitted for FLUENZ as recommended by the CHMP Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines (CPMP/SWP/465/95).

Reproduction Toxicity

An embryo-foetal development study was performed in ferrets. A 0.2 ml dose of refrigerated FLUENZ was administered intranasally to pregnant ferrets at 4 different time points during gestation (Days 3, 6, 13 and 22). The ferret study was designed to evaluate the effect of FLUENZ on maternal mortality, macroscopic pathology, clinical observations or body weight during gestations and on foetal development from before implantation throughout organogenesis. The study included the standard observations of maternal viability and behaviour and the standard observations for caesarean delivered foetuses. The results of this study showed that neither the vaccine nor the immune response induced by vaccination are associated with foetal or maternal toxicities in ferrets.

A pre-and postnatal toxicity study (terminated earlier than standard, i.e. on postnatal day 21, and including maternal function) was performed in rats. This study was conducted with a frozen formulation of FLUENZ and was designed to evaluate ICH Harmonized Tripartite Guideline Stages C through E, in order to detect adverse effects of the vaccine from before mating through implantation and lactation, on gestation, parturition, lactation and maternal behaviour in female rats and on the development of the offspring. Thus, approximately equal numbers of vaccine and placebo recipients were assigned to caesarean delivery on Day 21 of gestation, or to natural delivery. Delivered pups were sacrificed on Day 21 of life. Results indicated that exposure to 250 µl of FLUENZ once prior to mating and once during pregnancy (on gestation day 6) did not produce any maternal toxicity or affects the reproductive capacity of the dam. These exposures also did not produce any embryo-foetal toxicity in the F1 generation near term foetuses (no effect on weight or external, soft tissue and skeletal alterations) or F1 generation pups evaluated for 21 days postpartum.

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.

Local Tolerance

Evaluation of local tolerance at the administration site is included in the repeated dose toxicity study with the evaluation of the nasal mucosa.

The potential for ocular toxicity resulting from the inadvertent instillation of FLUENZ into the eye was evaluated in two ocular toxicity studies 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 specific studies were performed.

2.3.5. Ecotoxicity/environmental risk assessment

Environmental safety studies were conducted with FLUENZ to evaluate the tropism of the vaccine for nonhuman species. Studies in 21 different animal species showed that influenza vaccine strains did not replicate in any of the investigated species except hamsters, guinea pigs and ferrets which are all known to be capable of being experimentally infected with human influenza virus. The vaccine strains did not replicate in any avian species and the overall results demonstrated that the vaccine strains have no novel tropism for nonhuman species.

Evaluation of a number of experimentally created reassortants (genetic reassortment between wt and vaccine strains) in a ferret model indicate that such reassortment is not likely to create viruses with new properties compared with either progenitor and more likely that the reassortant will be attenuated.

FLUENZ formulation contains live attenuated viruses which are prepared by reverse genetics techniques therefore it is considered a GMO (see also quality section of this AR). The overall risk posed by this GMO to human health and the environment is considered low or negligible. FLUENZ does not replicate freely in the environment and moreover it is specific to humans and a few mammalian species (see above); it does not carry a toxic transgene, does not integrate and therefore it is very unlikely to transfer genes to any other species; finally it is well tolerated in vaccinated individuals at the recommended dose.

This assessment is also relevant for future seasonal strain updates, in the sense that there will be no need to submit an environmental risk assessment at each seasonal strain update procedure. However this situation might change in the future based on new scientific information that shall be published on this topic.

2.3.6. Discussion on non-clinical aspects

The primary pharmacology study comparing the frozen and refrigerated formulations in ferrets demonstrated that the patterns of shedding were indistinguishable, and immunity generated by either of the two formulations was similar as measured by haemagglutination inhibition (HAI), and neutralization titres in sera. Immunization with either the refrigerated or frozen formulation prevented replication of a wt virus in the lung tissues of animals and significantly decreased the level of replication of the challenge virus in the upper airways. This study demonstrated that the performances of the two formulations were comparable with respect to vaccine infectivity and replication, immune response induction, and protection of animals from a challenge infection with wt virus. The safety pharmacology study to evaluate the neurovirulence of monovalent and trivalent FLUENZ vaccine strains utilized a murine model and a well characterized neurovirulent strain of influenza as a control. Intranasal administration of the H1N1 neurovirulent strain (A/NWS/33) resulted in replication of the virus in the nasopharynx, followed by dissemination and replication in the central nervous system (CNS), ultimately leading to disease and death. In contrast, FLUENZ viruses, in both monovalent and trivalent formulations, did not exhibit any neurotropism or neurovirulence. As advised by the CHMP in the scientific advice "Intranasal Influenza Virus Vaccine, Trivalent, Types A and B, Live, att, ca (CAW-T influenza vaccine trivalent-Fluenz), EMEA/CHMP/SAWP/258103/2007 and EMEA/SA/H/408/1/FU/1/2007/111", the applicant is recommended to use a model with direct neuroinvasion (e.g. intracranial or intraspinal administration) for influenza neurovirulence testing (see

also: Neurovirulence of Influenza A virus. Ward A.C. J. Neurovirol., 1996), because this model allows neurovirulence detection regardless of neuroinvasion, thereby making it more likely to detect potential neurovirulence. Vaccine strains of novel subtypes of virus that have not been previously tested for neurovirulence will need to be tested in the future, since little changes in the amino-acid composition of NA may significantly alter neurovirulence.

2.3.7. Conclusion on the non-clinical aspects

Non-clinical data with FLUENZ revealed no special hazard for humans based on conventional non-clinical studies of repeated does toxicity, reproduction and developmental toxicity, local tolerance and neurovirulence.

2.4. Clinical aspects

2.4.1. Introduction

A total of 73 clinical and postmarketing studies of the frozen or refrigerated formulations of FLUENZ have been conducted: 63 studies conducted by 3 different company sponsors (Aviron, Wyeth, or MedImmune), and 10 conducted by independent investigators (non-company sponsored studies, e.g., those initiated by individual investigators or by USA governmental entities). Of the 63 company-sponsored studies, 57 have been completed (21 by Aviron, 24 by Wyeth, and 12 by MedImmune) and 6 were ongoing at the time of submission. Of these, data from 43 studies evaluated the clinical efficacy/effectiveness or immunogenicity of FLUENZ. Of these 43 studies, 31 included paediatric subjects (6 months through 17 years of age; 8 of these studies also included subjects ≥ 18 years of age) and 12 studies that enrolled only adult subjects (≥ 18 years of age). More than 64,000 subjects, ranging in age from 6 months to 97 years, have been entered in these 43 studies. Of the 31 studies that included pediatric subjects, 15 were designed to evaluate the efficacy/effectiveness of FLUENZ. Of the 12 studies that enrolled only adult subjects, 4 were designed to evaluate the efficacy/effectiveness of FLUENZ. Thus, a total of 19 studies have been conducted on the efficacy/effectiveness of FLUENZ in subjects 6 months of age through adulthood (> 65 years), of which 14 were TIV or placebo controlled and 5 were supportive studies. The immunogenicity of FLUENZ was assessed in 33 of the 43 efficacy/effectiveness/immunogenicity studies in the clinical program, 22 of which included paediatric subjects and 11 of which enrolled only adult subjects.

GCP

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

A GCP inspection was conducted on data management issues. However, during the process of the inspection, it was detected that the site of CRFs archiving had burned in 2006. The CHMP considered that through the loss of some data the validity of the Marketing Application was compromised. The applicant was therefore requested by the CHMP to recover the CRFs from the investigator sites. The procedure was stopped until CRF recovery could be performed. In April 2010, while the CRF recovery was still running the CHMP has nevertheless considered that in view of the recovery already achieved for the pivotal studies and the applicant's willingness to comply with the EMA request for CRFs recovery, the procedure could be re-started.

In July 2010 CHMP performed a thorough check between the computerized data and recovered CRFs. Two hundred and fifty CRF's were checked within 8 studies, regarding safety, demography and vaccination criteria. On the basis of this check, the CHMP concluded that the data collected in the clinical trial database are the "picture" of the CRF's provided by the investigator sites. The CRF recovery was therefore considered as being satisfactorily addressed.

- Tabular overview of main clinical efficacy/effectiveness studies

Table 8. Pediatric studies

Study number/ location/date	Design Main study objectives	Tests products	Number of subjects Randomized	Age
Dose response study				
D153-P513 Thailand The Philippines 2002	Randomized, double blind, Placebo controlled Two dose regimen ***Primary Efficacy Objective*** To evaluate dose trend in terms of incidence rates over the surveillance period of CCII caused by community-acquired subtypes antigenically similar to those contained in the vaccine To identify the dose(s) with clinically significant efficacy against CCII caused by community-acquired subtypes antigenically similar to those contained in the vaccine. ***Secondary Efficacy Objectives*** To evaluate dose trend, in terms of incidence rates over the surveillance period of CCII caused by any community-acquired subtypes To identify the dose(s) with clinically significant efficacy against CCII caused by any community-acquired subtypes. To evaluate dose trend, in terms of incidence rates of acute otitis media (AOM), febrile OM and influenza-associated OM To identify the dose(s) with clinically significant efficacy against AOM, febrile OM, and influenza-associated OM	Refrigerated FLUENZ 10^7 10^6 10^5 Placebo: 0.2 ml per dose	Total: 2172 543 546 546 537	6-<36 months
Main studies				
TIV controlled studies				
MI-CP111 USA Asia Europe Middle East 2004-05	Randomized, double blind, active controlled One or two dose regimen ***Primary Efficacy Objective*** to estimate the relative efficacy and assess the safety of FLUENZ compared to TIV on incidence of culture-confirmed CDC-Influenza-Like illness caused by matched strains. ***Secondary objectives*** To estimate the relative effectiveness of R-FLUENZ compared to TIV To assess the tolerability of FLUENZ compared to TIV	Refrigerated FLUENZ 0.2 ml TIV 6-35 months : 0.25 ml >35 months : 0.5 ml	Total: 8475 4243 4232	6-59 months
D153-P514 Europe Israel	Randomized, open label, controlled trivalent inactivated vaccine (TIV) Two dose regimen ***Primary Objectives***	Refrigerated FLUENZ 0.2 ml TIV	Total: 2187 1101 1086	6-71 months

2002-03	Non inferiority of FLUENZ vs TIV against culture-confirmed influenza-illness (CCII) caused by community-acquired subtypes antigenically similar to those contained in the vaccine, in children with a history of recurrent respiratory tract infections (RTIs) ***Secondary Objectives*** Non inferiority of FLUENZ vs TIV against CCII of any type Non inferiority against otitis media (OM), febrile OM and influenza-associated OM	6-35 months : 0.25 ml >35 months : 0.5 ml		
D153-P515 Europe Israel 2002-03	Randomized, open label, controlled trivalent inactivated vaccine (TIV) One dose regimen ***Primary Objectives*** Non inferiority of FLUENZ vs TIV against culture-confirmed influenza-illness (CCII) caused by community-acquired subtypes antigenically similar to those contained in the vaccine, in children with asthma ***Secondary Objectives*** Non inferiority of R-FLUENZ vs TIV against CCII of any type To compare the efficacy over a defined surveillance period against asthma exacerbations, asthma medication, clinic visits, hospitalizations, days off school (pharma-economic measures) associated with influenza-like illness	Refrigerated FLUENZ 0.2 ml TIV 0.5 ml	Total: 2229 1114 1115	6-17 years
Placebo controlled studies				
AV006 Y1 USA/1996-97	Randomized, double blind, Placebo controlled One or two-dose regimen ***Primary objective*** (revised) To demonstrate that children receiving a two-dose primary vaccination regimen of FLUENZ are protected from cultured-confirmed influenza illness (CCII) caused by community-acquired subtypes antigenically similar to those contained in the vaccine ***Secondary objectives*** (revised) (ITT) Two-dose regimen, as randomized. To demonstrate that children enrolled in a two-dose primary vaccination regimen of FLUENZ are protected from CCII. Core efficacy study cohort. To demonstrate the efficacy of either a one- or two-dose primary vaccination regimen of FLUENZ to protect children against CCII. One-dose regimen. To estimate the efficacy of a one-dose primary vaccination regimen of FLUENZ to protect children against CCII. Follow-on study cohort. To demonstrate the efficacy of a second year's single dose of FLUENZ to protect children who received a one- or two-dose primary vaccination in the previous year against CCII.	Frozen FLUENZ Placebo 0.5 ml	Total: 1602 1070 532	15-71 months
AV006 Y2 USA/1997-98	Randomized, double blind, Placebo controlled Re-vaccination in children enrolled in AV006 Y1 One dose regimen	Frozen FLUENZ Placebo	Total: 1358 917 441	26-85 months

	Primary Efficacy Objective Efficacy of a second year's single re-vaccinating dose of FLUENZ against CCII caused by subtypes antigenically similar to those contained in the vaccine in children who received a one- or two-dose primary vaccination regimen in the previous year ***Secondary Efficacy Objectives*** Protection of children enrolled in the two-dose regimen in the first year re-vaccinated in Year Two. Protection of children enrolled in the one-dose regimen in the first year re-vaccinated in Year Two. Protection against all community-acquired viral subtypes.			
D153-P501 China Taiwan India Southeast Asia 2000-2002	Year 1: randomized, double blind, Placebo controlled Two dose regimen Year 2 : randomized One dose regimen ***Primary Efficacy Objective*** Efficacy over one season against CCII caused by community-acquired subtypes <u>antigenically</u> similar to those contained in the vaccine ***Secondary Efficacy Objectives*** (cf narratives)	 Refrigerated FLUENZ Placebo Refrigerated FLUENZ Placebo 0.2 ml	Total Year 1 3174 1900 1274 Total Year 2 2947 1477 1470	<u>≥</u>12-<36 months
D153-P502 EU Israel 2000-2002	Randomized, double blind, Placebo controlled Two dose regimen in Year 1, one dose regimen in Year 2 ***Primary Efficacy Objective*** Efficacy over one season against CCII caused by community-acquired subtypes <u>antigenically</u> similar to those contained in the vaccine ***Secondary Efficacy Objectives*** (cf narratives)	 Refrigerated FLUENZ Placebo Refrigerated FLUENZ Placebo 0.2 ml	Total Year 1 1784 1059 725 Total Year 2 1119 658 461	<u>6</u>-<36 months
D153-P504 South Africa Brazil Argentina 2001-2002	Randomized, double blind, Placebo controlled One or two dose regimen in Year 1, one dose regimen in Year 2 ***Primary Efficacy Objective*** Efficacy over first season against CCII caused by community-acquired subtypes <u>antigenically</u> similar to those contained in the vaccine: Whether administration of 1 dose of FLUENZ resulted in superior efficacy compared to the placebo Whether administration of 2 doses of FLUENZ resulted in superior efficacy compared to the placebo ***Secondary Efficacy Objectives*** Efficacy over first season against CCII caused by any community-acquired subtypes Efficacy over 2nd season against CCII caused by community-acquired subtypes <u>antigenically</u> similar to those contained in the vaccine Efficacy over 2nd season against CCII	 Refrigerated FLUENZ (2 doses) Refrigerated FLUENZ (1 dose) Excipient Placebo Saline Placebo Refrigerated FLUENZ Refrigerated FLUENZ Placebo Placebo 0.2 ml	Total Year 1 3200 1064 1067 543 526 Year 2 2202 735 732 365 370	6-<36 months

	caused by any community-acquired subtypes Efficacy in the 1st and 2d seasons, against AOM, febrile OM, and influenza-associated OM Efficacy against hospitalization and pneumonia			
Study number/ location/date	Design Main study objectives	Tests products	Number of subjects Randomized	Age
Placebo controlled studies (continued)				
D153-P522 Europe Southeast Asia Hong Kong Mexico Bangladesh 2002-03	Randomized, double blind, Placebo controlled Two dose regimen ***Primary Efficacy Objective*** To determine if R-FLUENZ interferes with the immune response to MMR vaccine administered concomitantly ***Secondary Efficacy Objectives*** Efficacy over one season against CCII caused by community-acquired subtypes antigenically similar to those contained in the vaccine Efficacy over one season against CCII caused by any community-acquired subtypes Efficacy against AOM, febrile OM, and influenza-associated OM	Refrigerated FLUENZ (0.2 ml)+MMR Placebo (0.2 ml)+MMR	Total: 1233 819 414	11-<24 months

Table 9. Adults studies

Study number/ location/date	Design Main study objectives	Tests products	Number of subjects Randomized	Age
TIV controlled studies				
D153-P516 South Africa 2002	Randomized, open label, controlled trivalent inactivated vaccine (TIV) One dose regimen ***Primary Efficacy Objective*** Non inferiority of FLUENZ vs trivalent inactivated vaccine (TIV) over surveillance period against CCII caused by community-acquired subtypes antigenically similar to those contained in the vaccine ***Secondary Efficacy Objectives (main)*** Non inferiority of FLUENZ vs trivalent inactivated vaccine (TIV) against culture-confirmed influenza-illness of any type To compare the efficacy over a defined surveillance period against influenza-like illness, clinic visits, hospitalizations, confirmed pneumonia, and death associated with influenza-like illness	Refrigerated FLUENZ: 0.2 ml IN TIV 0.5 ml IM injection	Total: 3009 1508 1501	≥ 60 years
AV003 US/1995-96	Randomized, double blind, TIV and Placebo controlled challenge study One dose regimen ***Primary objectives*** Efficacy against laboratory-documented influenza illness compared to TIV and placebo Immunogenicity	Frozen FLUENZ TIV (Fluvirin) Placebo 0.5 ml IN 0.5 ml IM	Total : 103 36 33 34	18-40 years

	Viral shedding following challenge			
Placebo controlled studies				
D153-P507 South Africa 2001	Randomized, double blind, Placebo controlled One dose regimen ***Primary Efficacy Objective*** Efficacy over one season against CCII caused by community-acquired subtypes antigenically similar to those contained in the vaccine ***Secondary Efficacy Objectives (main)*** Efficacy over one season against culture-confirmed influenza-illness of any type Efficacy over one season against culture- and/or PCR confirmed influenza-illness of any type Efficacy over one year against influenza-like illness, clinic visits, hospitalizations, confirmed pneumonia, and death associated with influenza-like illness	 Refrigerated FLUENZ Placebo 0.2 ml	Total: 3242 1620 1622	≥ 60 years
AV009 USA 1997-98	Randomized, double blind, Placebo controlled One dose regimen ***Primary Effectiveness Objective*** to show that a smaller proportion of FLUENZ participants have any febrile illness (AFI) during influenza outbreaks than placebo participants.	 Frozen FLUENZ Placebo 0.5 ml	Total: 4561 3041 1520	Healthy working adults 18-64 years

2.4.2. Clinical pharmacology

FLUENZ is a live, attenuated virus vaccine composed of 3 reassortant influenza viruses that replicate locally in the mucosa of the upper respiratory tract and 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.

Pharmacokinetics

The initial deposition and clearance of frozen and refrigerated vehicle (i.e. excipient only) formulations of FLUENZ were evaluated in a randomized, open-label, 2-way crossover study in 21 adults (**Scintigraphy Study PPL-1014**). Vehicle formulations were mixed with the radiolabelled marker 99mTechnetium diethylenetriaminepentaacetic acid (99mTc- DTPA) prior to intranasal administration via the Accuspray™ device, and in vivo distribution was determined using standard 2-dimensional gamma scintigraphy nuclear imaging. In summary, in vivo distribution studies in adults have shown that the majority of the dose of a radiolabelled refrigerated vaccine vehicle, delivered by the same device that is used to deliver live attenuated virus-containing vaccine, 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.

Moreover the smaller volume of the refrigerated FLUENZ compared with the frozen formulation dose results in even larger median droplet size, making it more unlikely for refrigerated FLUENZ to deposit in the lower respiratory tract.

Viral shedding in humans was evaluated in three clinical studies and described in the safety section of this AR.

Pharmacodynamics

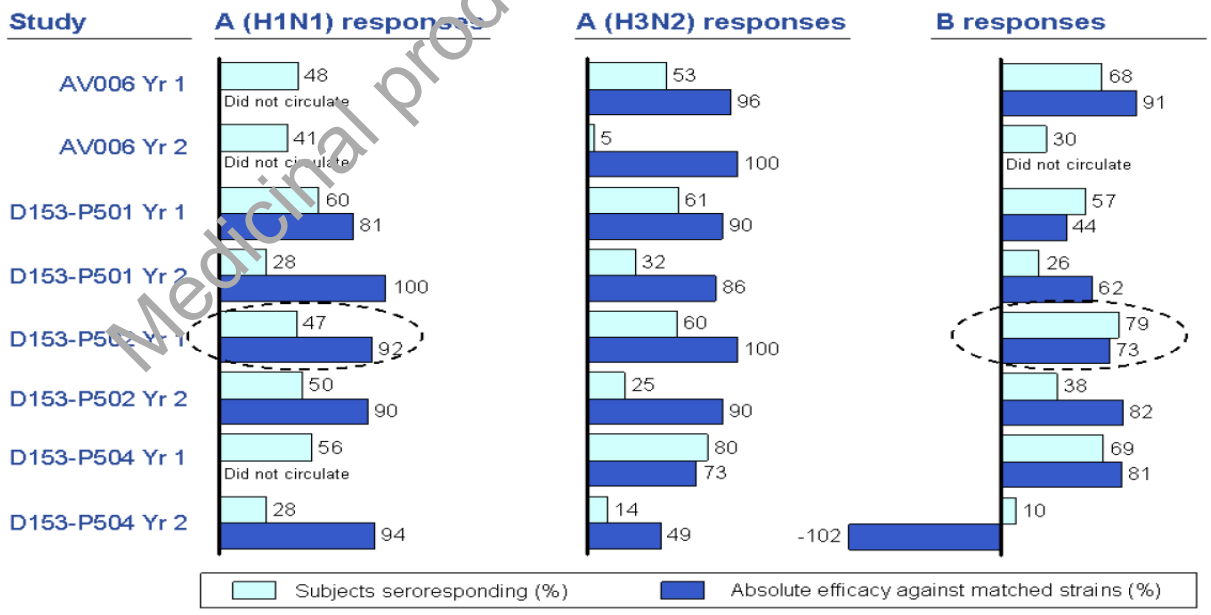
The pharmacodynamics of a vaccine relate to its interaction with the immune system.

The immunogenicity of FLUENZ was evaluated in 33 studies in the clinical development program, 22 of which included pediatric subjects and 11 of which enrolled only adult subjects. In general, evaluation of the immunogenicity of FLUENZ focused on serum antibody responses measured by HAI assay and compared pre- and postvaccination antibody levels (e.g. 4-fold or greater rise in HAI titers, HAI GMTs, and HAI geometric mean fold-rises [GMFRs]); one of the pediatric studies evaluated immunogenicity via an assay for cell-mediated immunity and did not collect HAI data. In addition, 3 of the pediatric studies evaluated the immune response seen with concomitant administration of FLUENZ and other live viral vaccines (see interaction studies).

A phase III TIV-controlled efficacy study (**MI-CP111**), conducted in children 6 to 59 months of age during the 2004-2005 influenza season (October through May), evaluated comparative efficacy using the same vaccine strains that were studied in **MI-CP123** (designed to compare the level of serum HAI antibody response to FLUENZ with that to TIV against influenza virus strains that were antigenically matched or mismatched to the vaccine strains). The combined results of immunogenicity in MI-CP123 and efficacy in MI-CP111 suggested the lack of a clear association between serum HAI responses and relative efficacy in young children for influenza strains other than the mismatched A/H3N2 strain.

This lack of correlation between immunogenicity and efficacy was further confirmed in other studies (as illustrated in the figure below), so that overall the immunogenicity data contributed poorly to the assessment of FLUENZ' clinical benefit. This issue had further repercussion on the criteria to be used for assessing the yearly strain change for seasonal vaccination (see discussion on clinical efficacy).

Lack of Correlation of Immune Response (Assessed by Seroresponse Rate) with Efficacy



Interaction studies

Three placebo controlled studies (D153-P522, AV018 and D153-P511) evaluated the safety and immunogenicity of concomitant administration of FLUENZ with other live viral vaccines (measles, mumps, rubella [MMR] vaccine; varicella [VAR] vaccine; and oral poliovirus [OPV] vaccine). All 3 studies assessed whether concomitant FLUENZ compromised the immunogenicity of the other vaccines. Studies AV018 and D153-P511 also assessed the effect of concomitant administration on FLUENZ immunogenicity, while Study D153-P522 assessed the effect of concomitant administration on FLUENZ efficacy against culture-confirmed influenza illness. Subjects in each study received 2 doses of either FLUENZ or placebo, and a single dose of the other standard vaccines.

In **Study D153-P522** both safety and efficacy of FLUENZ was demonstrated in children 11 to < 24 months of age when concomitantly administered with a commercially available combination MMR vaccine. Efficacy was 78% (95% CI: 50.9%, 91.3%) against influenza illness caused by antigenically matched strains and 64% (95% CI: 36.2, 79.8) against all strains regardless of antigenic match. No statistically significant interference occurred between the measles, mumps, and FLUENZ components. A reduction in the rubella serum antibody response rates was demonstrated at a threshold titer of 15 IU/ml (78% in FLUENZ plus MMR vs. 84% in placebo plus MMR); however, 15 IU/ml is higher than an internationally accepted standard of 10 IU/ml. Rubella responses rates were noninferior when the 10 IU/ml threshold was applied (90% vs. 93%). The seroresponse to rubella suggests some immunological interference with concurrent rubella vaccination; however, the clinical implication appears minimal because rubella titers exceeded the accepted threshold for protection from clinical illness. Thus, concomitant administration of FLUENZ with MMR was well tolerated and showed no clinically meaningful interference in immune responses to the measles, mumps, or rubella antigens in this study.

In **Study AV018** safety and equivalent immunogenicity were demonstrated in children 12 to 15 months of age when FLUENZ was concomitantly administered with 2 commercially available vaccines: MMR and VAR. Seroresponse rates and GMTs to MMR and VAR were similar with concurrent administration of FLUENZ or placebo (seroresponse rates: ≥ 97% to MMR and ≥ 83% to VAR in both groups). HAI antibody GMTs and seroconversion rates to influenza strains in FLUENZ were similar when FLUENZ was administered alone (seroconversion rates of 98%, 92%, and 44%, to A/H3N2, B, and A/H1N1 strains, respectively) or concomitantly with MMR and VAR (seroconversion rates of 98%, 96%, and 43%, respectively). Concurrent administration of FLUENZ with MMR and VAR vaccines was well tolerated and provided equivalent immunogenicity to all 3 live viral vaccines and their components compared with separate administration of each live viral vaccine.

In **Study D153-P511** safety and non-inferior immunogenicity were demonstrated for FLUENZ in children 6 to < 36 months of age when concomitantly administered with commercially available OPV. Seroresponse rates to each of the 3 poliovirus types were high (96% to 99%), and responder rates among subjects who received OPV concurrently with FLUENZ were statistically noninferior to those who received OPV concurrently with placebo. For each of the 3 influenza strains, serum HAI responses were statistically non-inferior in subjects who received the first dose of FLUENZ concurrently with OPV vs. those who received the first FLUENZ dose alone. Concurrent administration of FLUENZ with OPV was well tolerated and provided non-inferior OPV immunogenicity compared with separate administration in children 6 to < 36 months of age in this study.

In summary, the safety and immunogenicity profiles of the respective vaccines (FLUENZ, MMR, VAR, and OPV) were not altered when administered concomitantly or separately.

Frozen formulation versus refrigerated formulation

Two studies compared the frozen and refrigerated formulations (Studies MI-CP112 and D153- P500).

The clinical study that established immunologic equivalence between the frozen and refrigerated formulations was **Study MI-CP112**, a Phase III, randomized, double-blind, active controlled study in children and adults 5 to 49 years of age. The refrigerated and frozen formulations demonstrated equivalent immunogenicity in both paediatric and adult subjects based on strain-specific serum haemagglutination inhibition (HAI) geometric mean titres (GMTs) following vaccination, rates of seroconversion/ seroresponse (≥ 4- fold rise in HAI titer) and the proportions of subjects with post vaccination HAI GMT ≥ 32.

Early in the development of refrigerated FLUENZ, **Study D153-P500** was conducted by Wyeth in children 12 to <36 months of age in South Africa. It demonstrated immunological equivalence using the endpoint of seroconversion/seroresponse but only for the A/ H3N2 strain. Given the unexpected differences in immunogenicity for the other strains between the refrigerated and frozen formulations of FLUENZ used in this study, manufacturing and stability data were reviewed. The report noted that the refrigerated formulation used in the study was the first clinical lot manufactured by Wyeth, and it was found to be atypical compared to subsequent lots manufactured at Wyeth with respect to the high variability in potency and shelf-life of the B strain. The report concluded that the non-representative nature of the refrigerated FLUENZ lot in question (7- 6146- 001A) may have been responsible for the differences in immune responses observed with the 2 formulations.

Subsequent efficacy studies have confirmed the clinical efficacy of the refrigerated formulation.

Lot Consistency

Due to poor relevance of the usual immunogenicity criteria for this LAIV vaccine, reassurance on the lot consistency is mainly to be obtained from the quality dossier, through the measures the applicant has settled to ensure a reproducibility of the production (see the quality section).

2.5. Clinical efficacy

2.5.1. Dose response studies

Study AV002 (US), AV002-2 (Chile)

This is a phase I/II randomized, double-blind, placebo controlled, dose escalation in healthy children from 18 to 71 months of age. The study was conducted in three stages. For each stage, subjects were randomized to receive either FLUENZ or placebo. Stage 1 tested doses of 10^4 and 10^5 $TCID_{50}$ (50% tissue culture infectious dose), Stage 2 tested a dose of 10^6 $TCID_{50}$ and Stage 3 tested a dose of 10^7 $TCID_{50}$.

Given the poor relevance of immunogenicity criteria the study results will not be detailed.

Study D153-P513

Design: phase III, prospective, randomized, double-blind, placebo controlled in healthy children > 6 months and <36 months of age. Study centres: Thailand (4 sites), The Philippines (2 sites). Study period: February 2002-Novembre 2002.

Efficacy Objectives

See table 8.

Results

The estimated efficacy against culture-confirmed influenza illness caused by any strain antigenically similar to a vaccine strain by treatment group was:

- for FLUENZ 10^7: 62.2% (95% CI: 43.6, 75.2)
- for FLUENZ 10^6: 34.7% (95% CI: 8.7, 53.6)
- for FLUENZ 10^5: 12.2% (95% CI: -19.5, 35.6)

The estimated efficacy against antigenically similar A/H3N2 by treatment group was:

- for FLUENZ 10^7: 64.0% (95% CI: 45.7, 76.6)
- for FLUENZ 10^6: 35.1% (95% CI: 9.0, 54.0)
- for FLUENZ 10^5: 12.3% (95% CI: -19.6, 35.8)

The estimated efficacy for any community subtype was:

- for FLUENZ 10^7: 48.6% (95% CI: 28.8, 63.3)
- for FLUENZ 10^6: 24.6% (95% CI: -0.9, 43.8)
- for FLUENZ 10^5: 5.5% (95% CI: -24.3, 28.1)

These results showed a strong dose-dependent positive effect on efficacy. The efficacy against any community subtypes was only demonstrated in the 10^7 FEU group. For any type of strain, the highest dose only appeared as efficacious (10^7).

2.5.2. Main studies

Efficacy was assessed in 14 main clinical studies : 10 paediatric studies (7 placebo controlled and 3 TIV controlled), 3 adults studies (1 placebo controlled and 2 TIV controlled) and 1 placebo controlled study of clinical effectiveness in adults covering 7 different northern hemisphere seasons and 2 southern hemisphere seasons from 1995 to 2005.

Methods

In the clinical studies reviewed in this application **absolute efficacy** refers to the percentage reduction in cases of culture-confirmed influenza illness among vaccine recipients compared to background (ie, placebo) rates, **relative efficacy** refers to the percentage reduction in cases of culture-confirmed influenza illness among FLUENZ recipients compared to TIV recipients, and **effectiveness** refers to the percentage reduction in cases of illness, such as influenza-like illness (ILI) or acute febrile illness, without viral culture assessment, among FLUENZ recipients compared to background (ie, placebo) rates.

Influenza vaccine benefit was assessed in three ways:

1) assessment of efficacy by comparison of culture-confirmed influenza infection rates;

2) assessment of effectiveness by observations of clinical events;

3) serologic assessments of immune response (immunogenicity).

Table 10 lists the definitions for culture-confirmed or laboratory-documented influenza illness used by each of the 3 sponsors.

Table 10. Definitions of Culture-Confirmed or Laboratory-Documented Influenza Illness Used in FLUENZ Efficacy Studies

Sponsor and Study Numbers	Definition
Aviron AV006 Year 1 AV006 Year 2	**Culture-confirmed influenza illness** was defined as illness that occurred at least 15 days after receiving the first dose of vaccine or placebo and that was defined by a positive viral culture of a wild-type virus subtype antigenically similar to one contained in the vaccine.
Aviron AV003	**Laboratory-documented influenza illness** was defined as any of the following illnesses with laboratory evidence of viral infection: • Respiratory illness: One or more respiratory symptoms (nasal stuffiness, earache, runny nose, sore throat, cough, or breathing difficulty) of severity Grade 2 or 3 on any day, or 2 or more respiratory symptoms of severity Grade 1 or higher on any day, or at least 1 respiratory symptom of severity Grade 1 or higher on 2 consecutive days. • Systemic illness: Muscle aches or chills of severity Grade 2 or Grade 3. • Febrile illness: Oral temperature > 100°F. • Influenza-like illness: Febrile and either respiratory or systemic illness or both. • Any illness: Any febrile, respiratory, or systemic illness as defined above.
Wyeth D153-P501 D153-P502 D153-P504 D153-P513 D153-P514 D153-P515 D153-P516 D153-P522 D153-P507	**Culture-confirmed Influenza Illness** A culture-confirmed case of influenza illness was defined by an influenza-positive nasal swab culture of a wild-type virus. Nasal swab samples were obtained from subjects with either 1 sign, symptom or condition from Category A; or at least 2 signs, symptoms or conditions from Category B during the surveillance period: Category A: fever (≥ 38°C rectal temperature or ≥ 37.5°C axillary temperature), wheezing, shortness of breath, pulmonary congestion, pneumonia, or ear infection [AOM], suspected or diagnosed). Category B: runny nose or nasal congestion (rhinorrhea), sore throat (pharyngitis), cough, muscle aches, chills, headache, irritability, decreased activity, or vomiting. Viral cultures could also have been obtained if, in the opinion of the investigator, the symptom complex so warranted. For Study D153-P507, the criteria for obtaining nasal and throat swabs for viral culture was any one of the following: feeling of "feverishness", oral temperature ≥ 37.2°C, sore throat, new or increased cough, tiredness, muscle aches.

Sponsor and Study Numbers	Definition
MedImmune MI-CP111	**Culture-confirmed modified CDC-ILI** was defined as a positive culture for a community-acquired wild-type influenza virus associated within ± 7 days of modified CDC-ILI. **Modified CDC-ILI** was defined as fever (temperature ≥ 100°F [37.8°C] oral or ≥ 100.6°F [38.1°C] rectal/tympanic or ≥ 99.6°F [37.6°C] axillary) plus cough, sore throat, or runny nose/nasal congestion on the same or consecutive days. The addition of runny nose/nasal congestion accounts for the modification (for pediatrics) to the standard definition.

CDC = Centers for Disease Control and Prevention; ILI = influenza-like illness

Treatments

Study vaccine

Refrigerated formulation (liquid formulation)

Each dose of liquid formulation contained approximately 10^7 median tissue culture infectious dose ($TCID_{50}$) / 10^7 fluorescent focus units (FFU) of each of the 6:2 influenza reassortant virus strains. The total volume of 0.2 mL was administered intranasally with a spray applicator (approximately 0.1 ml into each nostril).

Frozen formulation

Each dose contained approximately 10^7 $TCID_{50}$ of each of the three cold-adapted reassortants. A total volume of 0.5 mL was administered intranasally with a spray applicator (approximately 0.25 mL into each nostril).

The virus strains contained in the FLUENZ vaccine formulations differed in each study contributing to the assessment of efficacy and immunogenicity of FLUENZ: there were a total of 11 different formulations and the HA and NA antigens of the wildtype influenza strains used to generate the type A/H1N1, A/H3N2, and B vaccine reassortants for FLUENZ were antigenically representative of the virus recommended by the World Health Organization (WHO) for the relevant hemisphere/influenza season.

Inactivated vaccines

FluShield (Wyeth)

TIV (Aventis-Pasteur, France)

Licensed TIV vaccine

Fluvirin (Medeva Evans)

Placebo

Physiological Saline

Vaccination schedules

Pediatric studies: One dose or two doses given at interval which varied according to studies.

Adult studies: One dose

Objectives

See tables 8 and 9.

Endpoints

The efficacy studies involving pediatric subjects were typically designed with culture-confirmed endpoints. Study designs and endpoints varied from study to study, but culture-confirmed or laboratory-documented influenza illness caused by strains that were antigenically matched to the vaccine was the primary efficacy endpoint for 13 of the 14 controlled efficacy/effectiveness studies discussed in this section. The primary endpoint of the remaining controlled study (AV009) was assessment of the effectiveness of FLUENZ with regard to any febrile illness (AFI).

An important shared secondary endpoint of these studies was efficacy against all influenza strains regardless of match. The secondary endpoint in pediatric studies was culture-confirmed influenza illness caused by any community-acquired antigenic subtype.

The efficacy of the vaccine against acute otitis media (AOM) associated with culture-confirmed influenza illness was a secondary efficacy endpoint that was evaluated during development. A subject met the criteria for this endpoint if the subject had AOM and if the associated influenza illness was confirmed by an influenza-positive nasal culture.

As specified by the Applicant, study AV012 was not intended to support the efficacy/effectiveness of the product but it was only submitted for safety.

Other secondary and exploratory efficacy/effectiveness endpoints evaluated included efficacy against lower respiratory illness (LRI), efficacy against febrile illness, efficacy against ILI, efficacy against symptomatic influenza infection, incidence of asthma exacerbations, severity of illness, and pharmaco-economic assessments.

Blinding

In open label trials, although investigators and parents knew which influenza vaccine was administered, sponsor representatives remained partially blinded during the data cleaning phase. Samples processed by the laboratories were identified using bar codes and accession numbers without reference to treatment. Both treatments, FLUENZ and placebo, were supplied in single-dose identically packaged sprayers, thus enabling a double-blind study design.

Due to the different administration route between FLUENZ (intranasal) and TIV (intramuscular), subjects randomized to receive FLUENZ concurrently received intramuscular placebo injection, and subjects randomized to receive TIV concurrently received intranasal placebo mist. FLUENZ and placebo intranasal sprayers were supplied as identically packaged, single-dose units to all study sites. TIV and placebo pre-filled syringes were supplied as identically packaged, single-dose units.

To maintain study vaccine blinding and to harmonize study vaccine administration across all sites worldwide, study products administrators who were not otherwise involved in the conduct of the study (i.e., were not involved in the collection, reporting, or assessment of efficacy and safety endpoint data) prepared and administered the study vaccines at each study site.

Statistical methods

While there were differences across studies, in general, the following definitions were used.

Per-protocol: subjects must have received all vaccinations as randomized, satisfied all eligibility criteria and have not any major protocol violations.

Intent-to-treat efficacy populations: subjects who received at least one dose.

As-treated: the subjects who received all vaccinations in accordance with protocol with no major violations other than not receiving the treatment as assigned and receiving the other treatment (same treatment at both doses); treatment actually received.

According-to-Protocol (ATP) Population: subjects who were randomized into the trial, who had at least one surveillance contact on a specific date (based on the trial) that was also at least 14 days after the final required vaccination, and who did not experience a major protocol violation during the study. Subjects in the ATP Population were analyzed in the treatment group according to the active study vaccine actually received at Dose One.

Results

Pediatric studies

The 7 placebo-controlled pediatric efficacy studies are summarized in table 11.

Table 11. Observed efficacies in placebo controlled studies

Study Number	Region [a]	Age [b] Range	Number of Subjects in the Primary Analysis Population	Frozen or Refrigerated FluMist	Influenza Season	Efficacy (95% CI) Matched Strains	Efficacy (95% CI) All Strains Regardless of Antigenic Match
D153-P501	Asia/Oceania	12 to < 36 M	2,764	Refrigerated	2000-2001	72.9% (62.8, 80.5)	70.1% (60.9, 77.3)
					2001-2002	84.3% (70.1, 92.4) [c]	64.2% (44.2, 77.3) [c]
D153-P502	Europe	6 to < 36 M	1,616	Refrigerated	2000-2001	85.4% (74.3, 92.2)	85.9% (76.3, 92.0)
					2001-2002	88.7% (82.0, 93.2)	85.8% (78.6, 90.9) [c]
D153-P504	Africa, Latin America	6 to < 36 M	1,886	Refrigerated	2001	73.5% (63.6, 81.0)	72.0% (61.9, 79.8)
					2002	73.6% (33.3, 91.2) [c]	46.6% (14.9, 67.2) [c]
D153-P513	Asia/Oceania	6 to < 36 M	2,107	Refrigerated	2002	62.2% (43.6, 75.2) [d]	48.6% (28.8, 63.3) [d]
D153-P522	Europe, Asia/Oceania, Latin America	11 to 24 M	1,150	Refrigerated	2002-2003	78.4% (50.9, 91.3)	63.8% (36.2, 79.8)
AV006 Yr1	USA	15 to 71 M	1,259	Frozen	1997-1997	93.4% (87.5, 96.5) [e]	Not applicable
AV006 Yr2	USA	27 to 83 M	1,358 [f]	Frozen	1997-1998	100% (63.1, 100) [c]	87.1% (77.7, 92.6) [c]

[a] For purposes of study grouping, Europe includes Western and Eastern Europe, Scandinavia, Israel and Lebanon, while Asia/Oceania includes East Asia, Southeast Asia, South Asia, and Australia.
[b] Age range as described in the protocol for the study. M = months.
[c] Rates shown are for second-season revaccination.
[d] Efficacy for subjects in the 10^7 FFU group.
[e] Results for subjects in the 2-dose group (primary endpoint).
[f] All subjects in AV006 Year 2 were included in AV006 Year 1.

The 3 TIV-controlled pediatric efficacy studies are summarised in table 12.

Table 12. Observed efficacies in TIV controlled pediatric studies

Study Number	Region [a]	Age [b] Range	Number of Subjects in the Primary Analysis Population	Frozen or Refrigerated FluMist	Influenza Season	Relative Efficacy (95% CI) Matched Strains	Relative Efficacy (95% CI) All Strains Regardless of Antigenic Match
MI-CP111	USA, Europe, Asia/Oceania	6 to 59 M	7,852	Refrigerated	2004-2005	44.5% (22.4, 60.0) fewer cases than TIV	54.9% (45.4, 62.9) fewer cases than TIV
D153-P514	Europe	6 to < 72 M	2,085	Refrigerated	2002-2003	52.7% (21.6, 72.2) fewer cases than TIV	52.4% (24.6, 70.5) fewer cases than TIV
D153-P515	Europe	6 to 17 Y	2,211	Refrigerated	2002-2003	34.7% (3.9, 56.0) fewer cases than TIV	31.9% (1.1, 53.5) fewer cases than TIV

[a] For purposes of study grouping, Europe includes Western and Eastern Europe, Scandinavia, Israel, and Lebanon; and Asia/Oceania includes East Asia, Southeast Asia, South Asia, and Australia.
[b] M = months; Y = years. Age range as described in the protocol for the study.

Adult studies

Table 13 summarises the 4 main efficacy adult studies: two are TIV controlled studies (AV003 and D153-P516), one is a placebo-controlled study (D153-P507) and one is a placebo controlled clinical effectiveness trial (AV009).

Table 13. Efficacy in Controlled Studies in Adults

Study Number	Region	Age [a] Range	Number of Subjects	Frozen or Refrigerated FluMist	Influenza Season	Results
AV003	USA	18 to 40 Y	103	Frozen	1995-1996	FluMist efficacy against laboratory-documented illness after wild-type challenge: 85% (95% CI: 28, 100) TIV efficacy against laboratory-documented illness after wild-type challenge: 71% (95% CI: 2, 97)
AV009	USA	18 to 64 Y	4,561	Frozen	1997-1998	Effectiveness vs placebo for Any Febrile Illness: 9.7% (not statistically significant) Effectiveness vs placebo for other febrile illness definitions: 17% to 24% (each analysis was statistically significant)
D153-P507	Africa	≥ 60 Y	3,242	Refrigerated	2001	Efficacy vs placebo for matched strains: 42.3% (95% CI: 27.6, 57.8) Efficacy vs placebo for all strains regardless of antigenic match: 41.6% (95% CI: 20.9, 57.1)
D153-P516	Africa	≥ 60 Y	3,009	Refrigerated	2002	Influenza incidence too low to determine relative efficacy of FluMist vs TIV

[a] Age range as described in the protocol for the study. Y = years.

Results of individual studies

Nine pediatric studies and 4 adult studies will be described in detail in this section. For further information see section 'Analysis performed across studies' and tables 8, 9, 11, 12 and 13.

Placebo controlled pediatric studies

- **Study D153-P501**

This was a randomized, double-blind, placebo-controlled, two year crossover study designed to determine safety and efficacy of refrigerated formulation of FLUENZ (R-FLUENZ) in children aged 12 months to <36 months of age.

Study centres: China, Hong Kong, India, Malaysia, the Philippines, Singapore and Thailand.

Study periods: September 30, 2000-October 31, 2002.

Treatment

The liquid formulation (refrigerated) vaccine was used at a dosage strength/Strain: 10^7 $TCID_{50}$. Vaccine and placebo were two doses given intranasally, 28 to 56 days apart, at a total dose volume of 0.2 ml.

Objectives

See table 8.

Randomisation

During the first year, 3174 subjects were randomized at 3:2 ratio to receive 2 doses of either refrigerated vaccine or placebo, separated by 28-56 days. In the 2nd year, 2947 subjects were

randomized again at 1:1 ratio to receive a single dose of vaccine or placebo, irrespective of their year 1 treatment. The crossover design resulted in 4 treatment groups (year 1/year 2 = FLUENZ/FLUENZ, FLUENZ/Placebo, Placebo/FLUENZ, and Placebo/Placebo).

Numbers analysed

Year 1

Of 3174 randomized subjects, a total of 2764 subjects were included in the per protocol population (87.0% R-FLUENZ, 87.2% placebo). The greatest percentage of subjects was excluded from the per protocol population for "No second vaccination in year 1" (4.7% Indian sites, 7.2% non-Indian sites).

Year 2

Of 2947 subjects randomized, a total of 2731 (92.7%) were included in the per protocol population in year 1 and 2527 (85.7%) were included in year 2. The greatest percentages of subjects were excluded from the per protocol population for year 2 due to no vaccination in year 2 (8.6%) or a major protocol violation in year 1 (7.3%).

Outcomes and estimation

Primary efficacy endpoint (efficacy against culture-confirmed influenza-illness caused by strains antigenically similar to those in the vaccine)

The overall efficacy of 2 doses of vaccine administered in the first year against matched strains was 72.9% (95% CI: 62.8, 80.5), based on an efficacy of 80.9% (95% CI: 69.4, 88.5) and 90.0% (95% CI: 71.4, 97.5) versus A/H1 and A/H3 strains respectively. Against the B vaccine strain, efficacy was found to be 44.3% (95% CI: 6.2, 67.2).

Incidence of culture-confirmed influenza illness (CCII) by age-group in Year 1

The incidence rates in FLUENZ group (CAIV-T group in the table) ranged from 2.2% in subjects 18 to < 24 months of age to 4.5 % in the oldest range of age (30 to < 36 months of age).

	Incidence, %		Number of Subjects		Number of Cases[a]	
Age Group[b]	CAIV-T	Placebo	CAIV-T	Placebo	CAIV-T	Placebo
12 to <18	3.2	11.1	469	343	15	38
18 to <24	2.2	12.8	402	234	9	30
24 to <30	3.6	13.2	357	265	13	35
30 to <36	4.5	13.4	425	269	19	36

a: Cases due to wild-type virus antigenically similar to that in the vaccine. Only a subject's first such case was counted.

b: Age in months at first vaccination.

These results show a consistently lower incidence of CCII in the FLUENZ group compared with placebo across age strata.

Secondary efficacy endpoints

The overall efficacy in year 2 of a primary series of two doses of FLUENZ in year 1 (FLUENZ/Placebo treatment group) against viral subtypes antigenically similar to those in the vaccine compared to Placebo/placebo was 56.2% (95% CI: 30.5, 72.7). Efficacy against the A/H3 strain was 61.3% (95%

CI: 34.9, 77.4). Efficacy assessment was not possible against the A/H1 and B strains, 83.7% (95% CI -64.4, 99.7) and 8.9% (95% CI: -264.1, 75.1) respectively, due to an inadequate number of isolates.

Efficacy in the second year of the study in the FLUENZ/FLUENZ vs. FLUENZ/ Placebo treatment showed an overall estimate of efficacy against any influenza subtype antigenically similar to those in the vaccine of 64.2% (95% CI: 28.9, 83.2). Although positive estimates of efficacy were reported for each strain, the number of cases reported for A/H1 and B were insufficient to draw any conclusion. The estimate of efficacy against antigenically similar A/H3 subtypes was 64.6% (95% CI: 21.5, 85.4).

In year 2 of the study, efficacy comparison in the FLUENZ/FLUENZ vs. Placebo/Placebo treatment groups provided an evaluation of efficacy in a fully vaccinated population compared to an unvaccinated population. The overall estimate of efficacy against viral subtypes similar to those in the vaccine was 84.3% (95% CI: 70.1, 92.4). While the individual efficacy estimates were positive for each of the influenza strains, there were insufficient cases to accurately assess efficacy against antigenically similar A/H1 and B subtypes, 100.0% (95% CI: 2.9, 100.0) and 61.6% (95% CI:-97.6, 94.0) respectively. The estimate of efficacy for antigenically similar A/H3 subtypes was 86.3% (95%CI: 71.4, 94.1).

Analysis of efficacy in the second year of the study in the FLUENZ/FLUENZ vs. Placebo/FLUENZ treatment groups revealed an overall efficacy against subtypes antigenically similar to those in the vaccine of 60.9% (95% CI: 15.9, 82.6). Due to insufficient numbers of culture positive cases of antigenically similar A/H1 or B subtypes, an accurate assessment of efficacy cannot be determined. The estimate of efficacy against antigenically similar A/H3 subtype was 67.4% (95% CI: 23.5, 87.1).

Due to the paucity of episodes no conclusions of efficacy against AOM (acute otitis media) could be drawn.

- **Study D153-P502**

This was a, randomized, double-blind, placebo controlled, multi-center trial in children aged 6 months to <36 months.

Study centres: Europe (Belgium, Finland, Israel, Spain and the United Kingdom).

Study period: October 2, 2000 through May 31, 2002.

Treatment

The liquid formulation (refrigerated) vaccine was used at a dosage strength/Strain: 10^7 $TCID_{50}$. Vaccine and placebo were two doses given intranasally in Year 1, one dose in Year 2, 35 ± 7 days apart, at a total dose volume of 0.2 ml per dose.

Objectives

See table 8.

Randomisation

A total of 1,784 subjects were randomized in a 3:2 ratio to receive 2 doses in the 1st year, 35 ± 7 days apart, and a single dose in the 2nd year of either FLUENZ vaccine or placebo. In year 2, subjects received the same treatment they had received in the first year.

Numbers analysed

A total of 1616 (90.6%) subjects [951 (89.8%) FLUENZ subjects and 665 (91.7%), placebo subjects)] were included in per protocol population in the first season.

1090 subjects [640 (97.3%) FLUENZ subjects and 450 (97.6%) placebo subjects] were part of the second season per-protocol analysis population.

Outcomes and estimation

The overall efficacy in the first year against influenza virus subtypes antigenically matched to those in the vaccine was 85.4% (95% CI: 74.3, 92.2). Against individual subtypes the efficacies were as follows: 91.8% (95% CI: 80.8, 97.1) against A/H1N1 and 72.6% (95% CI: 38.6, 88.9) against B. A/H3N2 was only detected in one placebo subject. The vaccine also provided similar protection against all influenza strains regardless of match with an overall efficacy of 85.9% (95% CI: 76.3, 92.0).

In year 2 of the study, efficacy against strains matched to those in the vaccine was 88.7% (95% CI: 82.0, 93.2). Efficacy of FLUENZ in year two against each of the individual vaccine strains was found to be 90.0% (95% CI: 56.3, 98.9), 90.3% (95% CI: 82.9, 94.9) and 81.7% (95% CI: 53.7, 93.9) for the A/H1N1, A/H3N2 and B subtypes, respectively. Vaccine efficacy was found to be 85.8% (95% CI: 78.6, 90.9) against all strains (regardless of match). Vaccination with FLUENZ in both years provided efficacy against acute otitis media associated with a nasal culture positive for influenza virus.

- **Study D153-P504**

This was a randomized, double-blind, placebo-controlled, two year crossover designed to determine safety and efficacy of refrigerated formulation of FLUENZ (R-FLUENZ) in children 6 months and < 36 months of age.

Study centres: South Africa, Brazil and Argentina

Study periods: April 03, 2001-November 30, 2001 and March 28, 2002-November 30, 2002

Treatment

The liquid formulation (refrigerated) vaccine was used at a dosage strength/Strain: 10^7 FFU. Vaccine and placebo were two doses given intranasally in Year 1, one dose in Year 2, 35 ± 7 days apart, at a total dose volume of 0.2 ml.

Objectives

See table 8.

Randomisation

In the 1st year 3200 subjects were randomized to receive a primary series of either 1 or 2 doses of FLUENZ vaccine, or 2 doses of either excipient or saline placebo. The following year, 2202 subjects continued the study and received 1 dose of vaccine or saline placebo. Due to incorrect implementation of treatment allocations in the 2nd year, approximately half of the subjects randomized to FLUENZ received saline placebo, and approximately half of the subjects randomized to placebo received FLUENZ, making the overall year 2 per-protocol population 1,364 subjects (61.9%).

Numbers analysed

In season 1, of the 3,200 subjects randomized, 2,821 subjects were included in the per protocol efficacy population.

In season 2, of the 2,202 subjects participating, 1,364 were included in the per protocol efficacy population.

Outcomes and estimation

2 doses of FLUENZ given during the first year demonstrated a 73.5% (95%CI: 63.6, 81.0) efficacy against any antigenically similar strain, while 1 dose of FLUENZ demonstrated a 57.7% (95% CI: 44.7, 67.9) efficacy. Relative efficacy of 2 doses vs 1 dose was 37.3% (95% CI: 9.5, 56.9), but this could not be reproduced in the second year, ie 24.1% (95% CI: -104.2, 75.7). In year 2, for subjects whom received either 1 or 2 doses of FLUENZ in the first year, absolute efficacy against antigenically similar

strains was 65.2% (95% CI: 31.2, 82.8) and 73.6% (95% CI: 33.3, 91.2) respectively. Efficacy of 1 dose, in the 2nd year, of FLUENZ in subjects who received placebo in year 1 was 60.3% (95%CI: 10.9, 83.8), more or less equal to the estimate for 1 FLUENZ dose in year 1 (ie, 57.7% [95%CI: 44.7, 67.9]).

Second season efficacy in subjects who received 2 doses of FLUENZ in year 1 and placebo in year 2 was 57.0% (95%CI: 6.1, 81.7).

- **Study D153-P522**

This was a, randomized, double-blind, placebo controlled, multi-center trial in children aged 11 months to 24 months.

Study centres: Asia (Singapore, Hong Kong, Malaysia, Thailand, Korea, Philippines, Bangladesh) Europe (Finland, Poland, Lithuania, Belgium, Germany), Mexico (32 sites).

Study period: October, 2002 to May 31, 2003.

Treatment

The liquid formulation (refrigerated) vaccine was used at a dosage strength/Strain: 10^7 FFU. Vaccine and placebo were two doses given intranasally, 35 ± 7 days apart, at a total dose volume of 0.2 ml per dose.

Objectives

See table 8.

Outcomes and estimation

2 dose regime of FLUENZ was efficacious (78.4% [95% CI: 50.9, 91.3]) against influenza illness caused by strains antigenically matched to those contained in the vaccine. Efficacy for individual vaccine strains was greatest against the B strain, 81.7% (95% CI: 38.2, 95.8). The point estimate of efficacy against the A/H3 strain was 68.5% but the CI included zero (95% CI: -9.0, 91.9). Although the point estimate for efficacy against the A/H1 strain viruses was 100.0% (95% CI: -168.0, 100.0), there were too few cases to make an accurate assessment of efficacy against this strain (only 2 cases, both in the placebo group). Efficacy against all strains regardless of match was 63.8% [95% CI: 36.2, 79.8].

- **Study AV006 Yr1**

This was a, randomized, double-blind, placebo controlled, multi-centre trial in children aged 15 months to 71 months. The study was designed as a two-year study with a single cohort recruited in year one, to be re-vaccinated without re-randomization in year two.

Study centres: US.

Study period: August 21, 1996 to April 29, 1997.

Eligible participants were subjects not previously vaccinated against influenza. A total of 1,602 subjects were enrolled and randomized 2:1 to receive either frozen FLUENZ (N = 1070) or placebo (N = 532) in year 1. Furthermore, subjects were enrolled to receive either a 2-dose (N = 1314) or 1-dose (N = 288) primary vaccination regimen of either FLUENZ or placebo in year 1: No randomization according to number of doses took place.

Treatment

Frozen formulation vaccine was used; dosage strength/Strain: 10^7 $TCID_{50}$.

Placebo contained normal allantoic fluid.

Children were vaccinated in the two-dose schedule and received their second dose administered 46 to 74 days after dose one.

Objectives

See table 8.

Outcomes and estimation

In subjects who underwent a 2 dose regime, the efficacy of FLUENZ was estimated to be 93.4% (95% CI: 87.5, 96.5) for any matched strain, 96.0% (95% CI: 89.4, 98.5) for matched A/H3N2, and 90.5% (95% CI: 78.0, 95.9) for matched B (No A/H1N1 influenza strain circulated that season). Efficacy in protecting against culture-confirmed influenza caused by any matched strain among subjects enrolled to receive 1 dose was estimated to be 88.8% (95% CI: 64.5, 96.5).

FLUENZ also significantly reduced the occurrence of febrile illness and otitis media associated with culture-confirmed influenza [95.0% (95%CI 90.0, 97.5) and 97.5% (95%CI 85.5, 99.5) respectively].

- **Study AV006 Yr2**

The second part of the AV006 study, covering the second year.

Study period: September 02, 1997 to May 04, 1998.

All subjects whom completed the year 1 part of the study were encouraged to participate in the second part. Subjects received a single dose of the same treatment (FLUENZ or placebo) according to their randomization in Year 1.

The population was made up out of returning subjects, whom remained in the same treatment group, FLUENZ (N = 917) or placebo (N = 441), to which they had been randomized in a 2:1 ratio in the prior year.

Objectives

See table 8.

Outcomes and estimation

Efficacy of FLUENZ after revaccination in the 2nd year was 100% (95% CI: 63.1, 100) against antigenically matched strains and 87.1% (95% CI: 77.7, 92.6) against all strains (nearly all, 66 of 71, of the wild-type strains isolated were an antigenically drifted A/H3N2 strain mismatched to the vaccine strain).

Pediatric TIV controlled studies

- **MI-CP111**

This was a phase III, refrigerated FLUENZ versus TIV randomized, double-blind, active-comparator, multinational trial, enrolling children aged 6 months to 59 months.

Study centres : US (108 investigators-133 sites) Europe/Middle East (97 investigators-101 sites Belgium, Czech Republic, Finland, Iceland, Sweden, Germany, Italy, Spain, Greece, Lebanon, Israel, and the United Kingdom) and Asia (15 investigators/15 sites).

Study periods: October 20, 2004- August 31, 2005 (last subject completed day 180 follow-up).

Recruitment

A total of 8475 subjects were randomized at 249 sites in the U.S. and 15 countries in Asia and Europe/Middle East: 4117 subjects (48.6%) were randomized in the U.S. (133 sites), 542 (6.4%) in Asia (3 countries, 15 sites), and 3816 (45.0%) in Europe/Middle East (12 countries, 101 sites). After the U.S., the countries with the highest number of randomized subjects were Finland (725 subjects, 8.6%), Israel (653 subjects, 7.7%), the United Kingdom (563 subjects, 6.6%), and Belgium (459 subjects, 5.4%).

Treatments and regimen

Liquid formulation vaccine (Refrigerated FLUENZ): dosage strength/strain: 10^7±.0.5 FFU of A/NewCaledonia/20/99 (H1N1), A/Wyoming/03/2003(H3N2) and B/Jilin/20/2003 [B/Shangai/361/2002-like] given intranasally at a total dose volume of 0.2 ml.

Commercial TIV vaccine: dosage strength /strains/0.25 ml or 0.5ml: 7.5 µg or 15 µg each of HA of A/NewCaledonia/20/99 (H1N1), A/Wyoming/03/2003 (H3N2), B/Jiangsu/10/2003 [B/Shanghai/361/2002-like] (2004-2005 formula), depending on subject age.

Children who were previously vaccinated were to receive one dose of vaccine/placebo, whereas those who were not previously vaccinated received 2 doses of vaccine/placebo. Children receiving two doses were given each vaccination 28-42 days apart. All doses were administered prior to the influenza season.

Objectives

See table 8.

Sample size

A sample size range of 7000-8500 subjects provided >99% power for non-inferiority (lower bound of the 95% CI > −30%) and approximately 87-94% power for statistical superiority (relative efficacy >0%) to demonstrate the relative efficacy of FLUENZ vs. TIV on the rate of culture-confirmed influenza associated with the presence of modified CDC-ILI (primary endpoint). These calculations assumed a 3% attack rate in the TIV treatment group, a 40% true FLUENZ efficacy relative to TIV in this population, and 90% evaluation. Stratified enrolment to accrue approximately 4000 subjects 6-23 months of age was chosen to provide 95% power to demonstrate statistically significant efficacy in this age subgroup, assuming a 4% attack rate in the TIV group and a 50% true R-FLUENZ efficacy relative to TIV.

Randomization

A total of 8475 subjects were randomized 1:1 with 4243 subjects in the FLUENZ group and 4232 subjects in the TIV group. Randomization was stratified by age, country, history of prior influenza vaccination, and history of wheezing (defined as ≥3 wheezing illnesses requiring medical follow-up or hospitalization).

Participant flow

Approximately 93% of randomized subjects (ITT Population) completed the trial. The small number of subjects who did not complete the study was balanced between the two treatment groups. The proportion of subjects with protocol deviations was generally balanced between the treatment groups in each region and overall.

Subject disposition at study completion

	CAIV-T N=4243	TIV N=4232
Number of subjects who completed the study	3933 (92.7%)	3911 (92.4%)
Number of subjects who did not complete the study	310 (7.3%)	321 (7.6%)
Reason for not completing the study:		
Lost to follow-up	168 (4.0%)	173 (4.1%)
Withdrawal of consent	116 (2.7%)	118 (2.8%)
Other	26 (0.6%)	30 (0.7%)
Site error: termination telephone contact prior to Day 180 or 31/May/05	10 (0.2%)	17 (0.4%)
Site error: failed to contact subject's parent/guardian	3 (0.1%)	2 (0.0%)
Parent/guardian non-compliance	0 (0.0%)	1 (0.0%)
Subject expired prior to Day 180	1 (0.0%)	1 (0.0%)
Site error: subject randomized but never dosed	10 (0.2%)	6 (0.1%)
Subject moved out of area	2 (0.0%)	3 (0.1%)

CAIV-T = R-FLUENZ in the text
ITT Population

Conduct of the study

There were some major changes including increasing the number of sites needed for subject recruitment, excluding children with a history of severe asthma and increasing the sample size and corresponding power calculations.

In the U.S. and Asia, only the 0.25-ml pre-filled TIV syringes were available for distribution due to TIV vaccine shortages during the 2004-2005 influenza season. As a result, only children 6-35 months of age were able to be enrolled in the U.S. and Asia.

The protocol specified hexaplex PCR on influenza-positive samples obtained within 28 days after study vaccination was not routinely performed but was left at the discretion of the investigator.

Baseline data

In the ITT efficacy analysis population:

		CAIVT N=4243	TIV N=4232	Total N=8475
Age Category	6-23 months	2022 (47.7%)	2002 (47.3%)	4024 (47.5%)
	24-35 months	1393 (32.8%)	1398 (33.0%)	2791 (32.9%)
	36-59 months	828 (19.5%)	831 (19.6%)	1659 (19.6%)
	60 months	0 (0.0%)	1 (0.0%)	1 (0.0%)

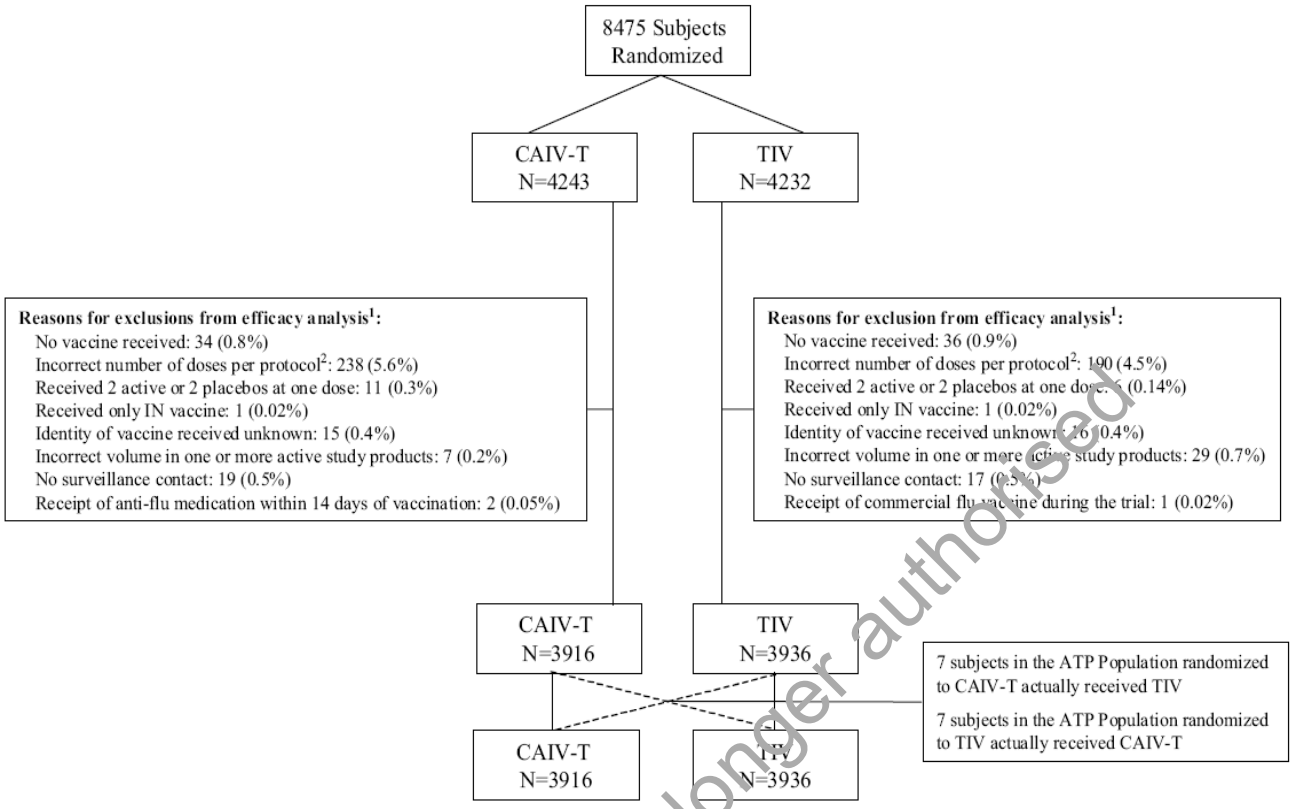
Subjects with Underlying Medical Conditions

The distribution of underlying medical conditions at baseline was similar between the R-FLUENZ and TIV treatment groups. Up to 9% of subjects had an underlying medical condition and 76% of all the underlying medical conditions identified were chronic lung disease.

Subjects with Prior Wheeze/Asthma History

The distribution of subjects by wheeze/asthma history was similar between treatment groups and among the ATP, ITT, and Safety populations. Approximately 21% were identified as having a past medical history of wheezing, 6% had a history of $\geq$3 wheezing illnesses that required medical follow-up or hospitalization, 4% had a medical diagnosis of asthma prior to the trial, 2% had a history of persistent cough related to asthma, and 18% had previously received medication for wheezing, asthma, or persistent cough related to asthma.

Numbers analysed



Summary of dose two administration

	CAIV-T N=4243	TIV N=4232
Received Dose One and indicated to receive Dose Two per baseline characteristics	3269	3247
Received Dose Two[a]	3032 (92.8%)	3061 (94.3%)
Did not receive Dose Two	237 (7.2%)	186 (5.7%)
Reasons for not receiving Dose Two:		
SAE/AE/Solicited event	37 (15.6%)	27 (14.5%)
Voluntary decision by parent/guardian	89 (37.6%)	67 (36.0%)
Lost to follow-up	14 (5.9%)	20 (10.8%)
Failed to meet continuing eligibility criteria	71 (30.0%)	53 (28.5%)
Other	26 (11.0%)	19 (10.2%)

CAIV-T=R-FLUENZ in the text
ITT Population
a. Subjects may not have received the same study products at Dose One and Dose Two

Outcomes and estimation

Administration of the FLUENZ vaccine resulted in a 44.5% (95% CI: 22.4, 60.6) higher reduction in influenza illness compared to TIV caused by virus strains antigenically matched to those used in the vaccine blend. The incidence of illness due to all possible strains showed a 55% larger reduction (95% CI: 45.4; 62.9), whereas efficacy against mismatched strains was 58.2% higher (95% CI: 47.4; 67.0). Results were driven by the relative efficacy for FLUENZ compared to TIV for circulating A/H1N1 strains (relative efficacy for B strains was not statistically significant in this study; no matched H3N2 strains were isolated in this study).

Relative efficacy of FLUENZ was also demonstrated against the symptomatic influenza infection (due to matched strains, mismatched strains, and all strains regardless of antigenic match) and against the

endpoints of acute otitis media (AOM) and lower respiratory illness (LRI) associated with positive nasal cultures for influenza.

Efficacy by-Stratification Factor Analyses of the Primary Endpoint

	CAIV-T N=3893			TIV N=3943				
	N	# of Cases	Crude Attack Rate (cases/N)	N	# of Cases	Crude Attack Rate (cases/N)	Relative Efficacy[a]	95% Exact CI for Relative Efficacy[a]
AGE								
6-23 months	1834	23	1.3%	1852	32	1.7%	29.1%	-21.2, 59.1
24-35 months	1311	17	1.3%	1301	24	1.8%	32.6%	-25.8, 64.5
36-59 months[b]	771	13	1.7%	783	37	4.7%	65.6%	36.3, 82.4
PRIOR INFLUENZA VACCINATION[c]								
Yes	929	18	1.9%	937	29	3.1%	39.3%	-9.2, 66.9
No	2987	35	1.2%	2999	64	2.1%	46.9%	20.0, 65.2
WHEEZING HISTORY[d]								
Yes	246	8	3.3%	216	9	4.2%	24.0%	-104.2, 72.1
No	3670	45	1.2%	3720	84	2.3%	46.9%	23.9, 63.3
GENDER								
Male	2008	24	1.2%	2017	43	2.1%	[illegible]	16.5, 70.4
Female	1908	29	1.5%	1919	50	2.6%	[illegible]	12.8, 65.6
RACE								
White/Non-Hispanic	3168	49	1.5%	3184	80	2.5%	40.3%	14.9, 58.4
Non-White	748	4	0.5%	752	13	1.7%	64.8%	-4.9, 90.2
Black	156	2	1.3%	140	2	1.4%	-124.7%	-6534, 82.9
Hispanic	225	0	0.0%	243	3	1.2%	100%	-65.1, 100.0
Asian	290	1	0.3%	297	5	1.7%	69.4%	-190.0, 98.8
Other	77	1	1.3%	72	3	4.2%	45.6%	-623.8, 98.2

ATP Population

a. Relative efficacy was adjusted for country, age, prior vaccination status, and wheezing history status.
b. One 60-month-old subject was counted in the 36-59 month stratum.
c. Subjects with an unknown vaccine history were counted as not having received prior influenza vaccination.
d. Positive wheezing history was defined as a history of ≥3 wheezing illnesses requiring medical follow-up or hospitalization. Subjects with an unknown wheezing history were counted as having a negative wheezing history.

- **D153-P515**

This was a randomized, open-label, Phase III, active-controlled, multinational, outpatient study enrolling children aged 6 years to 17 years of age with a clinical diagnosis of asthma.

Study centres: Europe (Belgium, Finland, Germany, Greece, Italy, the Netherlands, Norway, Poland, Portugal, Spain, Switzerland and the United Kingdom) and Israel (145 sites).

Study periods: October 04, 2002 to May 31, 2003.

Treatment

Liquid formulation vaccine (Refrigerated FLUENZ): dosage strength/strain: 10^7±0.5 FFU of A/NewCaledonia/20/99 (H1N1), A/Panama/2007/99 (H3N2) and B/Hong Kong/330/01 given intranasally at a dose volume of 0.2 ml.

Commercially available TIV, inactivated influenza vaccine (Split Virion) (Aventis Pasteur MSD, Lyon, France) dosage strength/dose: 15 µg each of HA of A/New Caledonia/20/99 – IVR-116, A/Panama/2007/99 – RESVIR-17, and B/Shangdong/7/97 given intramuscularly at a dose volume of 0.5 ml.

Objectives

See table 8.

Sample size

Approximately 2,200 subjects were planned to be enrolled in this study. The study was sized to have at least 90% power to demonstrate non-inferiority for the primary efficacy comparison between

FLUENZ and TIV and at least 80% power to demonstrate equivalence for the primary safety comparison between FLUENZ and TIV, based on the following assumptions: the culture-confirmed influenza attack-rate in unvaccinated asthmatic children is at least 12%; efficacy relative to placebo is 75% for TIV (attack rate was 3%); efficacy relative to placebo is 85% for FLUENZ (attack rate was 1.8%); the incidence of asthma exacerbation, defined as acute wheezing illness associated with hospitalization, any unscheduled clinic visit, or any new prescription is 7.6% for TIV.

Randomization

Subjects were randomly assigned to receive either refrigerated FLUENZ or TIV as they were enrolled at a ratio of 1:1.

Participant flow

A total of 2311 subjects between the ages of 6 and 17 years were enrolled in the study, 2229 subjects were randomized on a 1:1 ratio (1114 FLUENZ, 1115 TIV) and a total of 2220 (99.6%) subjects completed the study.

Numbers analysed

During the trial, 1114 and 1115 subjects were randomized to the FLUENZ and TIV groups respectively; 5 in the FLUENZ group and 13 in the TIV group were excluded from the per protocol efficacy population for major protocol violations.

Outcomes and estimation

4.1% of the subjects in the FLUENZ group and 6.4% of those in the TIV group showed an incidence of influenza illness caused by a strain that was antigenically similar to one of the strains contained in the vaccines. Relative efficacy of FLUENZ was determined to be 34.7% (95% CI: 3.9, 56.0).

Efficacy of FLUENZ relative to TIV against individual antigenically similar strains was as follows: 100.0% (95% CI: -8.4, 100) for the A/H1 strains [0/1109 cases in the FLUENZ group (0.0%) and 5/1102 in the TIV group (0.5%)]; 0.6% (95% CI: -141.8, 59.2) for the A/H3 ones [12/1109 cases in the FLUENZ group (1.1%) and 12/1102 in the TIV group (1.1%)] and 36.3% (95% CI: 0.1, 59.8) against the B strains [34/1109 cases in the FLUENZ group (3.1%) and 53/1102 in the TIV group (4.8%)]. Non-inferiority of FLUENZ relative to TIV was not demonstrated for the A/H3 strain.

Efficacy against all virus strains, regardless of antigenic match, for FLUENZ versus TIV was as follows: 50 FLUENZ recipients (4.5%) and 73 (6.6%) TIV recipients were infected, resulting in a relative efficacy of FLUENZ of 31.9% (95% CI: 1.1, 53.5). Relative non-inferiority of FLUENZ was demonstrated for the A/H1 (100.0%, 95% CI: 15.6, 100.0) and B (36.8%; 95% CI: 1.6, 59.8) strains. The majority of subjects having non-antigenically matched A/H3 isolates were FLUENZ recipients. The non-inferiority of FLUENZ relative to TIV in efficacy against any A/H3 strain could thus not be demonstrated (-29.9%; 95% CI: -190.9, 40.6).

- **Study D153-P514**

This was a randomized, open-label, Phase III, active-controlled, multinational, outpatient study enrolling children aged 6 to 71 months who had ≥ 2 documented respiratory tract infections during the 12 months before vaccination participated.

Study centres: Europe (Belgium, Czech Republic, Finland, Germany, Italy, Poland, Spain, Switzerland, and United Kingdom) and Israel.

Study periods: October 04, 2002 to June 02, 2003.

Treatment

Liquid formulation vaccine (Refrigerated FLUENZ): dosage strength/strain: 10^7±0.5 FFU of A/NewCaledonia/20/99 (H1N1), A/Panama/2007/99 (H3N2) and B/Hong Kong/330/01 given intranasally at a dose volume of 0.2 ml.

Commercially available TIV, inactivated influenza vaccine (Split Virion) BP (Aventis Pasteur MSD, Lyon, France) dosage strength/dose: 7.5 µg or 15 µg each of HA of A/Moscow/10/99 (H3N2)-like strain (A/Panama/2007/99 – RESVIR–17), A/New Caledonia/20/99 (H1N1)-like strain (A/New Caledonia/20/99 – IVR-116), and B/Hong Kong/330/2001-like strain (B/Shangdong/7/97), depending on subject age.

Objectives

See table 8.

Sample size

Approximately 2,200 subjects were planned to be enrolled in this study. The study was sized to have at least 90% power to demonstrate non-inferiority for the primary efficacy comparison between R-FLUENZ and TIV and at least 80% power to demonstrate to detect frequency differences, between the R-Fluenz and TIV groups, ranging from 3.4% to 6.7%, depending on the frequency in the TIV group, based on the following assumptions: the culture-confirmed influenza attack rate in unvaccinated asthmatic children is at least 12%; efficacy relative to placebo is 75% for TIV (attack rate was 3%); efficacy relative to placebo is 85% for R-FLUENZ (attack rate was 2.0%).

Randomization

Subjects were randomly assigned to receive 2 doses (35 +/- days apart) of either refrigerated FLUENZ or TIV as they were enrolled at a ratio of 1:1.

Participant flow

A total of 2187 children aged 6 months to less than 72 months were enrolled and randomized (1101 FLUENZ, 1086 TIV). A total of 2137 (97.7%) subjects completed the study. Of the 2187 subjects enrolled, 2114 (96.7%) received the second dose.

Outcomes and estimation

The relative efficacy against CCII caused by community-acquired subtypes of influenza virus antigenically similar to those in the vaccine in children in the per-protocol population was 52.7% (95% CI: 21.6, 72.2). Efficacies for individual strains were 100.0% (95% CI: 42.3, 100.0) against A/H1 [0/1050 cases in the FLUENZ group (0.0%) and 8/1035 in the TIV group (0.8%)] and 68.0% (95% CI: 37.3, 84.8) against B [12/1050 cases in the FLUENZ group (1.1%) and 37/1035 in the TIV group (3.6%)]. Against A/H3 strains, FLUENZ was apparently not more effective than TIV.

The efficacy of FLUENZ against all community acquired influenza strains was also superior to that of TIV, with an efficacy of 52.4% (95% CI: 24.6, 70.5).

There was no statistically significant difference demonstrated between FLUENZ and TIV against AOM associated with a nasal culture positive for influenza virus antigenically similar to that in the vaccine.

Placebo and TIV controlled adult studies

There are three main clinical efficacy/effectiveness studies in adults (see tables 9 and 13): Study D153-P516, Study AV009, Study 153-P507 and one supportive challenge study (AV003).

- **Study D153-P516** is a randomized open label non-inferiority trial comparing FLUENZ and TIV in 3009 patients aged ≥ 60 years. The non-inferiority could not be proven statistically therefore this study was not conclusive. This was due to the too low incidence of influenza during the study.
- **Study D153-P507** was a prospective, randomized, double-blind placebo controlled study designed to evaluate efficacy, safety, and immunogenicity of FLUENZ in 3242 patients aged ≥ 60 years (mean age of 69.5) years in South Africa in 2001. 70% of the patients were Caucasian, 25% were Cape-Coloured, 4% were Black. The results on the primary endpoint (1 year: culture confirmed influenza due to matched strains) were as follows: FLUENZ 4.3% and placebo 7.5% leading to a statistically significant reduction of 42.3% FLUENZ versus placebo (95% CI: 21.6, 57.8).

Study	FLUENZ Formulation	Age Range	No subjects	Study Year	FLUENZ absolute efficacy (95% CI)	
					Matched Strains	All Strains Regardless of Match
D153-P507	Refrigerated	≥ 60 Y	3136	2001	42.3% (21.6, 57.8)	41.6% (20.9, 57.1)

The study met its primary efficacy endpoint, demonstrating that FLUENZ provided statistically superior ($p < 0.001$) protection compared with placebo against culture-confirmed influenza illness caused by influenza subtypes matched to those in the vaccine. For the A/H1N1 strain, no estimate of efficacy was possible, as no cases involving this subtype were detected during the study. Efficacy against the matched A/H3N2 strain was estimated to be 52.5% (95% CI: 32.1, 67.2). For B strains, the incidence of influenza illness in subjects who received vaccine was not reduced compared with those who received placebo.

- **Study AV009** was a double-blind, randomized, placebo controlled, multicenter study designed to assess the safety, tolerability, and effectiveness of a single intranasal dose of FLUENZ compared with placebo in working subjects 18 to < 65 years of age. A total of 4,561 participants were randomized 2:1 to receive FLUENZ (n=3,041) or placebo (n= 1,520) at 13 study centres. 84% of the patients were Caucasian, 10% were Black. The primary endpoint (any febrile illness) was reached by 13.2% of the patients in the FLUENZ group and by 14.6% in the placebo group leading to a statistically non significant reduction of 9.7% FLUENZ vs. placebo (p=0.19). Although this study fails to reach any demonstration on the primary endpoint, some degree of efficacy is acknowledged on the basis of the secondary endpoints (severe febrile illness, absenteeism…).
- **Study AV003**

  This is a prospective, randomized, double-blind, placebo and TIV controlled challenge study in 103 subjects aged 18-40 years. The enrolled subjects were serosusceptible (HAI ≤ 8) to at least 1 of the 3 influenza virus types in the FLUENZ vaccine (A/ H1N1, A/ H3N2, or B). 52% of the entire cohort was white and 40% was black. The remainder of participants were Hispanic (4%) and Asian/Pacific Islander (4%). The results on the primary endpoint (laboratory documented influenza after challenge) were as follows: FLUENZ 7%, TIV 13% and placebo 45%. FLUENZ resulted in an 85% reduction (95%CI: 28, 100) vs. placebo and TIV resulted in a 71% reduction vs. placebo (95%CI: 2, 97). The difference FLUENZ vs. TIV was not statistically significant. However, no formal conclusion could be drawn on this small sample sized study, far from the real life.

Ancillary analyses

Effect of paracetamol on vaccine efficacy

The effects on vaccine efficacy or immunogenicity of paracetamol use against fever were not endpoints in any of FLUENZ' studies. **Study MI-CP111** was chosen for post hoc analysis of the efficacy of FLUENZ based on subjects' paracetamol use, because it was the largest controlled study conducted

during clinical development. A post-hoc analysis of the MI-CP111 concomitant medication database identified 3 subgroups of subjects based on subjects' use of paracetamol containing medications during the study (up to 42 days post last dose):

- Definitive or possible paracetamol use (N = 4,307+4,314 respectively): Subjects who listed paracetamol or a concomitant medication as possibly containing paracetamol as a concomitant medication.
- No paracetamol use (N = 3,538): Subjects who did not list any concomitant medication that possibly contained paracetamol.

Table 14. Relative Efficacy of FLUENZ Against Culture-Confirmed Modified CDC-ILI Caused by Wild-Type Strains (Study MI-CP111)

Tylenol Subgroup	FluMist			TIV			% Relative Efficacy[a]	95% Exact CI for Relative Efficacy
	N	No. of Cases	Crude Attack Rate, % (Cases/N)	N	No. of Cases	Crude Attack Rate, % (Cases/N)		
All Matched Strains								
Definitive Tylenol Use	2,088	32	1.5	2,219	63	2.8	48.7	(21.5, 67.0)
No Tylenol Use	1,823	21	1.2	1,715	30	1.7	35.5	(-13.2, 63.7)
All Mismatched Strains								
Definitive Tylenol Use	2,088	74	3.5	2,219	178	8.0	54.1	(39.8, 65.3)
No Tylenol Use	1,823	28	1.5	1,715	67	3.9	60.3	(38.1, 75.0)
All Strains Regardless of Antigenic Match								
Definitive Tylenol Use	2,088	105	5.0	2,219	240	10.8	52.9	(40.7, 62.8)
No Tylenol Use	1,823	48	2.6	1,715	98	5.7	53.8	(34.6, 67.7)

a Relative efficacy was adjusted for country, age, prior vaccination status, and wheezing history status.
* the table contains 'Tylenol', please note that paracetamol is meant here.

In summary, FLUENZ efficacy was demonstrated against culture-confirmed modified CDC influenza-like illness in subjects with and without paracetamol use during the study. Based on these data, the use of paracetamol might have no impact on the relative efficacy of the vaccine. However, considering that fever, if any, will only occur 2-3 days after administration of this live attenuated vaccine, by the time the viral replication will take place, the resort to the prophylactic use of paracetamol is not entirely supported.

Effectiveness by age strata in adults

Overall the efficacy in the older adults (>50 years) was not found to be unequivocally proven during evaluation and moreover it was expected that the immunogenicity would be lower in this age category. Study D153-P516 was inconclusive because of the low incidence of influenza. Study AV009 did not show a statistically significant result on the primary endpoint (any febrile illness). Only study D153-P507 (≥ 60 years) provided evaluable results showing a 42.3% reduction compared to placebo in culture confirmed influenza by matched strains. Therefore the Applicant was requested to reanalyse the results stratifying by age categories (30-49, 50-60, 60-65, 65-70, etc…) to examine if the efficacy diminished with increasing age.

Post hoc subgroup analyses of the clinical effectiveness of FLUENZ in reducing the occurrence, number of episodes, days of illness, days of work missed, number of healthcare visits and days of antibiotic use were performed by age according to the following strata: < 30, 30 to 39, 40 to 49, 50 to 59, and ≥ 60- years for study AV009.

Figure 1. Effectiveness by Age for Any Febrile Illness in Study AV009

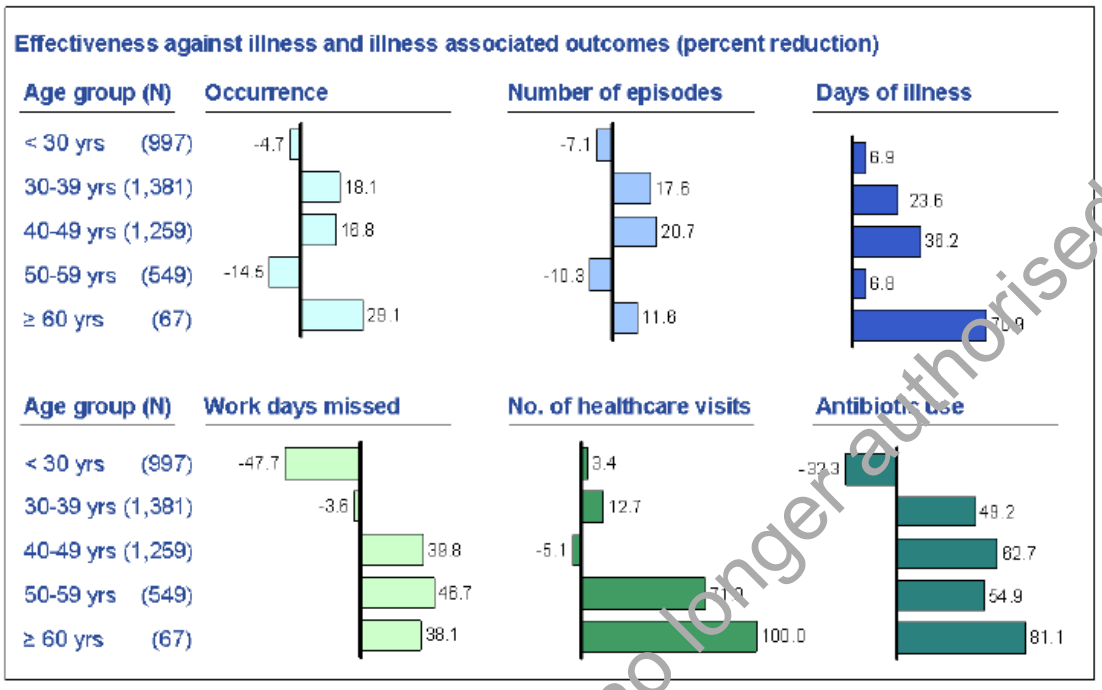


Figure 2. Effectiveness by Age for Severe Febrile Illness in Study AV009

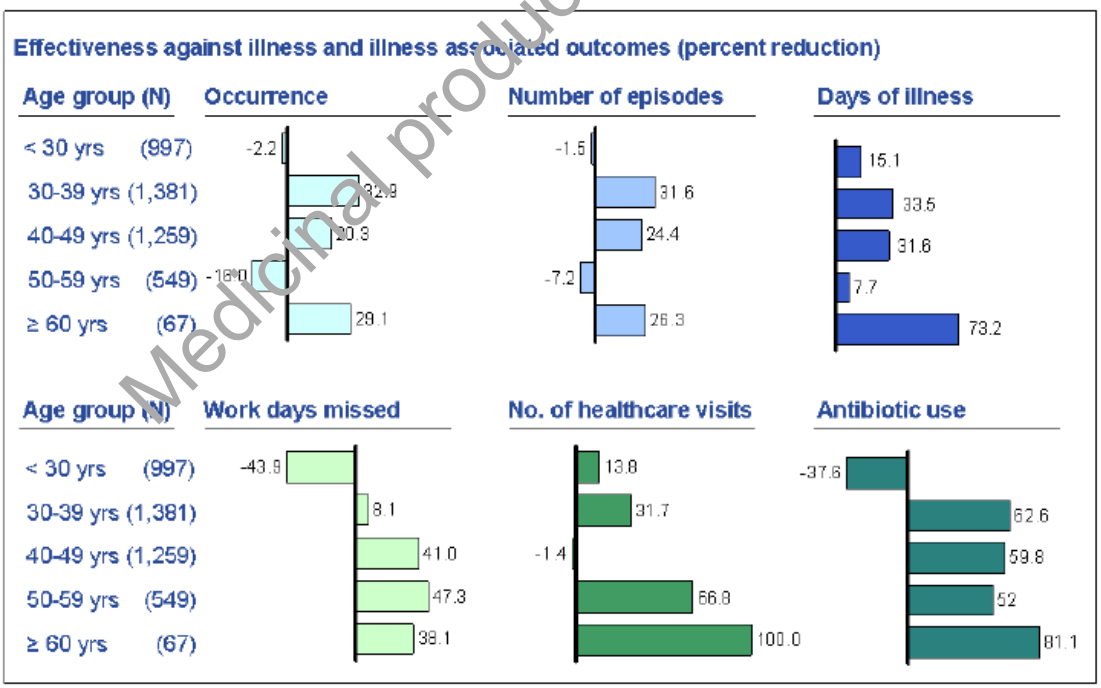
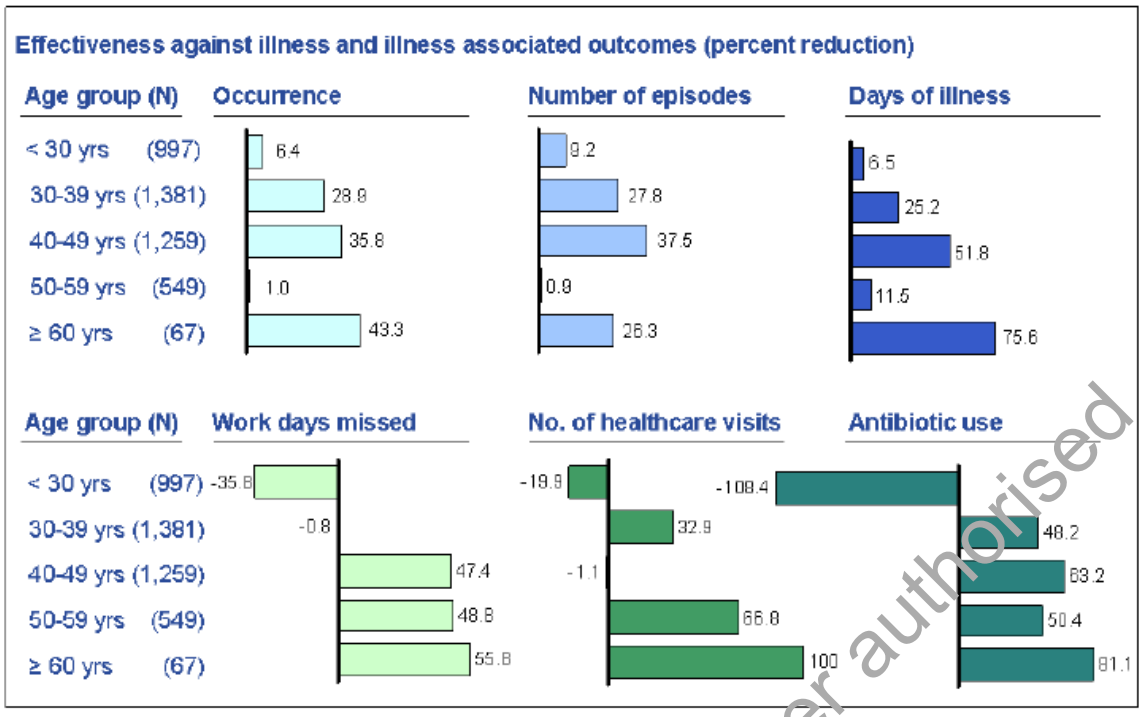


Figure 3. Effectiveness by Age for CDC-defined ILI in Study AV009



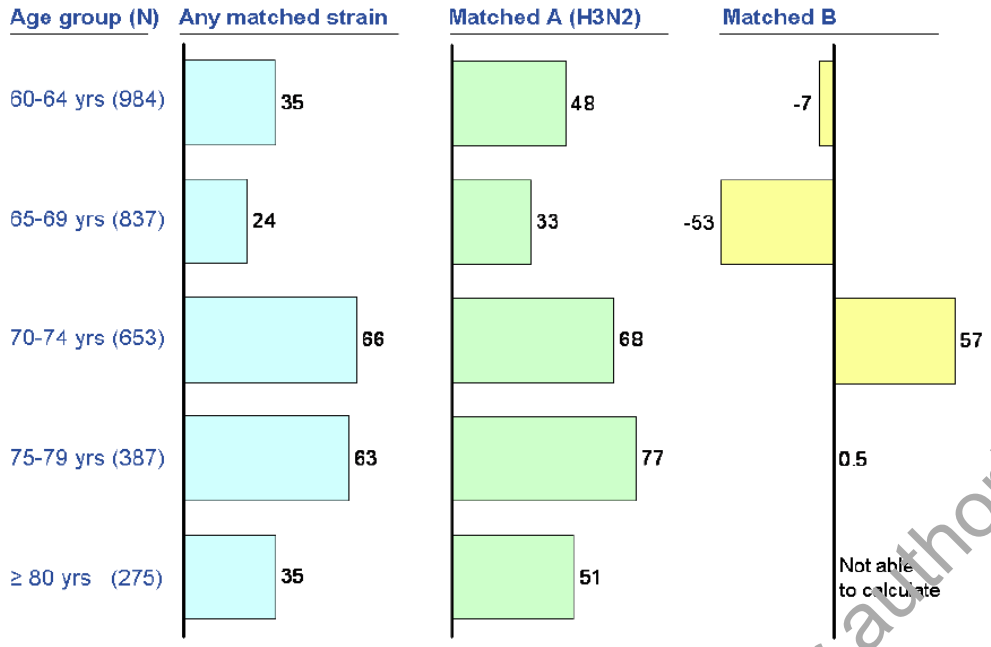
Moreover, a post hoc subpopulation analyses of the clinical effectiveness of FLUENZ in reducing the occurrence, number of episodes, and days of illness events were performed by age group (< 50 and ≥ 50 years of age) and the results are displayed the following table:

Table 15. Percentage of Subjects Having 1 or More Illness Events by Age Group in Study AV009

Occurrence of:	Age < 50 years			Age ≥ 50 years		
	FluMist N = 2,411	Placebo N = 1,226	Percent Reduction	FluMist N = 422	Placebo N = 194	Percent Reduction
Any Febrile Illness	13.7	15.4	10.9	10.0	9.3	-7.3
Severe Febrile Illness	10.4	12.9	19.5	8.3	7.7	-7.3
Febrile Upper Respiratory Illness	8.8	11.6	23.7	6.4	6.2	-3.4
CDC-Defined ILI	11.0	14.6	24.4	8.5	9.3	8.1
DOD-Defined ILI	10.7	13.6	21.1	8.8	13.9	37.0

As regards study D153-P507, the applicant has also provided post-hoc Age Subgroup Analysis. FLUENZ efficacy was explored in 5 age cohorts: 60 to 64, 65 to 69, 70 to 74, 75 to 79, and ≥ 80 years of age as described below:

Figure 4. Absolute Efficacy (Percent) of FLUENZ Against Culture-Confirmed Influenza, by Strain and Age Subgroup in Study D153-P507



Analysis performed across trials

Pediatric studies

Compared to placebo, the clinical efficacy of a two doses regimen in primary series has been repeatedly shown better:

- against matched strains ranging from 62% to 93% (see table 11),
- against all strains regardless of antigenic match ranging from 49% to 86% (see table 11),
- against specific matched subtypes of influenza virus varied by studies (see table 16).

Table 16. Strain-Specific Efficacy of FLUENZ Against Antigenically Matched Strains

Study Number	Efficacy (95% CI) A/H1N1	Efficacy (95% CI) A/H3N2	Efficacy (95%CI) B
D153-P501 Year 1	80.9% (69.4, 88.5)	90.0% (71.4, 97.5)	44.3% (6.2, 67.2)
D153-P502 Year 1	91.8% (80.8, 97.1)	100% (-2,627, 100.0) [a]	72.6% (38.6, 88.9)
D153-P504 Year 1	NC	72.7% (60.7, 81.5)	81.4% (64.2, 91.2)
D153-P513	100% (-3,733.1, 100.0) [a]	64.0% (45.7, 76.6)	NC
D153-P522	100% (-168.0, 100.0) [a]	68.5% (-9.0, 91.9)	81.7% (38.2, 95.8)
AV006 Year 1	NC	96.0% (89.4, 98.5)	90.5% (78.0, 95.9)

NC = not computable due to 0 cases of culture-confirmed illnesses caused by the specific strain in the placebo group (D153-P504 and D153-P513) or 0 cases of culture-confirmed influenza caused by the specific strain in both treatment groups (AV006 Year 1).
[a] Efficacy based on culture-confirmed illness from a single isolate (D153-P502 and D153-P513) or from 2 isolates (D153-P522).

Against culture-confirmed influenza-associated acute otitis media due to matched strains, the estimated efficacy suggested a superiority of FLUENZ over placebo and reached statistical significance in several studies (see table 17).

Table 17. FLUENZ Efficacy Against Acute Otitis Media Associated with Culture-Confirmed Influenza due to Matched Strains in Placebo Controlled Studies

Study Number	Acute Otitis Media Attack Rate		Efficacy (95% CI)
	FLUENZ n/N (%)	Placebo n/N (%)	
D153-P501 Year 1	2/ 1,649 (0.1%)	2/ 1,105 (0.2%)	33.0% [a]
D153-P501 Year 2	0/ 770 (0.0%)	1/ 494 (0.2%)	100% [a]
D153-P502 Year 1	3/ 951 (0.3%)	22/ 664 (3.3%)	90.5% (68.3, 98.2)
D153-P502 Year 2	1/ 639 (0.2%)	23/ 450 (5.1%)	96.9% (81.1, 99.9)
D153-P504 Year 1	14/ 944 (1.5%)	52/ 941 (5.5%)	73.2% (50.9, 86.3)
D153-P504 Year 2	2/ 338 (0.6%)	5/ 342 (1.5%)	59.5% (-147.2, 96.1)
D153-P513	1/ 521 (0.2%)	5/ 515 (1.0%)	80.2% (-76.7, 99.6)
D153-P522	0/ 624 (0.0%)	4/ 312 (1.3%)	100% [a]
AV006 Year 1	1/ 1,070 (0.1%)	20/ 532 (3.8%)	97.5% (85.5, 99.6)
AV006 Year 2 [b]	2/ 917 (0.2%) [b]	17/ 441 (3.9%) [b]	94.3% (78.1, 98.5) [b]

[a] Confidence interval not calculated in the study report analysis.
[b] Data for all strains regardless of antigenic match is shown because, in this season, mismatched strains predominantly circulated.

In conclusion, FLUENZ always performed better than placebo and differences can vary according to studies and with the reference to geographical area specificities of viral attacks (strength, strains).

Compared to TIV, the relative efficacy of a two doses regimen in primary series has been repeatedly shown better:

- against culture-confirmed influenza illness caused by wild-type virus strains antigenically matched to those in the vaccine, reducing the number of cases by 35% to 53% (see table 12);
- against all strains regardless of antigenic match, reducing the number of cases by 32% to 55% (see table 12);
- against specific matched subtypes of influenza virus varied by studies (see table 18 and 19).

Table 18. Strain-Specific Relative Efficacy of FLUENZ versus TIV Against Antigenically Matched Strains

Study Number	Efficacy (95% CI) A/H1N1	Efficacy (95% CI) A/H3N2	Efficacy (95%CI) B
D153-P514	100% (43.2, 100.0)	-97.1% (-540.2, 31.5)	68.0% (37.3, 84.8)
D153-P515	100% (-8.4, 100.0)	0.6% (-141.8, 59.2)	36.3% (0.1, 59.8)
MI-CP111	89.2% (67.7, 97.4)	NC	27.3% (-4.8, 49.9)

NC = not computable because no antigenically matched A/H3N2 strain was isolated.

Table 19. Strain-Specific Relative Efficacy of FLUENZ versus TIV Against all Strains Regardless of match

Study Number	Efficacy (95% CI) A/H1N1	Efficacy (95% CI) A/H3N2	Efficacy (95%CI) B
D153-P514	100% (56.0, 100.0)	-47.9% (-236.5; 32.6)	68.9% (39.2, 85.2)
D153-P515	100% (15.6, 100.0)	-29.9% (-190.9, 40.6)	36.8% (1.6, 59.8)
MI-CP111	89.2% (67.7, 97.4)	79.2 (70.6, 85.7)	16.1% (-7.7, 34.7)

Against culture-confirmed influenza-associated acute otitis media due to matched strains, the results do not demonstrate any difference between FLUENZ and TIV. However in Study MI-CP111, the fact that FLUENZ demonstrates a significant relative efficacy against influenza related AOM regardless of antigenic match is in favour of a benefit of FLUENZ.

Table 20. Relative efficacy Against Acute Otitis Media Associated with Culture-Confirmed Influenza due to Matched Strains

Study Number	Acute Otitis Media Attack Rate		Relative Efficacy
	FLUENZ n/N (%)	TIV n/N (%)	
D153-P514	2/ 1,048 (0.2%)	6/ 1,034 (0.6%)	67.1% (90% CI: -47.8, 95.2) fewer cases than TIV
MI-CP111	10/ 3,916 (0.3%)	10/ 3,936 (0.3%)	0.4% (95% CI: -146, 59.6) fewer cases than TIV
	26/ 3,916 (0.7%) [a]	54/ 3,936 (1.4%) [a]	50.6% (95% CI: 21.5, 69.5) fewer cases than TIV [a]

[a] Efficacy for all strains regardless of antigenic match is also shown because, in this season, mismatched strains predominantly circulated.

Clinical studies in special populations

Recurrent respiratory tract infections

While clinical development studies conducted for FLUENZ generally enrolled pediatric and adult subjects without chronic underlying medical conditions, 2 studies enrolled children with a history of recurrent respiratory tract infections and children with stable medically treated asthma (D153-P514 and D153-P515, respectively). Data from **Study D153-P514** indicated that, in this population of children with recurrent respiratory tract infections, FLUENZ was safe with superior efficacy to TIV against culture-confirmed influenza illness. In **Study D153-P515** FLUENZ also demonstrated superior relative efficacy compared with TIV, with no associated increase in post vaccination asthma exacerbation rates in subjects 6 to 17 years of age with a diagnosis of asthma prior to study enrolment.

Asthma/Wheezing

Study MI-CP111 provided some information about the efficacy of FLUENZ in subjects 6 to 59 months of age with and without a pre-existing history of wheezing or asthma. In brief, over the entire study period post-immunization, children 24 to 59 months of age with a pre-existing history of wheezing or asthma who had received FLUENZ showed a significantly reduced rate of culture-confirmed modified CDC-ILI, medically-attended culture-confirmed influenza and culture-confirmed influenza when

compared to children who had received TIV. No children in this age group with pre-existing asthma or wheezing had hospitalized culture-confirmed influenza. See table 21.

Information about efficacy of FLUENZ in children 6 to 17 years of age with pre-existing asthma or wheezing was obtained by **Study D153-P515**. Results demonstrated statistically superior relative efficacy of FLUENZ compared to TIV against community-acquired influenza illness, whether caused by matched strains (34.7% efficacy) or all strains regardless of match (31.9% efficacy). The incidence of first episode of asthma exacerbation after vaccination was similar for FLUENZ and TIV recipients (31.2% vs. 29.6%, respectively). Rates of first episode of hospitalization for asthma were 0.7% in both groups. In summary, FLUENZ demonstrated superior relative efficacy against community-acquired influenza compared to TIV, in subjects 6 to 17 years of age with asthma.

Table 21. Study MI-CP111, Efficacy Measures, Entire Study Period, in Subjects 24 to 59 Months of Age

Endpoint	FluMist n/N (%)	TIV n/N (%)	Rate Difference / p value
Culture confirmed mCDC ILI			
Overall	102/2,187 (4.7%)	216/2,198 (9.8%)	-5.2 / 0.000
History of Any Wheeze/Asthma	38/572 (6.6%)	73/573 (12.7%)	-6.1 / 0.000
No History of Any Wheeze/Asthma	64/1,615 (4.0%)	143/1,625 (8.8%)	-4.8 / 0.000
Medically attended culture confirmed influenza			
Overall	30/2,187 (1.4%)	63/2,198 (2.9%)	-1.5 / 0.001
History of Any Wheeze/Asthma	12/572 (2.1%)	25/573 (4.4%)	-2.3 / 0.030
No History of Any Wheeze/Asthma	18/1,615 (1.1%)	38/1,625 (2.3%)	-1.2 / 0.008
Culture confirmed influenza			
Overall	137/2,187 (6.3%)	252/2,198 (11.5%)	-5.2 / 0.000
History of Any Wheeze/Asthma	53/572 (9.3%)	88/573 (15.4%)	-6.1 / 0.002
No History of Any Wheeze/Asthma	84/1,615 (5.2%)	164/1,625 (10.1%)	-4.9 / 0.000
Hospitalized culture confirmed influenza (+/- 7 day culture window)			
Overall	1/2,187 (0.0%)	2/2,198 (0.1%)	-0.0 / 0.567
History of Any Wheeze/Asthma	0/572 (0.0%)	0/573 (0.0%)	0.0 / 1.000
No History of Any Wheeze/Asthma	1/1,615 (0.1%)	2/1,625 (0.1%)	-0.1 / 0.567

Note: "Culture confirmed" influenza endpoints measured against all wild type strains regardless of match beginning with the first dose
Note: Rate differences and 95% CIs calculated on crude basis. Rate difference was FLUENZ minus TIV expressed as a percentage point.
Note: Analyses subject-based rather than event-based

Immunodeficiency

The studies that have evaluated FLUENZ in children with HIV and with immunodeficiency not-HIV related have been safety and immunogenicity studies (**Study DMID 99-012** to evaluate immunogenicity data in children 1 to 7 years of age with HIV infection; **Study MI-CP114** to evaluate safety and immunogenicity in immunocompromised children 5 to 17 years of age; **Study PACTG1057** to evaluate safety and immunogenicity in HIV-infected children and adolescent 5 to 17 years of age). These studies were not designed to evaluate efficacy. In study DMID 99-012 the proportion of children achieving at least a 4-fold increase in titre from baseline to post Dose 2 was as high in the HIV-

infected as in the non-HIV-infected children. Importantly, immune responses were observed in both HIV status groups for those children who were seronegative at baseline. Study PACTG1057 overall demonstrated that FLUENZ was immunogenic in HIV-infected children who, at the time of study screening, did not meet the World Health Organization criterion for severe immunosuppression, i.e., CD4% < 15 (WHO, 2007).

Pre-existing systemic illnesses

Overall sufficient clinical data has not been obtained to permit an analysis of FLUENZ efficacy in this specific population of individuals from 6 months to 17 years of age with systemic illnesses at enrolment. Study **MI-CP11**1 is the only study in which the presence of pre-existing systemic illnesses was documented. In that study, of 8,475 subjects 6 to 59 months of age, FLUENZ recipients included 25 with chronic cardiac disease, 1 with diabetes, 6 with other chronic metabolic disorders, 5 with haemoglobinopathy, 8 with renal disease and 35 with other unspecified chronic diseases. These numbers are too small to permit meaningful analysis of efficacy in these groups. FLUENZ recipients included 180 subjects with chronic lung disease, which specifically included asthma in its definition. Within this group, non-asthmatics were not distinguished from asthmatics; results for nonasthmatics are therefore not available.

Published studies

Adults

There are several independent published studies which provided relevant information on the efficacy of live attenuated influenza vaccines (LAIV) and trivalent influenza vaccines (TIV). These are discussed below.

- The study from Eick et al. 2009 compared the absolute and relative efficacy of LAIV and TIV vaccines in recruits and non recruits 17 to 49 years of age with the following results:

  i) In non recruits, the ILI incidence among LAIV recipients was higher than among TIV recipients for the two seasons 2005-2006 and 2006-2007.

  ii) Unlike the non recruit population, recruit LAIV recipients had a statistically significant lower ILI incidence rates than TIV recipients. This finding was consistent for both seasons.

  The authors tentatively explained these findings by the fact that recruits subjects may have an immune system that is relatively naïve to influenza and therefore have a different immune response compared to that of more seasoned service members who are likely to have received multiple annual influenza immunisations. As discussed by the authors pre-existing antibody from multiple years of influenza may be playing a role in reducing the replication and antibody response to the LAIV. On the other hand the authors find in non-recruits an inverse relationship between LAIV to TIV IRRs and age that is counter to the hypothesis that older age groups would have received more influenza immunisations and therefore have greater interference of pre-existing antibody with LAIV. Indeed, this is in the youngest stratum (17-19 years of age) that the highest ILI incidence rate ratio FLUENZ vs. TIV (i.e. inferiority of LAIV to TIV) is the most marked.

  Overall, the authors conclude that these findings really point out that "the interaction between LAIV and the immune system is multifaceted and requires more detailed investigations of the innate and adaptive immune responses to influenza immunizations." It is noteworthy that although the interpretation of the authors is cautious, the applicant uses this unexpected finding to make an optimistic assumption of an increase of LAIV efficacy over increasing age.

- **Ohmit SE et al.** (Prevention of antigenically drifted influenza by inactivated and live attenuated vaccines. N Engl J Med. 2006) have explored the efficacy of LAIV and TIV vaccines in adult subjects (18-46 years of age) over a seasonal period (2004-2005). Unlike for the TIV vaccine, no statistically different efficacy over the placebo arm is observed with LAIV. According to the authors, the difference in efficacy between the two vaccines appeared to be related mainly to a reduced protection against type B viruses induced by the live attenuated vaccine.
- **Wang Z. et al.** (Live attenuated or inactivated influenza vaccines and medical encounters for respiratory illnesses among US military personnel. JAMA 2009): data are available on three seasonal vaccinations in a population of military personnel (highly immunized). Although a statistical superiority over non immunisation is consistently shown for the TIV over the three seasonal periods, this is only shown for one seasonal period for the LAIV vaccine. The comparison of TIV and LAIV over the three seasons yields a 20 to 40% difference on the vaccine effect (primary diagnosis consistent with pneumonia or influenza).

  The authors concluded that in a highly immunized adult population, TIV may be more effective than LAIV for the prevention of pneumonia and influenza related morbidity.
- **Monto et al** (NEJM, September 2009) describes the results of a randomized, double blind, placebo-controlled trial of LAIV versus TIV in 1952 healthy subjects from 18 to 49 years of age. The primary endpoint was a case of symptomatic illness that was confirmed by cell culture or PCR. As illustrated in the table below, there was a 50% relative reduction in laboratory-confirmed influenza among subjects who received inactivated vaccine as compared with those given live attenuated vaccine.

Table 2. Estimated Absolute and Relative Efficacies of the Trivalent Inactivated and Live Attenuated Influenza Vaccines.*

Confirmation of Symptomatic Influenza†	Cumulative Incidence of Influenza			Relative Risk (95% CI)			Percent Relative Reduction (95% CI)‡		
	TIV (N=813)	LAIV (N=814)	Placebo (N=325)	TIV vs. Placebo	LAIV vs. Placebo	TIV vs. LAIV	Absolute Efficacy, TIV vs. Placebo	Absolute Efficacy, LAIV vs. Placebo	Relative Efficacy, TIV vs. LAIV
	no. of participants (%)								
Positive culture	21 (2.6)	38 (4.7)	31 (9.5)	0.27 (0.15–0.49)	0.49 (0.30–0.81)	0.55 (0.31–0.97)	73 (51–85)	51 (19–70)	45 (3–69)
Positive PCR	28 (3.4)	56 (6.9)	35 (10.8)	0.32 (0.19–0.54)	0.64 (0.41–1.00)	0.50 (0.31–0.80)	68 (46–81)	36 (0–59)	50 (20–69)
Positive culture, positive PCR, or both	28 (3.4)	56 (6.9)	35 (10.8)	0.32 (0.19–0.54)	0.64 (0.41–1.00)	0.50 (0.31–0.80)	68 (46–81)	36 (0–59)	50 (20–69)

* The study population included all 1952 enrolled participants who were randomly assigned to a vaccine or a placebo group and who actually received vaccine or placebo. The trivalent inactivated influenza vaccine (TIV) used was Fluzone (Sanofi Pasteur), and the trivalent live attenuated influenza vaccine (LAIV) used was FluMist (MedImmune). The placebo was physiologic saline administered as an intramuscular injection or as an intranasal spray. Exact 95% confidence intervals (CI) were calculated.

† Case-eligible episodes of symptomatic influenza-like illness were confirmed by culture, real-time polymerase-chain-reaction (PCR) assay, or both. Confirmation by culture was defined as isolation of virus by cell culture and subsequent identification by fluorescence antibody assay.

‡ The percent relative reduction in vaccine efficacy was defined as (1 − relative risk) × 100.

2.5.3. Discussion on clinical efficacy

One of the major concerns raised by the CHMP was the inconsistency in the efficacy results across studies in adults, which is probably linked to the other major concern that the efficacy of FLUENZ could wane over time as a result of pre-existing cross-reacting anti-influenza immunity.

It was observed that unlike in children where the efficacy is consistently shown over placebo, as well as superiority over the TIV vaccine, the adult data did indicate clearly some degree of efficacy over placebo but unexplained inconsistencies were observed between and within studies which precluded

any firm conclusion to be drawn on the benefit of this vaccine in the adult population. Along these lines, some published studies (see the section on 'Published studies') also suggested higher efficacy over placebo for TIV compared to LAIV. These elements are consistent with the *a priori* biological concern that pre-existing immunisation could alter the efficacy of a LAIV vaccine. Indeed, in contrast to trivalent inactivated vaccine, LAIV vaccines induce an immune response through viral replication. Therefore, pre-existing immunity may negatively affect the response to LAIV through the activity of existing neutralising antibodies on the vaccine's virus replication itself.

In light of this concern and even though in children this vaccine could offer better protection against flu as compared to TIV based on applicant's sponsored and published studies, the fact that in children the sustainability of the vaccine efficacy could only be judged over two seasonal periods in studies versus placebo (AV006, D153-P501, D153-P502, D153-P504), was further discussed by the VWP.

The VWP considered that no evidence was available on frequency and years of exposure needed in order to establish baseline immunity which, even if unspecific to surface antigens, will scavenge live attenuated influenza vaccine viruses before a specific immune response to seasonal HA and NA could effectively be mounted. Finally the VWP concluded that the data available did not indicate that this question might be as relevant for a paediatric and adolescent population as it might become for an adult and elderly population.

With regard to the adults' results, the Applicant was asked to provide reassurance on the maintenance of efficacy over time, especially in subjects previously vaccinated with FLUENZ, and to explain the variability observed in the adult population. Post-hoc analyses with different age strata were provided and the interaction Vaccine x Age tested (see Ancillary analysis section).

Based on results from study AV009 in adults 18 to <65 years of age, it was noted that overall for subjects <50 years of age the point estimates of the difference over the placebo arm are always in favour of superiority of LAIV whatever the endpoint. However, for subjects >50 years of age, as compared to placebo, an efficacy higher than placebo was only seen in 2 endpoints out of 5. Moreover, the effect sizes of the percent reductions as compared to the placebo arm for the two endpoints [CDC-Defined ILI (fever + cough or sore throat) and DOD-Defined ILI (cough + fever or chills)] are unexpectedly quite different: 8% and 37% respectively. Based on the items used for each endpoint and on the other efficacy results, it is likely that such difference is mainly driven by the non-specific chills. Finally when scrutinizing the results according to additional age strata (<30; 30-39; 40-49; 50-59; >60 years of age), it was observed that results generally show negative impact versus placebo for the youngest adult stratum (n=997), which was unexpected when considering the size of this age stratum and the fact that that this stratum would have lower pre-immunisation. As a consequence the concerns on the benefit of the vaccine in adult patients are not limited to the oldest age stratum.

In Study D153-P507 in adults ≥60 years of age, subgroup analyses according to 5 age strata from 60 years to >80 years of age were performed and didn't show any statistical interaction Vaccine x Age. However marked differences in the effect size in the different age strata (24% in the 65-69 years age, and 66% in the 70-74 years age) were observed.

In both studies no statistical interaction Vaccine x Age or even a trend were observed. This generated more difficulties in the interpretation due to:

- the observation that the effect size was highly variable across age strata, as above discussed for studies AV009 and D153-P507;
- the efficacy data were not analysed according to the pre-immunisation status in adults so that there might not be strict correlation between age and pre-immunisation;

- the sharp distinction observed between the paediatric and adult data. Unlike for adults, superiority was consistently shown over TIV in the paediatric population.

When considering the independent published studies which provided comparative data versus TIV, on top of the placebo controlled data provided in the dossier, they reported an overall higher efficacy of TIV versus LAIV over placebo and scientifically acknowledged the theoretical concern of the influence of pre-existing immunity on LAIV efficacy (Ohmit et al, 2006; Wang et al, 2009). Most importantly inconsistencies in the results within and among studies were reported depending on age stratum, viral strain and seasonal period, which further underlined the uncertainties regarding the mechanism of action of a LAIV vaccine. The findings from the Eick et al. study (2009) were judged by the authors unexpected and led them to conclude that "the interaction between LAIV and the immune system is multifaceted and requires more detailed investigations of the innate and adaptive immune responses to influenza immunizations" and "(investigation) of the role of annual influenza immunisations in the adult population on the immunogenicity and viral replication of LAIV".

Another study included in the evaluation was Ohmit SE et al. (Prevention of symptomatic seasonal influenza in 2005-2006 by inactivated and live attenuated vaccines. J Infect Dis. 2008), which was considered not relevant due to the very low attack rate as reflected by the large confidence intervals.

In conclusion, the data accumulated through independent published studies further weakened the clinical results in adults. Taken altogether these uncertainties hampered the ability of the clinical dossier to support the requested indication in adults and uncovered a gap in knowledge on the determinants of efficacy for live attenuated influenza vaccination in adults, which did not allow for clear delineation of the conditions under which the clinical benefit of FLUENZ could be optimal in the adult population.

Annual strain update

The clinical dossier to support annual update of TIV vaccines relies on the adequate demonstration of immunogenicity according to the CHMP criteria. However for this LAIV usual immunogenicity criteria are not considered relevant. This was a crucial issue which needed to be resolved before a final opinion could be given on FLUENZ. Therefore the Applicant was requested to propose a more suitable approach to validate the yearly strain update for FLUENZ. This question was addressed by the Applicant during the oral explanation at the September 2010 CHMP meeting.

The applicant has proposed to address the annual update through immunogenicity data in ferret.

However, the applicant reasoning was judged disputable as it would amount to consider that the immunogenicity criteria in ferret would better predict the clinical efficacy of this vaccine in human (despite the viral strain change through annual update) than would by themselves the immunogenicity data in human.

Moreover, it was difficult to perceive how the applicant could both claim a correlation between immunogenicity and challenge in ferret and argue against the correlation between immunogenicity and efficacy in humans without questioning the very validity of the animal model.

As suggested by the VWP, data on clinical effectiveness spanning several years of post-marketing usage (and therefore covering a series of strain changes) could be collected. It was suggested that the Applicant should update the CHMP with such data on an annual basis prior to changing the strain(s). The CHMP would review these data each year until when the CHMP may consider that it is no longer necessary. The Applicant proposed to submit pediatric clinical effectiveness data gathered from a recently completed U.S. investigator-sponsored study (1998-2010; Piedra et al) in response to this request. This study was a multi-year, community-based, nonrandomized, open-label study designed to evaluate the safety and effectiveness of FLUENZ in children. During the timeframe of the study 5 new

H1N1 strains, 7 new H3N2 strains and 9 new B strains were incorporated into the vaccine. As a result, the study provides robust data on the clinical effectiveness of FLUENZ that spans multiple years of usage and covers numerous strain changes. The CHMP agreed with this proposal as effectiveness but also safety data over time might indeed provide further reassurance on the stability of the vaccine efficacy despite the viral strain change and would be supportive of the challenge study in ferret. The applicant is asked to ensure that the timelines for the provision of these data will match the timelines for annual update (i.e. June 2011 for the 2011-2012 flu season), so that the timelines of submission and analysis of the full study protocol of the Piedra study will be adapted to the vaccine release.

In conclusion the CHMP recommended that the annual strain update should include:

- a challenge study in ferrets with any new virus strains incorporated into the vaccine;
- historical effectiveness and safety data with change in viral strains;
- active monitoring of vaccine failure in the paediatric population as part of routine pharmacovigilance practices.

These requirements might change in the future (pending VWP revision of guidelines for LAIV development).

2.5.4. Conclusions on clinical efficacy

Adult indication

The Applicant further addressed the adult indication in written responses and during the oral explanation at the September 2010 CHMP meeting. The Applicant failed to convince the CHMP about the acceptability of the adult indication. Given the biological plausibility that pre-existing immunity may negatively affect the efficacy of this intranasal live attenuated vaccine, there are theoretical grounds that adults might not be optimal candidates for this vaccine. This concern is reinforced by the sharp distinction of the efficacy data in children and in adults. Indeed, whereas in children the efficacy of this vaccine is consistently shown across studies (versus placebo and active comparator), the same does not apply to adult efficacy data. In adults, the intra (notably across age subgroup strata) and inter studies inconsistencies do not allow for clear delineation of the conditions under which the clinical benefit of FLUENZ could be adequate in adults. Considering the above, the CHMP was of the opinion that an indication of this LAIV in adults could only be considered on the basis of an additional efficacy study versus TIV with an adequate sample size enabling informative analyses of efficacy among strata of increasing age, as well as among increasing pre-vaccination level of immunization through prior exposure to influenza virus and/or vaccines.

Children indication

FLUENZ consistently performed better than placebo and than TIV; an acceptable degree of variability was observed according to studies and to geographical area specificities of viral attacks (strength, strains). The efficacy of FLUENZ in children is considered established.

2.6. Clinical safety

In several studies, Solicited adverse events (SEs) were monitored by diary cards for 10 to 14 days post any vaccination in the paediatric population and for mostly 7 days post vaccination in adults. The difference in collection periods is based on the original hypotheses that titre and duration of vaccine virus shedding after dosing would be greater in children than in adults and that SEs would be temporally related to vaccine virus shedding, as suggested by data from clinical studies. However, this

short surveillance period from any post Dose precludes any possibility to detect late adverse events detectable up to day 28 post dose (some of those late events are taken in account in the efficacy studies as Influenza-Like Illness (ILI) symptoms from the classical start D14/D15 post vaccination surveillance for efficacy data). Obviously, due to the live attenuated character of the FLUENZ vaccine, SEs and AEs mimic an influenza illness.

Other adverse events (AEs) were recorded for 14 to 42 days following each dose. Globally, all serious adverse events (SAEs) were recorded during the study period from the day of vaccination through Day 42 post last dose since it was the most common data collection period and from day of vaccination until Day 180 post last dose (that is, 180 days post Dose 1 if only 1 dose was administered or 180 days post Dose 2 if 2 doses were administered).

Reactogenicity could be evaluated after repeated vaccination in more than half of paediatric studies (two doses in the same year or one yearly single dose up to three annual revaccinations). In order to allow an optimum traceability of the cohort, each subject retained the same participant number that was used in the first year of the trial. In general, returning participants also remained in the same treatment group to which they had been randomized in the prior year.

Patient exposure

Safety data derive from over 141,000 subjects who have received the frozen (F) or refrigerated (R) liquid formulations of FLUENZ in 73 clinical and postmarketing studies conducted over more than a decade (from 1994 to 2008) in multiple regions of the world.

Of the 73 total FLUENZ studies, 57 studies contribute to FLUENZ exposure in 123,834 subjects. A total of 44,102 of these subjects received the R-FLUENZ for which authorization is being sought in subjects aged from >12 months to older, whilst the rest received the original F-FLUENZ. Among these 57 studies: 39 studies included more than 39,000 children aged 7 weeks to 17 years and, 18 studies included more than 8,500 adults aged 18 years to 97 years. Two of the 57 studies are postmarketing studies.

Additionally, more than 10 million doses of FLUENZ (mainly R-FLUENZ) have been distributed commercially in the USA from initial licensure in 2003 until the end of the 2007-2008 influenza season. The frozen formulation was replaced by the refrigerated formulation for the 2007/2008 influenza season; from that season onwards only the refrigerated formulation of FLUENZ was produced and initiated. Wyeth was the distributor of FLUENZ during the 2003/2004 season. MedImmune distributed FLUENZ during the succeeding years. The number of FLUENZ doses distributed in the USA per seasonal year from the 2003/2004 season through 2007/2008 is provided in table 22.

Table 22. FLUENZ distribution in the USA

Influenza Seasonal Year	FluMist Formulation		Total Doses/Seasonal Year
	Frozen	Refrigerated	
2003/2004	500,000 [a]	0	500,000 [a]
2004/2005	2,123,956	0	2,123,956
2005/2006	1,735,360	0	1,735,360
2006/2007	2,600,180	0	2,600,180
2007/2008	0	3,864,240	3,864,240
Total Number of Doses	6,959,496	3,864,240	10,823,736

[a] Approximate number of doses; FluMist was distributed by Wyeth during the 2003/2004 influenza season.

Adverse events

Overall, based on the available data from individual studies (stratified analysis by age groups:<6 to <36 months, >2 years or >36 months of age to 6 years, >6 to 9 years, >9 to 18 years, >18 to 49 years and >60 years of age) and pooled studies (1-17 years of age, >18 years of age), R- FLUENZ vaccine was considered safe and well tolerated with a safety profile similar to that of the comparator treatment group (TIV and placebo).

Individual studies

Based on individual studies, the use of antipyretics (for the children group) was more frequent in the FLUENZ group compared to the control group (TIV or placebo) with rate differences in some studies ≥ 2.0 percentage points. Reactogenicity of FLUENZ was generally higher after the first dose of R-FLUENZ than after the second dose or after the yearly revaccination (up to 4 years). In fact in all age groups for R-FLUENZ, TIV, placebo groups, the incidence of reactions showed a tendency to decrease post the second dose and the yearly revaccination (the rate of events being then in between those of post Dose 1 and post Dose 2).

Pooled analysis in children

In subjects <18 years of age, among SEs runny/stuff nose was more commonly observed in the R-FLUENZ group than in either the TIV or placebo groups. Other SEs with rate differences ≥ 0.9 percentage points (FLUENZ>comparator) in both TIV and placebo controlled studies included: decreased appetite, irritability, headache and fever ≥ 38.0°C. High fever (≥ 39.5°C) was no more common in FLUENZ subjects than in subjects who received placebo or TIV.

The most important AEs by rate difference were generally similar to events defined as SEs (eg, rhinorrhoea and pyrexia). The most frequently reported AE that occurred at a higher rate in FLUENZ than TIV or placebo subjects was pyrexia. The incidence of rhinorrhoea and upper respiratory tract infection were also usually higher in the FLUENZ treatment group than in the comparator treatment group. The use of antipyretics (for the children group) was more frequent in the FLUENZ group compared to the control group (TIV or placebo) with rate differences ≥ 2.0 percentage points.

Pooled analysis in adults

In adults, among SEs, runny/stuffy nose was also more commonly observed in the R-FLUENZ group than in either the TIV or placebo groups. Other SEs with rate differences ≥ 2 percentage points (FLUENZ > comparator) in both TIV and placebo controlled studies included: sore throat, cough, headache and malaise. AEs that occurred in at least 1% of FLUENZ subjects with incidence greater in FLUENZ than in comparator in TIV and/or placebo controlled studies were rhinorrhoea, myalgia, pharyngolaryngeal pain, cough and nasopharyngitis.

Pooled analysis by age strata

Additional safety data were provided from pooled safety analysis performed in several age groups of children (12-23 months, 24-35 months, 36-59 months, 5-17 years of age) and elderly (60-64 years, 65-74 years, ≥75 years of age). The conclusions across strata for both children and elderly populations were similar to the above reported analysis: runny/stuffy nose was the primary factor contributing to the overall occurrence and distribution of SEs for FLUENZ subjects in both TIV and placebo controlled studies regardless of the dose.

Solicited Events in Subjects 12 Months to 17 Years of Age Occurring With a Rate Difference (FluMist Minus Comparator) Greater Than or Equal to 1.0 Percentage Point by Age Group - Days 0-10 Post Dose 1 in Year 1

Age Group Solicited Event	TIV Controlled Studies			Placebo Controlled Studies		
	FluMist n/N (%)	TIV n/N (%)	Rate Diff [a]	FluMist n/N (%)	Placebo n/N (%)	Rate Diff [b]
Subjects 12 to 23 Months of Age						
Runny/stuffy nose	1,003/1,516 (66.2)	775/1,465 (52.9)	13.3	2,744/3,892 (70.5)	1,417/2,371 (59.8)	10.7
Subjects 24 to 35 Months of Age						
Runny/stuffy nose	951/1,619 (58.7)	757/1,606 (47.1)	11.6	2,034/3,113 (65.3)	1,119/1,866 (60.0)	5.4
Subjects 36 to 59 Months of Age						
Runny/stuffy nose	589/1,209 (48.7)	505/1,219 (41.4)	7.3	9/11 (81.8)	3/4 (75.0)	6.8
Subjects 5 to 17 Years of Age						
Runny/stuffy nose	787/1,273 (61.8)	585/1,283 (45.6)	16.2	22/117 (18.8)	12/122 (9.8)	9.0

Solicited Events in Subjects 12 Months to 17 Years of Age Occurring With a Rate Difference (FluMist Minus Comparator) Greater Than or Equal to 1.0 Percentage Point by Age Group - Days 0-10 Post Dose 2 in Year 1

Age Group Solicited Event	TIV Controlled Studies			Placebo Controlled Studies		
	FluMist n/N (%)	TIV n/N (%)	Rate Diff [a]	FluMist n/N (%)	Placebo n/N (%)	Rate Diff [b]
Subjects 12 to 23 Months of Age						
Runny/stuffy nose	576/1,138 (50.6)	476/1,089 (43.7)	6.9	1,877/3,177 (59.2)	1,189/2,188 (54.3)	4.9
Subjects 24 to 35 Months of Age						
Runny/stuffy nose	478/1,040 (46.0)	393/1,038 (37.9)	8.1	1,318/2,496 (52.8)	858/1,696 (50.6)	2.2
Subjects 36 to 59 Months of Age						
Runny/stuffy nose	348/986 (35.3)	351/1,016 (34.5)	0.7	4/7 (57.1)	1/3 (33.3)	23.8

Solicited Events in Subjects Greater Than or Equal to 60 Years of Age Occurring With a Rate Difference (FluMist Minus Comparator) Greater Than or Equal to 1 Percentage Point - Days 0-6 Post Dose

Age Group Solicited Event	TIV Controlled Studies			Placebo Controlled Studies		
	FluMist n/N (%)	TIV n/N (%)	Rate Diff [a]	FluMist n/N (%)	Placebo n/N (%)	Rate Diff [b]
Adults greater than or equal to 60 to 64 years of age						
Runny/stuffy nose	167/513 (32.6)	110/498 (22.1)	10.5	225/544 (41/4)	101/518 (19.5)	21.9
Adults 65 to 74 years of age						
Runny/stuffy nose	235/715 (32.9)	148/720 (20.6)	12.3	314/764 (41.1)	160/806 (19.9)	21.2
Adults ≥ 75 years of age						
Runny/stuffy nose	96/306 (31.4)	55/314 (17.5)	13.9	106/353 (30.0)	59/344 (17.2)	12.9

Following administration of the first and second dose of FLUENZ, AEs were reported more frequently by FLUENZ subjects than by comparator subjects.

Adverse Events in Subjects 12 Months to 17 Years of Age Occurring in at Least 1% of FluMist Subjects and With a Rate Difference (FluMist Minus Comparator) of at Least 0.5 Percentage Point by Age Group - Days 0-10 Post Dose 1 in Year 1

Age Group MedDRA v 8.0 System Organ Class Preferred Term	TIV Controlled Studies			Placebo Controlled Studies		
	FluMist n (%)	TIV n (%)	Rate Diff [a]	FluMist n (%)	Placebo n (%)	Rate Diff [b]
Subjects 12 to 23 Months of Age	(N = 1,555)	(N = 1,495)	--	(N = 3,988)	(N = 2,417)	--
Subjects Reporting ≥ 1 Adverse Event	409 (26.3)	340 (22.7)	3.6	1,543 (38.7)	837 (34.6)	4.1
Subjects 24 to 35 Months of Age	(N = 1,650)	(N = 1,647)	--	(N = 3,149)	(N = 1,900)	--
Subjects Reporting ≥ 1 Adverse Event	335 (20.3)	312 (18.9)	1.4	952 (30.2)	547 (28.8)	1.4
Subjects 36 to 59 Months of Age	(N = 1,219)	(N = 1,247)	--	(N = 11)	(N = 4)	--
Subjects Reporting ≥ 1 Adverse Event	226 (18.5)	216 (17.3)	1.2	4 (36.4)	1 (25.0)	11.4

Subjects 5 to 17 Years of Age	(N = 1,278)	(N = 1,288)	--	(N = 118)	(N = 122)	--
Subjects Reporting ≥ 1 Adverse Event	299 (23.4)	227 (17.6)	5.8	19 (16.1)	11 (9.0)	7.1

Adverse Events in Subjects 12 Months to 17 Years of Age Occurring in at Least 1% of FluMist Subjects and With a Rate Difference (FluMist Minus Comparator) of at Least 0.5 Percentage Point by Age Group - Days 0-10 Post Dose 2 in Year 1

Age Group MedDRA v 8.0 System Organ Class Preferred Term	TIV Controlled Studies			Placebo Controlled Studies		
	FluMist n (%)	TIV n (%)	Rate Diff [a]	FluMist n (%)	Placebo n (%)	Rate Diff [b]
Subjects 12 to 23 Months of Age	(N = 1,169)	(N = 1,131)	--	(N = 3,201)	(N = 2,205)	--
Subjects Reporting ≥ 1 Adverse Event	249 (21.3)	195 (17.2)	4.1	1,040 (32.5)	669 (30.3)	2.1
Subjects 24 to 35 Months of Age	(N = 1,073)	(N = 1,068)	--	(N = 2,526)	(N = 1,731)	--
Subjects Reporting ≥ 1 Adverse Event	169 (15.8)	174 (16.3)	-0.5	615 (24.3)	460 (26.6)	-2.2
Subjects 36 to 59 Months of Age	(N = 997)	(N = 1,034)	--	(N = 7)	(N = 3)	--
Subjects Reporting ≥ 1 Adverse Event	158 (15.8)	155 (15.0)	0.9	1 (14.3)	0 (0.0)	14.3
Subjects 5 to 17 Years of Age	(N = 160)	(N = 168)	--	--	--	--
Subjects Reporting ≥ 1 Adverse Event	32 (20.0)	34 (20.2)	-0.2	NA	NA	NA

Adverse Events in Subjects Greater Than or Equal to 60 Years of Age Occurring in at Least 1% of FluMist Subjects and With a Rate Difference (FluMist Minus Comparator) Greater Than or Equal to 0.5 Percentage Point - Days 0-6 Post Dose

Age Group MedDRA v 8.0 System Organ Class Preferred Term	TIV Controlled Studies			Placebo Controlled Studies		
	FluMist n (%)	TIV n (%)	Rate Diff [a]	FluMist n (%)	Placebo n (%)	Rate Diff [b]
Subjects 60 to 64 years of age	(N = 513)	(N = 479)	--	(N = 545)	(N = 521)	--
Total Number of Subjects Reporting ≥ 1 AE	99 (19.3)	110 (22.0)	-2.7	38 (7.0)	36 (6.9)	0.1
Subjects 65 to 74 years of age	(N = 716)	(N = 720)	--	(N = 767)	(N = 808)	--
Total Number of Subjects Reporting ≥ 1 AE	151 (21.1)	118 (16.4)	4.7	73 (9.5)	44 (5.4)	4.1
Subjects ≥ 75 years of age	(N = 308)	(N = 314)	--	(N = 354)	(N = 344)	--
Total Number of Subjects Reporting ≥ 1 AE	50 (16.2)	40 (12.7)	3.5	23 (6.5)	15 (4.4)	2.1

In subjects aged 12 to 23 months:

- Low-grade fever occurred more frequently in FLUENZ subjects vs. comparator subjects post Dose 1.

Age Group Solicited Event	TIV Controlled Studies			Placebo Controlled Studies		
	FluMist n/N (%)	TIV n/N (%)	Rate Diff [a]	FluMist n/N (%)	Placebo n/N (%)	Rate Diff [b]
Subjects 12 to 23 Months of Age						
Fever ≥ 38.0°C	229/1,496 (15.3)	198/1,447 (13.7)	1.6	692/3,884 (17.8)	361/2,363 (15.3)	2.5

- Post Dose 1, the AE that was consistently elevated in FLUENZ subjects compared to placebo subjects was pyrexia.

In subjects aged 24 to 35 months:

- Post Dose 1, the AE that was consistently elevated in FLUENZ subjects compared to placebo subjects was pyrexia.

In subjects aged 36 to 59 months:

- Low-grade fever occurred more commonly in FLUENZ subjects than TIV subjects post Dose 1.
- Post Dose 2, irritability was the SE with the greatest rate difference between FLUENZ subjects than TIV subjects.

- In Year 2, the overall incidence of any SE was comparable between the FLUENZ and placebo groups, and decreased appetite was the SE with the greatest rate difference.
- The AEs with the greatest rate difference between FLUENZ subjects and TIV subjects were otitis media acute and rhinorrhoea post Dose 1.
- In Year 2, the AE with the highest rate difference was pyrexia.

In subjects aged 60 to 64 years:

- During Days 0-6 post dose, AEs were reported at a comparable or lower rate by FLUENZ subjects than by comparator subjects.
- The AEs that were consistently increased in FLUENZ subjects compared to placebo subjects were rhinorrhoea and myalgia.

Age Group MedDRA v 8.0 System Organ Class Preferred Term	TIV Controlled Studies			Placebo Controlled Studies		
	FluMist n (%)	TIV n (%)	Rate Diff [a]	FluMist n (%)	Placebo n (%)	Rate Diff [b]
Subjects 60 to 64 years of age	(N = 513)	(N = 499)	--	(N = 545)	(N = 521)	--
Total Number of Subjects Reporting ≥ 1 AE	99 (19.3)	110 (22.0)	-2.7	38 (7.0)	36 (6.9)	0.1
Rhinorrhoea	14 (2.7)	6 (1.2)	1.5	7 (1.3)	3 (0.6)	0.7
Myalgia	7 (1.4)	5 (1.0)	0.4	8 (1.5)	1 (0.2)	1.3

In subjects aged from 65 to 74 years:

- Following receipt of FLUENZ, AEs were reported more frequently by FLUENZ subjects than by comparator subjects.
- Headache and rhinorrhoea were the most frequently reported AEs and occurred at a higher rate in FLUENZ recipients than either TIV or placebo recipients.

Age Group MedDRA v 8.0 System Organ Class Preferred Term	TIV Controlled Studies			Placebo Controlled Studies		
	FluMist n (%)	TIV n (%)	Rate Diff [a]	FluMist n (%)	Placebo n (%)	Rate Diff [b]
Subjects 65 to 74 years of age	(N = 716)	(N = 720)	--	(N = 767)	(N = 808)	--
Total Number of Subjects Reporting ≥ 1 AE	151 (21.1)	118 (16.4)	4.7	73 (9.5)	44 (5.4)	4.1
Headache	60 (8.4)	48 (6.7)	1.7	21 (2.7)	13 (1.6)	1.1
Rhinorrhoea	14 (2.0)	4 (0.6)	1.4	13 (1.7)	7 (0.9)	0.8

In subjects aged ≥ 75 years:

- During Days 0-6 post dose, AEs were reported at a higher rate by FLUENZ subjects than by comparator subjects.

Age Group MedDRA v 8.0 System Organ Class Preferred Term	TIV Controlled Studies			Placebo Controlled Studies		
	FluMist n (%)	TIV n (%)	Rate Diff [a]	FluMist n (%)	Placebo n (%)	Rate Diff [b]
Subjects ≥ 75 years of age	(N = 308)	(N = 314)	--	(N = 354)	(N = 344)	--
Total Number of Subjects Reporting ≥ 1 AE	50 (16.2)	40 (12.7)	3.5	23 (6.5)	15 (4.4)	2.1

Viral shedding and transmission

Because FLUENZ contains live, attenuated influenza viruses that induce immune protection by the viruses replicating in cells lining the nasopharynx, isolation of vaccine virus from nasal secretions is expected to occur for some period after vaccination. A total of 13 studies contributed with data on

virus isolation from nasal wash or nasal swab specimens (i.e., shedding). Eight of these studies included only pediatric subjects (ages 6 months to 17 years), 3 included only adult subjects (ages ≥ 18 years), and 2 included both pediatric and adult subjects. Parameters that were typically evaluated, based on culture of nasal specimens, included incidence and number of days of shedding, genotypic characterization of influenza virus detected to distinguish vaccine-type from wild-type virus and phenotypic characterization of the influenza virus detected to confirm maintenance of the ca, ts, and att properties. In some studies, the quantity of shed virus was also assessed.

The most comprehensive analyses of shedding are provided in **Study D145-P500** for children 8 to < 36 months of age, **Study MI-CP129** for children 6 to < 60 months of age and **Study FM026** for children and adults 5 to 49 years of age. In general, the proportion of subjects who shed vaccine virus, the duration of shedding, and the amount of virus shed declined with increasing age of the subjects. Shedding was detected in up to 80% of children < 36 months of age after dosing. In studies of older age cohorts, the incidence of shedding was 44% in children 5 to 8 years of age, 27% in children 9 to 17 years of age, and 17% in adults 18 to 48 years of age. Younger children also appeared to shed virus for longer periods than did older children or adults. In both adults and children, shedding began early (Day 1-2) after vaccination, and peak incidence was generally detected on Day 2. Shedding typically ceased within 12 days of dosing, even among infants and children, but in some cases was detected as late as Day 21.

Moreover study D145-P500 of children 8 to < 36 months of age who attended day care showed that the shed isolates were genetically stable and exhibited no evidence of reversion to wild-type, i.e., they maintained the ca, ts, and att phenotypic properties (D145-P500 CSR; Vesikari et al, 2006; Buonagurio et al, 2006).

In Study FM026, a higher proportion of subjects who were baseline seronegative/serosusceptible to a strain were found to shed vaccine virus (up to 30%) compared to those who were baseline seropositive (up to 7%). In this study, peak titres of shed vaccine virus were > 100-fold lower than the dose administered (10^7 $TCID_{50}$ per strain). Mean titres of shed virus were generally lower in the oldest age group relative to the youngest. Influenza virus titres were below the detection limit of the assay after Day 6 for subjects 9 to 17 years of age and 18 to 49 years of age and after Day 11 for subjects 5 to 8 years of age.

In studies involving HIV-infected subjects (DMID 99-012, PACTG 1057, and DMID 98-005), the rates of shedding were similar to those described in non-HIV-infected subjects, and there was no evidence of prolonged vaccine virus shedding.

Study D145-P500 (prospective, randomized, double-blind, placebo controlled study) was performed in a child day care setting to assess the risk of transmission of vaccine viruses from a vaccinated individual to a non-vaccinated contact. A day care setting was selected to optimize the chance for transmission to occur. A total of 197 subjects 8 to < 36 months of age were randomized to receive 1 dose of FLUENZ (N = 98) or placebo (N = 99). Virus shedding was evaluated for 21 days by culture of nasal swab specimens. At least 1 vaccine strain was isolated from 80% of FLUENZ recipients. Ten influenza isolates (9 influenza A, 1 influenza B) were cultured from a total of 7 placebo subjects, but only the Type B isolate was confirmed to be vaccine virus. This isolate retained the ca, ts, and att phenotypes of the vaccine strain and had the same genetic sequence when compared to a Type B virus cultured from a vaccine recipient within the same playgroup. Four of the 9 influenza Type A isolates were confirmed as wild-type circulating H3N2 virus. The placebo subject from whom Type B vaccine virus was isolated had a similar spectrum of SEs as that described among the subjects who received FLUENZ. Statistical modelling estimated the probability of transmission to a subject in a contact group containing a single subject vaccinated with FLUENZ to be 0.58% (upper limit of 95% CI, 1.72)

(Vesikari et al, Jul 2006). For subjects in contact with 2, 3, 4, or 5 subjects vaccinated with FLUENZ, the probability of transmission was estimated to be 1.16%, 1.73%, 2.30%, or 2.87%, respectively.

Serious adverse event/deaths/other significant events

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 R- or F-FLUENZ recipients) was considered to be related to FLUENZ.

Safety in special populations

Immunocompromised individuals

Since the vaccine contains live influenza viruses, it was extremely important to have a clear view of its safety implications in subjects immunodeficient at the time of vaccination. Children with severe immunosuppression were excluded from all FLUENZ clinical studies. Studies **DMID 99-012, PACTG 1057**, and **MI-CP114** did include pediatric subjects with immunosuppressive conditions but each of these studies included eligibility criteria designed to exclude children with severe immunosuppression. Overall very limited safety data were provided in immunodepressed children (HIV infected or not).

HIV infected individuals

FLUENZ safety data in children 1 to 7 years of age with HIV infection are available from **Study DMID 99-012**. In brief, the safety of FLUENZ was compared in HIV-infected subjects who had either asymptomatic or mildly symptomatic HIV disease (CDC class N1-2 or A1-2) with subjects of similar age who were not infected with HIV. Twenty-five HIV-negative subjects and 24 HIV-infected subjects were enrolled. The study established the equivalence of post-immunization fever incidence among HIV-infected and non-HIV-infected subjects, based on the pre-specified criterion of an upper bound for the 95% CI no greater than 22.5%. Other SEs and AEs were no more common after FLUENZ vaccination in HIV-infected subjects than in HIV-negative subjects. Immunization of HIV-infected subjects did not lead to clinically significant changes in CD4 counts, HIV viral load, or routine blood tests. In summary, FLUENZ was generally safe and well tolerated when given to subjects 1 to 7 years of age with asymptomatic or mildly symptomatic HIV infection.

FLUENZ safety data in children 5 to 17 years of age with HIV infection are available from **Study PACTG 1057** (Levin et al, 2008). This study, conducted by the Pediatric AIDS Clinical Trials Group (PACTG), evaluated the safety of FLUENZ compared with TIV in HIV-infected subjects, ≥ 5 to < 18 years of age. With the exception of nasopharyngeal symptoms, which were more frequent after FLUENZ vaccination, rates of all other AEs, including pulmonary signs and symptoms, were similar between the 2 groups and did not vary significantly with the immunological status of the subjects. Overall, the safety profiles were similar between the FLUENZ and TIV treatment groups. FLUENZ had no effect on markers of HIV progression.

Non-HIV related immunodeficient individuals

Sufficient clinical data has not been obtained to permit an analysis of FLUENZ safety in this specific population. A post-marketing study, **MI-MA175**, intended to assess the effectiveness of the Applicant's risk minimization plan for use of FLUENZ among children < 5 years of age, identified a total of 12 children 24 to 59 months of age with possible underlying immunosuppression. No safety events related to the use of the vaccine were reported.

Except for limited data pertaining to children with mild to moderate immunodeficiency resulting from treatment of cancer, sufficient clinical data has not been obtained to permit an analysis of FLUENZ safety in this specific population. The limited data available was from **Study MI-CP114**. This study was a Phase 1, randomized, double-blind, placebo controlled study in subjects 5 to 17 years of age who had cancer and who were actively receiving chemotherapy and/or radiation therapy. The primary objective was to describe the safety of FLUENZ compared with placebo in mild to moderately immunocompromised subjects with cancer as indicated by measures of SEs and AEs occurring during the 42-day post-dosing period. Overall, FLUENZ was well tolerated in this population, and its safety profile was comparable to that seen in the general population.

Children below 24 months of age

A significant increase in wheezing events was observed in subjects younger than 24 months of age (**Study MI-CP111** including children aged from 6 to 59 months) but this risk seemed to be confined to those with a pre-existing history of wheezing or asthma. No such increase in rates of wheezing was seen in subjects ≥ 24 months of age in this study.

Study MI-CP111 Safety Events, Days 0-42, in Subjects 6 to 23 Months of Age

Endpoint	FluMist n/N (%)	TIV n/N (%)	Rate Difference
MSW			
Overall	117/1,992 (5.9%)	75/1,975 (3.8%)	2.1
History of Any Wheeze/Asthma	51/332 (15.4%)	28/295 (9.5%)	5.9
No History of Any Wheeze/Asthma	66/1,660 (4.0%)	47/1,680 (2.8%)	1.2
RE/AE Wheeze			
Overall	198/1,992 (9.9%)	141/1,975 (7.1%)	2.8
History of Any Wheeze/Asthma	78/332 (23.5%)	42/295 (14.2%)	9.3
No History of Any Wheeze/Asthma	120/1,660 (7.2%)	99/1,680 (5.9%)	1.3
MSW or RE/AE Wheeze			
Overall	216/1,992 (10.8%)	158/1,975 (8.0%)	2.8
History of Any Wheeze/Asthma	91/332 (27.4%)	50/295 (16.9%)	10.5
No History of Any Wheeze/Asthma	125/1,660 (7.5%)	108/1,680 (6.4%)	1.1
Medically attended RE/AE Wheeze			
Overall	167/1,992 (8.4%)	106/1,975 (5.4%)	3.0
History of Any Wheeze/Asthma	66/332 (19.9%)	30/295 (10.2%)	9.7
No History of Any Wheeze/Asthma	101/1,660 (6.1%)	76/1,680 (4.5%)	1.6

AE: Adverse event. An AE was any adverse change from the study subject's baseline condition that occurred following the first administration of study vaccine that was not captured as an RE.
MSW: Medically Significant Wheezing. Medically significant wheezing was defined as the presence of wheezing on physical examination and accompanied by at least 1 of the following:
- Signs of respiratory distress: tachypnea, retractions, or dyspnea
- Hypoxemia (O2 saturation < 95%)
- New prescription for daily bronchodilator therapy (not on an "as needed" basis)

RE: Reactogenicity Event. Reactogenicity events were a subset of solicited events that included fever, runny/stuffy nose, sore throat, cough, wheeze, vomiting, headache, muscle aches, chills, decreased activity, irritability, abdominal pain, decreased appetite, injection site pain, injection site swelling, injection site redness.
RE/AE: RE/AE wheezing refers to those events that were collected as solicited REs (reported by parent/guardian on safety worksheets) and/or as AEs on case report forms. Wheezing synonyms included wheezing, bronchospasm, asthma, bronchiolitis.

Study MI-CP111 Safety Events, Days 0-180, in Subjects 6 to 23 Months of Age

Endpoint	FluMist n/N (%)	TIV n/N (%)	Rate Difference

MSW			
Overall	223/1,992 (11.2%)	185/1,975 (9.4%)	1.8
History of Any Wheeze/Asthma	82/332 (24.7%)	58/295 (19.7%)	5.0
No History of Any Wheeze/Asthma	141/1,660 (8.5%)	127/1,680 (7.6%)	0.9
RE/AE Wheeze			
Overall	338/1,992 (17.0%)	305/1,975 (15.4%)	1.5
History of Any Wheeze/Asthma	116/332 (34.9%)	78/295 (26.4%)	8.5
No History of Any Wheeze/Asthma	222/1,660 (13.4%)	227/1,680 (13.5%)	-0.1
MSW or RE/AE Wheeze			
Overall	367/1,992 (18.4%)	334/1,975 (16.9%)	1.5
History of Any Wheeze/Asthma	127/332 (38.3%)	89/295 (30.2%)	8.1
No History of Any Wheeze/Asthma	240/1,660 (14.5%)	245/1,680 (14.6%)	-0.1
Medically attended RE/AE Wheeze			
Overall	296/1,992 (14.9%)	256/1,975 (13.0%)	1.9
History of Any Wheeze/Asthma	102/332 (30.7%)	66/295 (22.4%)	8.4
No History of Any Wheeze/Asthma	194/1,660 (11.7%)	190/1,680 (11.3%)	0.4

Moreover rates of hospitalization for any cause (mostly respiratory and gastrointestinal diagnoses) among children 6 to 11 months of age were 6.1% in FLUENZ recipients vs. 3.6% in TIV recipients (p = 0.002) from day of randomization through 180 days after the last vaccine dose. The aetiology of this phenomenon is unclear.

Individuals with systemic illnesses

Due to the small number of children with pre-existing systemic illnesses that were analysed, no conclusion could be drawn about the safety of FLUENZ in this population.

Individuals with asthma or wheezing

6 months to 2 years of age

Study MI-CP111 provides information about the safety of FLUENZ in subjects 6 to 23 months of age with and without a pre-existing history of wheezing or asthma. The Safety Population included subjects who received both study products (intramuscular injection and intranasal spray) and had any safety follow-up. Subjects in the Safety Population were analyzed in the treatment group according to the active study vaccine received at Dose 1 (i.e. as-treated not as-randomized). In brief, in Study MI-CP111, in children 6 to 23 months of age with a prior history of wheezing or asthma, receipt of FLUENZ was associated with increases in risk of wheezing over the first 42 days after immunization compared with receipt of TIV. Compared to TIV, in this population, receipt of FLUENZ was also associated with a greater risk of subsequent medically-attended lower respiratory tract infection (occurred in 27.4% of FLUENZ recipients vs. 18.0% of TIV recipients), and REs (occurred in 96.4% of FLUENZ recipients vs. 91.9% of TIV recipients). REs are defined as Reactogenicity events, a subset of solicited events that included fever, runny/stuffy nose, sore throat, cough, wheeze, vomiting, headache, muscle aches, chills, decreased activity, irritability, abdominal pain, decreased appetite, injection site pain, injection site swelling, injection site redness.

2 to 5 years of age

In brief, in Study MI-CP111, in children 24 to 59 months of age with a prior history of wheezing or asthma, receipt of FLUENZ was not associated with significant increases in subsequent wheezing, hospitalizations, SAEs, or REs over 180 days after immunization, compared with receipt of TIV.

An integrated summary of 20 FLUENZ studies (AV006 Year 1, AV006 Year 2, AV009, AV019, D145-P500, DMID 98-005, AV001, AV002, AV002-2, AV003, AV004, AV005, AV007, AV008, AV010, AV012

Years 1 and 2, AR001, AV011, AV014, and AV015) identified 1,302 subjects 25 to ≤ 71 months of age with a history of pre-existing asthma or wheezing. Of these, 997 received FLUENZ and 305 received placebo. Within this total group of 1,302 subjects, 3 SAEs occurred that were identified as asthma/wheezing reactive airways/shortness of breath events.

In Study AV019, 10 asthma/RAD/wheezing/shortness of breath events occurred in 598 FLUENZ recipients 24 to 71 months of age, and 3 events occurred in 295 placebo recipients in this age group.

Post-marketing Study MI-MA175 evaluated children who did not meet indications to receive FLUENZ, but who had received this vaccine. This study identified 325 children who met the criteria for asthma, who had received FLUENZ, and 308 children who met the criterion for wheezing, but not asthma, who had received FLUENZ. In addition, 12,843 children with asthma and 4,880 with wheezing who had received TIV were identified. Among the combined total (N = 633) of these FLUENZ-vaccinated children with asthma or wheezing, a list of all primary discharge diagnoses associated with any emergency room (ER) visit or hospitalization within 42 days after a FLUENZ vaccination was compiled. A total of 27 separate ER visits (2 of which subsequently included hospitalization) and 4 additional hospitalizations occurred for a total of 31 events involving ER visit or hospitalization. The event rate was 49.0 (95% CI: 33.5, 68.8) per 1,000 vaccinations. Of the combined total (N = 17,723) of children with asthma or wheezing who received TIV, there were 1,489 events (diagnoses associated with a hospitalization or ER visit) after the first TIV vaccination, for an event rate of 84.0 (95% CI: 80.0, 88.2) per 1,000 vaccinations. Of FLUENZ vaccinated children, 7/633 (1.1%) visited the ER or were hospitalized for a lower respiratory condition known to complicate asthma or wheezing within 42 days after vaccination, versus 2.1% of TIV-vaccinated children. Within the limits imposed by its retrospective character and the process for subject identification, this study did not identify any greater risk of AEs in general, or of respiratory AEs in particular, after immunizing children 24 to 59 months of age with asthma or wheezing with FLUENZ, as compared with TIV immunization.

5 to 17 years of age

An integrated summary of 20 FLUENZ studies (AV006 Year 1, AV006 Year 2, AV009, AV019, D145-P500, DMID 98-005, AV001, AV002, AV002-2, AV003, AV004, AV005, AV007, AV008, AV010, AV012 Years 1 and 2, AR001, AV011, AV014, and AV015) identified 1,988 subjects > 71 months to 17 years of age with a history of pre-existing asthma or wheezing: no SAEs occurred that were identified as asthma/wheezing reactive airways/shortness of breath events.

Studies AV010, AV012 Year 1, AV012 Year 2, AV012 Year 3, AV012 Year 4, D153-P514, and D153-P515 were reviewed to identify subjects 5 to 17 years of age with pre-existing asthma or wheezing, who developed SAEs from Days 0-42 and Days 0-180 post dose. In brief, the number of SAEs across all studies was very small and showed no significant association with FLUENZ.

Study FM005, a post-marketing study, obtained safety data on asthmatic children 5 to 18 years of age who received FLUENZ. This study separated asthmatic children into 2 age cohorts, 1028 individuals 5 to 8 years of age and 1621 individuals 9 to 17 years of age. Post vaccination event rates were collected and analyzed for Days 0 to 21 after immunization (FLUENZ rate), and separately for Days 22 to 42 after immunization (control rate). Neither asthma/reactive airway disease nor wheezing/shortness of breath occurred at rates that were significantly different in the risk period compared to the control period in any analysis.

Individuals with severe asthma or active wheezing

The applicant indicated that >4400 paediatric subjects 6 months to <18 years of age and >2200 adults with a history of respiratory illness were exposed to FLUENZ without increased risk of post-vaccination respiratory illness compared to TIV. As few data are available for severe asthma or active

wheezing patients (**study AV010** enrolled 48 children 9 to 17 years of age with moderate to severe asthma), the CHMP agreed with the addition of this risk as important missing information in the RMP and to further address it in section 4.4 of the SmPC.

Safety related to drug-drug interactions and other interactions

Concomitant administration of FLUENZ and other live viral vaccines (MMR, VAR and OPV) appears well tolerated, comparable to the rate of events observed with each of these other viral vaccines in the studies submitted by the applicant. This interaction is described in the SmPC (section 4.5).

Discontinuation due to adverse events

Overall, the study withdrawal rates due to AEs for each of the studies were very low (<1%).

Post marketing experience

More than 10 million doses of FLUENZ have been distributed commercially in the USA from initial licensure in 2003 to the end of the 2007-2008 influenza season and the safety profile has been consistent with the clinical study safety database.

A total of 462 AEs have been reported from 263 unique case reports received from postmarketing study sources. Of these AEs, 71.2% were received from the postmarketing **study FM025**, and the remaining 28.8% were received from other non-company-sponsored postmarketing studies (**FLU008-04, FLU003-03, MI-MA004,** and **FLU019-07**). The distribution of all AEs by SOC received from postmarketing study sources through the period ending 01 April 2008 showed that overall SOCs with the most commonly reported AEs were Injury, Poisoning and Procedural Complications (n = 70), Infections and Infestations (n = 64), and Gastrointestinal Disorders (n = 44).

Of the 462 AEs received, 27.3% were considered by the investigator to be related to administration of FLUENZ (59 from FM025 and 67 from non-company-sponsored postmarketing studies). The remaining AEs (72.7%) were classified as not related to FLUENZ administration (270 from FM025 and 66 from non-company-sponsored postmarketing studies). The most commonly reported AEs coded by MedDRA preferred term from postmarketing studies were drug exposure during pregnancy (n = 31), pregnancy (n = 25), mental disorder (n = 17), injury (n = 16), appendicitis (n = 10), and abdominal pain (n = 10). The majority of these events were considered by the investigators to be unrelated to FLUENZ administration.

In conclusion, no major safety concern was identified from the post-marketing data analysis. However Guillain-Barré syndrome, facial palsy and asthma (particularly in at-risk populations) should be closely monitored in the context of the risk management plan.

Review of postmarketing studies has not identified data bearing on the safety, immunogenicity or efficacy of FLUENZ in severely immunocompromised patients.

2.6.1. Discussion on clinical safety

Based on the safety data provided by the Applicant, the most common side effects observed in clinical trials in all ages are nasal congestion/rhinorrhoea, decreased appetite, headache, malaise, myalgia and pyrexia. Only one major concern was identified: the increased risk of wheezing in children aged <24 months, but only in those with a medical history of wheezing/asthma (study MI-CP111). This major

objection was solved by the Applicant's withdrawal of this pediatric subset from the proposed indication. Warning statements have been introduced in sections 4.2, 4.4 and 4.8 of the SmPC.

Other studies (AV010, D153-P514 and D153-P515) demonstrated that FLUENZ is safe in children >24 months of age, including children with mild/moderate asthma. However a potential risk of increase in severe wheezing/asthma events in subjects older than 24 months of age with a pre-existing history of wheezing or asthma cannot be totally excluded based on very limited data, therefore FLUENZ is not recommended in children and adolescents with severe asthma or active wheezing. Relevant warnings have been introduced in the SmPC (sections 4.4 and 4.8).

Based on the very limited safety data provided in immunodepressed children (HIV infected or not) due to the small number of subjects, no conclusion could be drawn about FLUENZ' safety in this specific population. As FLUENZ is a live-attenuated vaccine, the CHMP decided as a precautionary measure to contra-indicate this vaccine in all clinically immunodeficient patients regardless of the degree of severity, as outlined in section 4.3 of the SmPC.

It is not known whether there could be an increased risk of live attenuated vaccine virus dissemination in the brain of children with unrepaired craniofacial malformations following intranasal administration. A warning statement was introduced in section 4.4 of the SmPC.

A statement was introduced in section 4.8 of the SmPC to reflect that data are limited in children with pulmonary diseases other than mild to moderate asthma, or in children with chronic cardiovascular, metabolic or renal diseases. In studies of adults in which a high percentage of individuals had underlying chronic medical conditions, the safety profile of FLUENZ was comparable to the safety profile observed in individuals without these conditions.

The proportion of subjects who shed vaccine virus, the duration of shedding, and the amount of virus shed declined with increasing age of the subjects. Shedding began early (Day 1-2) after vaccination, and peak incidence was generally detected on Days 3-8. Shedding typically ceased within 12 days of dosing, even among infants and children, but in some cases was detected as late as Day 21. Peak titres of shed vaccine virus were >100-fold lower than the dose administered. The mean titres of shed virus were generally lower in the oldest age group relative to the youngest age group. Shedding in HIV-infected subjects was similar to shedding in non-HIV-infected subjects. One study on the risk of transmission of the vaccine virus showed that 1 placebo subject shed vaccine virus as a result of transmission of the Type B vaccine virus strain from a FLUENZ subject. Clinically significant illness did not occur in this subject. Thus, even in a population and setting that optimize the chance for transmission to occur, the observed transmission rate was low. Since the marketing of FLUENZ in the USA in 2003 and the distribution of over 10 million doses to date, there have been no confirmed postmarketing reports of FLUENZ virus transmission or of illness associated with FLUENZ virus transmission.

2.6.2. Conclusions on clinical safety

Overall, FLUENZ appears safe and well tolerated in subjects from 24 months of age onwards.

2.7. Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAH submitted a risk management plan (version 1.04)

Table Summary of the risk management plan

Safety concerns	Proposed Pharmacovigilance Activities (Routine and Additional)	Proposed Risk Minimisation Activities (Routine and Additional)
Identified Risks		
Medically significant wheezing in children < 24 months of age	• Passive surveillance Figure: 1. Targeted questionnaires	Children < 24 months of age will be excluded from the indicated population for FLUENZ. Routine Risk Communication Tools (e.g., SPC and Package Leaflet) will be provided to healthcare practitioners and vaccine recipients. This is included in the "Special warnings and precautions for use" and "Undesirable effects" section of the proposed SPC and also included in the Package Leaflet
Hypersensitivity disorders including Anaphylaxis	• Passive surveillance Figure: 2. Targeted questionnaires • Postmarketing studies Figure: 3. FM025 Figure: 4. MI-MA162 Figure: 5. MI-MA194	Routine Risk Communication Tools (e.g., SPC and Package Leaflet) will be provided to healthcare practitioners and vaccine recipients. This is a class effect that is included in the "Contraindications" section, and is listed in the "Undesirable Effects" section of the proposed SPC and also included in the Package Leaflet. The following statement is also in the "Special warnings and precautions for use" section of the proposed SPC: "As with most vaccines, appropriate medical treatment and supervision should always be readily available in case of an anaphylactic event following the administration of FLUENZ."
Potential Risks		
Guillain-Barré syndrome	• Passive surveillance Figure: 6. Targeted questionnaires • Postmarketing studies Figure: 7. FM025 Figure: 8. MI-MA162 Figure: 9. MI-MA194	Routine Risk Communication Tools (e.g., SPC) will be provided to healthcare practitioners and vaccine recipients. Stated in "Undesirable Effects" section of the proposed SPC that very rare reports of GBS have been observed in the post-marketing setting.

Safety concerns	Proposed Pharmacovigilance Activities (Routine and Additional)	Proposed Risk Minimisation Activities (Routine and Additional)
Bell's palsy	• Passive surveillance Figure: 10. Targeted questionnaires • Postmarketing studies Figure: 11. FM025 Figure: 12. MI-MA162 Figure: 13. MI-MA194	No risk minimisation activities are deemed necessary as this is not a confirmed identified risk.
Secondary transmission in severely immunocompromised patients	• Passive surveillance Figure: 14. Targeted questionnaires	Routine Risk Communication Tools (e.g., SPC and Package Leaflet) will be provided to healthcare practitioners and vaccine recipients. Included in "Special warnings and precautions for use" section of proposed SPC. Information is also found in the proposed Package Leaflet.
Inadvertent administration to immunocompromised patients	• Passive surveillance Figure: 15. Targeted questionnaires • Postmarketing studies Figure: 16. MI-MA162 Figure: 17. MI-MA194	Routine Risk Communication Tools (eg, SPC and Package Leaflet) will be provided to healthcare practitioners and vaccine recipients. Included in "Contraindications" section of proposed SPC. Information is also found in the proposed Package Leaflet.

Safety concerns	Proposed Pharmacovigilance Activities (Routine and Additional)	Proposed Risk Minimisation Activities (Routine and Additional)
Seizures/Convulsions	• Passive surveillance Figure: 18. Targeted questionnaires • Postmarketing studies Figure: 19. FM025 Figure: 20. MI-MA162 Figure: 21. MI-MA194	No risk minimisation activities are deemed necessary as this is not a confirmed identified risk.
Encephalitis	• Passive surveillance Figure: 22. Targeted questionnaires • Postmarketing studies Figure: 23. FM025 Figure: 24. MI-MA162 Figure: 25. MI-MA194	No risk minimisation activities are deemed necessary as this is not a confirmed identified risk.
Neuritis	• Passive surveillance Figure: 26. Targeted questionnaires • Postmarketing studies Figure: 27. FM025 Figure: 28. MI-MA162 Figure: 29. MI-MA194	No risk minimisation activities are deemed necessary as this is not a confirmed identified risk.
Vasculitis	• Passive surveillance Figure: 30. Targeted questionnaires • Postmarketing studies Figure: 31. FM025 Figure: 32. MI-MA162 Figure: 33. MI-MA194	No risk minimisation activities are deemed necessary as this is not a confirmed identified risk
Vaccination failure (lack of efficacy)	• Passive surveillance Figure: 34. Routine pharmacovigilance including lot/batch analysis	Routine Risk Communication Tools (e.g., SPC) will be provided to healthcare practitioners and vaccine recipients. Efficacy from controlled clinical studies is provided in "Pharmacodynamic properties" section of the proposed SPC.
Important Missing Information		

Safety concerns	Proposed Pharmacovigilance Activities (Routine and Additional)	Proposed Risk Minimisation Activities (Routine and Additional)
Severe Asthmatics	• Passive surveillance Figure: 35. Routine pharmacovigilance, • Postmarketing studies Figure: 36. MI-MA194	FLUENZ should not be administered to children and adolescents with severe asthma or active wheezing. Routine Risk Communication Tools (e.g., SPC and Package Leaflet) will be provided to healthcare practitioners and vaccine recipients. Included in "Special warnings and precautions for use" section of proposed SPC. Information is also found in the proposed Package Leaflet.
Pregnant and lactating women	• Passive surveillance Figure: 37. Routine pharmacovigilance • Postmarketing studies Figure: 38. FM025 Figure: 39. MI-MA194	FLUENZ is not recommended for use in women who are pregnant. FLUENZ should not be used during breastfeeding. Routine Risk Communication Tools (e.g., SPC and Package Leaflet) will be provided to healthcare practitioners and vaccine recipients. Included in "Fertility, pregnancy and lactation" section of proposed SPC. Information is also found in the proposed Package Leaflet.
Immunocompromised Vaccine Recipients	• Passive surveillance Figure: 40. Targeted questionnaires • Postmarketing studies Figure: 41. MI-MA162 Figure: 42. MI-MA175 Figure: 43. MI-MA194	FLUENZ is contraindicated in individuals that are clinically immunodeficient due to conditions and immunosuppressive therapy such as those listed in "Contraindications" section of SPC. Routine Risk Communication Tools (e.g., proposed SPC and Package Leaflet) will be provided to healthcare practitioners and vaccine recipients. Information is included in the "Contraindications" section of proposed SPC. Information is also found in the proposed Package Leaflet.
Children < 6 months of age	• Passive surveillance Figure: 44. Routine pharmacovigilance	FLUENZ is not indicated for use in recipients who are < 24 months of age. Routine Risk Communication Tools (e.g., proposed SPC and Package Leaflet) will be provided to healthcare practitioners and vaccine recipients. Included in "Special warnings and precautions for use," "Therapeutic indications," and "Undesirable effects" sections of proposed SPC. Information is also found in the proposed package leaflet.

Safety concerns	Proposed Pharmacovigilance Activities (Routine and Additional)	Proposed Risk Minimisation Activities (Routine and Additional)
Elderly people	• Passive surveillance Figure: 45. Routine pharmacovigilance activities	FLUENZ is not indicated for use in recipients who are18 years of age and older. Routine Risk Communication Tools (e.g., proposed SPC and Package Leaflet) will be provided to healthcare practitioners and vaccine recipients. Information is included in "Therapeutic indications" and "Pharmacodynamic properties."
Serious Chronic Disease	• Passive surveillance Figure: 46. Routine pharmacovigilance • Postmarketing studies Figure: 47. MI-MA194	No specific risk minimisation activities are deemed necessary for this population.

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.8. Benefit-Risk Balance

Benefits

- Beneficial effects

In children, the overall data gathered for FLUENZ are adequately convincing as both applicant's sponsored studies and independent published studies have consistently shown efficacy over placebo, as well as superiority over the TIV vaccine. Moreover the vaccine is well tolerated in subjects from 2 years of age onwards.

- Uncertainty in the knowledge about the beneficial effects.

As regards adult data, even though the data are suggestive of some degree of efficacy over placebo, unexplained inconsistencies (according to age strata, viral strain, seasonal period) are observed between and within studies which, along with a strong suggestion of inferiority over TIV in comparative published studies, preclude any firm conclusion to be drawn on the benefit of this vaccine in the adult population. These elements are strengthened by an a priori biological concern on the grounds that pre-existing immunisation could alter the efficacy of this LAIV vaccine. Indeed, in contrast to trivalent inactivated vaccine, LAIV vaccine induces an immune response through viral replication. Therefore, pre-existing immunity may negatively affect the response to live attenuated vaccine since existing neutralising antibodies may counteract the replication LAIV vaccine has to undergo in order to express its activity.

The *a priori* biological concern that pre-existing immunity or immunisation could alter the efficacy of LAIV was also discussed for the vaccine re-administration over time in the paediatric population. However the VWP has considered that based on the data available, this issue might not be as relevant for a paediatric and adolescent population as it might become for an adult and elderly population.

There are no efficacy data in individuals that are clinically immunodeficient but a decreased immunogenicity could be expected in this group.

Risks

- Unfavourable effects

The use of this vaccine does no raise any major safety concern.

The salient aspect of FLUENZ' safety profile is the risk of wheezing of particular concern in young children (below 24 months of age). Therefore the indication was restricted to individuals above 2 years of age.

- Uncertainty in the knowledge about the unfavourable effects.

A potential risk of increase in severe wheezing/asthma events in subjects under than 24 months of age at risk for complicated influenza (notably with a pre-existing history of wheezing or asthma) cannot be excluded. FLUENZ is therefore not recommended in children and adolescent with severe asthma or active wheezing.

It is not known whether there could be an increased risk of live attenuated vaccine virus dissemination in the brain of children with unrepaired craniofacial malformations following intranasal administration. Data are limited in children with pre-existing systemic illnesses.

Benefit-Risk Balance

- Discussion on favourable and unfavourable effects

In children:

This intranasal vaccine offers particular convenience over existing TIV vaccines, the efficacy is regarded as satisfactorily substantiated and the safety profile is considered as not raising any major issue under the precautionary measures taken by the applicant (restriction above 2 years of age, not recommended in patients with severe asthma or active wheezing).

In adults:

Given the biological plausibility that pre-existing immunity might negatively affect the efficacy of this intranasal live attenuated vaccine, there are theoretical grounds that adults might *a priori* not be an optimal candidate for this vaccine. This concern is reinforced by the sharp distinction of the efficacy data in children and in adults. Indeed, whereas in children the efficacy of this vaccine is consistently shown across studies (versus placebo and active comparator), in adults the intra (notably across age subgroup strata) and inter studies inconsistencies do not allow any conclusion to be made on the clinical benefit of FLUENZ and rather suggest that the efficacy of LAIV in this population would be lower than that of the TIV and overall would need further investigation.

- Benefit-risk balance

The overall benefit-risk balance of FLUENZ is considered positive only in individuals aged 24 months to less than 18 years.

2.8.1. Risk management plan

A risk management plan was submitted (version 1.04). The CHMP, having considered the data submitted, was of the opinion that pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns. No additional risk minimisation activities were required beyond those included in the product information.

2.9. Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of FLUENZ in the prophylaxis of influenza in individuals 24 months to less than 18 years of age and older was favourable and therefore recommended the granting of the marketing authorisation.